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BACTERIAL DEPURATION BY THE AMERICAN OYSTER (*CRASSOSTREA VIRGINICA*) UNDER CONTROLLED CONDITIONS

VOLUME I

BIOLOGICAL AND TECHNICAL STUDIES

by

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Principal Investigators for this project were Dexter S. Haven and Frank O. Perkins. Both small-scale and commercial-scale depuration experiments were designed and carried out by Reinaldo Morales-Alamo and Dexter S. Haven. Special bacteriological studies and monitoring of the depuration experiments were conducted by Martha W. Rhodes and Frank O. Perkins. The engineering design of depuration tanks and plants was prepared by Bruce Neilson.

The findings and conclusions are presented in two separate Volumes:

Volume I. Biological and Technical Studies

Volume II. Practical Considerations and Plant Design

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INTRODUCTION

Increasing acreages for oyster-growing bottoms in the United States are being made unfit for the profitable culture of shellfish because they are restricted for shellfish marketing. In Virginia, in 1975, there were 178,732 acres restricted. Much of this acreage is capable of supporting one or more of the following species: oysters, *Crassostrea virginica*; hard clams, *Mercenaria mercenaria*; soft clams, *Mya arenaria*; and the brackish water clam *Rangia cuneata*.

Of these four molluscs listed, the eastern oyster is the most important commercially. In 1975, about three million bushels of oysters worth about $4.70 per bushel were landed from Chesapeake Bay. In view of the volumes of oysters cultured in Chesapeake Bay and because of the ever increasing threat of bacterial contamination, emphasis was placed on this species.

In Virginia, oysters from restricted areas may be marketed after being held for 15 days in approved waters at temperatures above 10°C (50°F) under the supervision of an officer from the Virginia Marine Resources Commission. Relaying is expensive since there are two harvests; seldom can more than 80% of that which was originally placed on the bottom be recovered. Because of these aspects, depuration in tanks under controlled conditions is an attractive alternate to relaying under natural conditions. All oysters would be recovered in a depuration plant and holding time need not exceed 48 hours in most cases. Furthermore, improved regulatory control would ensure marketing of a safer product.

This final report summarizes the essential details of a three-year study begun at the Virginia Institute of Marine Science in 1973 to determine rates of depuration under the environmental conditions existing in Chesapeake Bay and to consider certain aspects of plant design. Not all the data collected were used in this report. The exclusions, as explained in the appropriate sections, were necessary because they involved techniques which make the data unreliable. The results of all our studies have been previously reported in eight triannual progress reports to the FDA. These sources contain detailed descriptions of the experimental design and other details not presented here. Where necessary, reference is made to those progress reports.

Depuration under controlled conditions has been accepted by the regulatory agencies of at least five states: Maine, Massachusetts, New York, New Jersey and Delaware. Facilities are presently being used in these areas to depurate soft clams and hard clams. Bacterial depuration of many species of bivalve molluscs is an accepted practice in England and other European countries.

As used throughout this report, the term depuration implies a reduction in the number of coliform bacteria found in oyster meats when samples of oysters from the same batch are analyzed through a progression of time intervals, usually of 24 hours each. The term is never used to imply that oysters have been cleaned to a specific or acceptable health standard. The term is also used to identify the processes and apparatus used to accomplish such a reduction in coliform bacteria.

This investigation was completed in two phases. First, laboratory studies were conducted using small, shallow plastic trays which held 25 to 36 oysters in a single layer with about two inches between them. Experiments in these trays provided information on depuration of oysters under conditions which excluded the crowding in large tanks. After completion of these experiments, tests were conducted in large tanks under conditions associated with commercial plant operations.

Distribution of this report does not preclude publication of selected portions elsewhere in a revised form.

SUMMARY

Oysters contaminated in nature depurated fecal coliform bacteria with consistency and a high degree of predictability. MPN levels below 50/100 g were achieved in 48 hours or less over a wide range of environmental conditions typical of the lower Chesapeake Bay region. Temperature was found to be the most critical environmental factor in the ranges normally found in the region.

At temperatures higher than 14°C groups of 25 to 36 oysters held in shallow trays reduced fecal coliform levels to an MPN less than 50/100 g in 24 hours from initial levels as high as 79,000/100 g. The same results were obtained at temperatures between 11.8 and 13.5°C, but mean initial MPN was never higher than 230/100 g because at those temperatures higher levels were not found in oysters contaminated in nature.

In four types of commercial-size tanks tested, holding from two to five bushels of oysters, oysters depurated fecal coliform bacteria to approximately 50/100 g in 24 hours from initial levels as high as 3,000/100 g. In the 2 x 8-foot tank, oysters depurated to under 50/100 g from a level of 39,000/100 g in 48 hours. Thus, time required to reduce fecal coliform numbers to a specified level was influenced by the initial fecal coliform level. Between 10.1 - 11.5°C, oysters with initial MPN levels of 200/100 g or less also depurated themselves to an MPN under 50/100 g in 48 hours and to an MPN of 25/100 g or less in 72 hours. Oysters with initial contamination levels higher than 200/100 g were not found at the times and temperatures at which these experiments were conducted.

Oysters contaminated in the laboratory with cultures of _Escherichia coli_ were able to reduce the concentration of that bacterium in their meats from a level as high as 500,000/100 g to less than 50/100 g in 24 hours at temperatures between 10.4 and 12.3°C. However, applicability of data based on artificial contamination with _E. coli_ to depuration of oysters contaminated in nature is questionable, because it is not known if results under these two conditions are comparable. They, nevertheless, point out to a high degree of depuration activity in oysters at those temperatures.

At dissolved oxygen concentrations of 1.8 mg/l and higher, oysters depurated themselves of fecal coliforms to MPN levels of 50/100 g or lower

in 24 hours in laboratory experiments in shallow trays. Oxygen concentrations between 1.1 and 0.6 mg/l slowed depuration rates, but did not prevent depuration in 72 hours. In commercial-size tanks, oxygen concentrations ranging from 2.8 mg/l (at a mean temperature of 27.4°C) to 9.6 mg/l (at a mean temperature of 10.5°C) did not have any differential effects on depuration. On the basis of these results it is recommended that dissolved oxygen concentrations in depuration tanks be maintained at levels higher than 2 mg/l if depuration is required in a period no longer than 72 hours.

Water flow rate into depuration tanks is a major factor contributing to maintenance of adequate oxygen levels. Although most of our depuration runs were conducted at flow rates around 2 gal/min/bu (GPM/Bu), results from several runs at 1 GPM/Bu gave similar results. It is advised that water flows not be lower than 1 GPM/Bu.

Turbidity levels ranging up to a mean of 77 mg/l over a three-day period did not have differential effects on depuration. Neither did mean salinities ranging between 14.0 and 21.4°/oo.

Depuration did not appear to be correlated with pumping rate and biodeposition activity of oysters. Oysters depurated equally well at low and high pumping rates and levels of biodeposition activity.

Oysters infected with the pathogens *Dermocystidium marinum* and *Minchinia nelsoni* (MSX) depurated as rapidly as uninfected ones.

Oyster size, meat quality and chlorophyll levels did not affect depuration in the ranges included in the study.

Tray-location in commercial size tanks did not influence depuration because water circulation patterns left no dead spots in the tanks.

All four commercial-size tanks of different design tested in these studies were found to be satisfactory for depuration of oysters in a period of 48 hours. One of the four, a narrow and relatively shallow flume, showed a bounce in MPN level at the end of 72 hours which was not present in the others and which was not attributable to factors other than tank design. Because of this, such a design is not recommended. The other three tanks performed well. One of them is recommended over the others only on the basis of simplicity of design and operation. It was constructed of 3/4-inch plywood and measured 8 feet in length, 4 feet in width and 18 inches in depth (water depth was held at 14½ inches).

At temperatures above 15.0°C oysters concentrated coliforms over levels present in the surrounding estuarine water with the greatest concentration of both total and fecal coliforms occurring between 15.1-20.0°C. Poor correlation existed between coliform levels in oysters and in shellfish-growing waters. However, as coliform levels increased in the harvest waters, oysters required a longer period of depuration to reach specified levels.

Biodeposits contain high levels of total and fecal coliforms, but apparently their accumulation in the tanks does not have a detrimental effect on the depuration process. Coliform levels in oyster wastes decrease with time but remain relatively high after 72 hours, posing a potential threat to successful depuration if they were to be agitated or resuspended.

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

The Medium A-1 test was superior to the elevated temperature coliform plate (ETCP) procedure for determination of fecal coliforms in oysters. When using the ETCP method, an inhibitory effect on the expression of fecal coliforms was observed which could be overcome by incubating oyster samples up to 72 hours. The Medium A-1 test resulted in a 24 hour determination of fecal coliform content with the occurrence of fewer false positives compared to the 72 hour standard procedure. In addition to the reduction of time and labor, Medium A-1 was also more economical in terms of cost of media and supplies.

DESCRIPTION OF GENERAL PROCEDURES

Description of certain techniques and procedures necessary to the understanding of this report are presented below. Further details may be obtained from the series of triannual progress reports prepared by us for the Food and Drug Administration.

Water Supply

Water was pumped from the York River to the laboratory where the depuration studies were conducted. The bacterial quality was monitored daily at the laboratory during the course of each experiment. The water always met the standards established by the U. S. Public Health Service for approved shellfish growing areas, i.e., <70 total coliforms/100 ml of water. Bacterial analyses of the water flowing out of the ultraviolet treatment units was also conducted daily. With very rare exceptions, the MPN level was indeterminate (<1.8/100 ml).

Source of Oysters and Preparation for Depuration

Oysters used in the experiments came from several locations: Tanyard Landing in the Poropotank River (a tributary of the York River), the York River proper, Pagan River (a tributary of the James River) and the Rappahannock River. The Virginia bushel, equal to 50 quarts, was the standard bushel measurement used. Oyster count averaged about 300 per bushel.

Often, oysters harvested from the above locations had very low coliform levels. Therefore, they were carried to Wormley Creek (a tributary of the York River located approximately 2.5 nautical miles downriver from Gloucester Point) where they were held in wire trays for periods ranging from two days to two weeks. The usual exposure period was two to three days. Coliform levels in oysters were usually high after that exposure period.

Artificial Contamination of Oysters

During periods when water temperatures in nature dropped below 14°C, we were unable to obtain oysters with fecal coliform levels high enough to provide an adequate test for our depuration experiments. To secure such oysters, we contaminated them in the laboratory with diluted sewage solutions from the Hampton Roads Sanitary District treatment plant at Menchville, Newport News, Virginia. The material collected had gone through the full treatment except for chlorination.

There was much difficulty in controlling the resulting level of coliforms in the oysters following addition of the sewage dilutions. In most cases, it was too high; in some, too low. The data obtained in depuration of oysters contaminated in this manner were also erratic. Therefore, only one of these experiments is included in this report.

Subsequently, oysters were also contaminated in the laboratory by addition of pure cultures of Escherichia coli to standing water in a tank. This contamination process was satisfactory and oysters depurated with more predictability.

Contaminated oysters brought to the laboratory for depuration were hosed down thoroughly with tap water and gapers and dead oysters removed before they were placed in the trays or tanks.

Procedures Used During Depuration

At the end of every 24 hour period of depuration, oysters were taken out, and both oysters and tanks or shallow trays were washed down with tap water. Gapers and dead oysters were removed. Water samples for bacteriological analysis were usually collected at these 24 hour intervals. In several experiments, the large tanks were drained and refilled at additional intervals between these 24 hour periods.

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.

Intensity of ultraviolet lamps was monitored weekly with a Model No. J225 Ultraviolet meter (Ultraviolet Products, Inc., San Gabriel, Calif.).

Bacteriological Analysis

Samples for bacteriological analysis usually consisted of six to eight oysters. They were usually analyzed as a pool but in several instances they were analyzed individually. Total and fecal coliform levels in these samples were determined using the MPN (Most Probable Number) technique (5 replicate tubes for dilution) according to the procedures outlined in APHA (1970). Levels below the limits of the technique as used were labelled indeterminate (ID) and were equivalent to <18/100 g for oysters and <1.8/100 ml for water.

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 throughout the text of this report, 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 a MPN value $\geq$ than a certain figure the resulting computed mean for the group would be preceded by the same symbol ($\geq$). When both ID and $\geq$ values were present, the mean is preceded by the symbols $\frac{>}{<}$.

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. When the symbol preceding the MPN value is $\frac{>}{<}$ then the plotted value has an arrow pointing downward and another 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 equal to 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.

The data were also summarized in terms of the percentage of samples with values equal to or less than given MPN levels of total and fecal coliforms. The MPN levels chosen for total coliforms were 500, 230 and 100. Chosen levels for fecal coliforms were 100, 50 and 20.

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.

A summary of the means for measurements recorded for environmental factors during individual experiments is given in Table 1.

PART I. OYSTER DEPURATION STUDIES USING SMALL NUMBERS OF OYSTERS UNDER LABORATORY CONDITIONS

Introduction

The initial phase of these studies involved laboratory experiments. They were designed to find out the effect of different environmental conditions on depuration of oysters. Oysters were subjected to ranges of one parameter while others were maintained at optimal levels. The acrylic plastic (Plexiglas(R)) trays used to hold the experimental molluscs measured 32 inches in length, 21 3/4 inches in width and 3 3/8 inches in height (Figs. 1 and 2). They held from 25 to 36 oysters with spaces of one to three inches between adjacent animals. Thus, the trays provided each oyster with more than adequate conditions in terms of spacing and water supply.

These studies in shallow trays give an indication of the intrinsic capability of individual oysters to depurate coliform bacteria. Subsequent tests in large tanks showed the extent to which such capability applied to the conditions encountered in a commercial-scale depuration plant.

A total of 79 experiments was carried out in shallow trays. Of these, 18 were conducted in smaller acrylic plastic trays which held 12 oysters each. These were the first tests made under this contract and are discussed in the progress report for the period 16 September 1973 to 15 February 1974. Only one of these experiments is included here. The rest were excluded because all but five of the experiments were conducted in a recirculating system. The other five using running water were also excluded because oysters used were contaminated with sewage in the laboratory. Furthermore, the initial MPN levels of fecal coliforms were very low. The one experiment used (Expt. 2) involved oysters infected with the pathogens Dermocystidium marinum and Minchinia nelsoni (MSX).

Sixty-one experiments were completed in the trays that held 25-36 oysters each. Only 44 of them are included here. The other 17 were conducted with oysters contaminated with sewage in the laboratory, and we wished to depend on data gathered from oysters contaminated in nature as much as possible. However, one experiment with oysters contaminated with sewage in the laboratory was included in the data used to analyze the effect of pathogenic organisms on depuration of oysters (Expt. 40).

All experiments in shallow trays were conducted at relatively high flows (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 could be assessed without concern for food and oxygen levels, except when the latter was intentionally lowered.

The ultraviolet lamp unit designed and constructed by us for use with these shallow trays was 48 inches long, 26 inches wide and 1 3/4 inches deep (Figs. 3 and 4). It held four 34 inch long UV lamps (effective length was 30½ inches).

Details of experimental procedures and apparatus used in these experiments appear in the progress reports for the periods 16 February to 15 June 1974, 16 June to 15 October 1974, 16 October 1974 to 15 February 1975 and 16 February to 15 May 1975.

Results of these studies were analyzed for the effect of different environmental factors and other conditions and are presented in seven separate sections in the pages that follow.

SECTION I

Temperature

Introduction

Temperature influences the rates of water transport through the gills, feeding, respiration and other physiological functions of oysters. Ciliary activity declines above 32°C and below 12°C. Long Island oysters cease filtration of solids from the water at temperatures below 7°C (Galtsoff, 1964). The quantity of feces and pseudofeces voided by oysters in Chesapeake Bay declines at 7°C to 85% of the production in summer months; at 3°C, volumes voided were nearly zero (Haven and Morales-Alamo, 1966).

In the Chesapeake Bay region, water temperatures range from near zero in winter to slightly over 32°C in late summer. Therefore, it was suspected that there would be a period of time in winter when depuration of oysters should not be attempted unless the water was heated. The effect of temperature on depuration of oysters was investigated under varying conditions of salinity, oxygen, turbidity and water flows past the oysters to test this hypothesis. Although these variables interact with each other, we have analyzed temperature in more detail than the other factors because its effect appeared to be the most critical.

Materials and Methods

Most of the experiments on the effect of water temperatures on the depuration of oysters were conducted at ambient water temperatures during different seasons in our laboratory at Gloucester Point. River water was heated in three experiments. In those, water from the heat exchangers was directed to a six foot long cascading trough where air was bubbled into two of the first compartments. This procedure eliminated supersaturation of the water with oxygen. Water temperature was lowered, when required, by passing the water through a coil immersed in a constant temperature water bath holding cold water.

Experiments were grouped according to mean initial MPN level in the oysters and mean water temperature. Initial level categories were chosen arbitrarily. Temperature measurements were divided into classes of five degrees each above and below a temperature of 19°C. This temperature was chosen as a pivotal point because spawning of oysters begins at a temperature of 20°C. Therefore, above and below 19°C there are differing physiological activities in the oyster which could conceivably affect the results obtained during the purification process.

Placement of an experiment into any of the temperature categories was based on the mean of all temperature measurements made during the three-day experiment. The initial coliform level in an experiment was represented by the geometric mean of all samples from the same batch of oysters analyzed at 0 hour.

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 because our analyses indicated that their effect was minimal in the ranges included. The low oxygen levels had a decided adverse effect on depuration and were considered separately.

Results

Total coliforms. Results in terms of total coliforms were erratic. In most cases, there was wide variation around the mean and the slopes of corresponding sections of different decay curves often diverged from each other.

At initial levels <1,000 (Fig. 5) MPN levels were reduced to 100 or less in 24 hours in the temperature ranges 9-14°C (actual range: 11.8-13.5°C), 14-19 (actual range: 14.8-17.9°C) and 19-24°C (one expt., mean temperature = 19.6°C). At a temperature range of 24-29°C (actual range: 25.8-27.6°C) the MPN level was slightly higher than 230 at 24 and 48 hours but was under 100 at 72 hours.

At initial levels between 1,000-10,000 (Fig. 6) MPN levels were reduced to less than 100 in 24 hours at temperature ranges 19-24°C (mean temperature = 21.9°C) and 24-29°C (actual range: 25.0-26.7°C). In the temperature range 9-14 (mean temperature = 10.4°C) the MPN level was never reduced below 490 and the 48- and 72-hour levels were higher than that at 24 hours. At temperatures between 14-19°C (actual range: 15.7-19.1°C) levels were slightly over 230 at 24 and 48 hours and under 200 after 72 hours.

At initial levels >10,000 (Fig. 7) MPN levels were reduced to less than 100 in 24 hours at temperatures between 24-29°C (actual range: 24.8-25.8°C). Levels remained under 100 at 48 and 72 hours. In the temperature range of 14-19°C experiments were divided into three different mean initial MPN levels. At the lowest (11,000, mean temperature = 17.8°C) the MPN level was reduced to 210 in 72 hours (no samples were collected at 24 and 48 hours). At the next highest level (130,000, mean temperature = 14.7°C) the MPN level decreased to less than 63 in 24 hours, was slightly lower at 48 hours but went up to 290 after 72 hours. At the highest initial level (920,000, mean temperature = 17.0°C) the MPN level was only slightly under 1,000 after 72 hours.

In summary, the erratic nature of the total coliforms MPN in shallow tray experiments resulted in wide variation around the mean and in a lack of consistency of most of the data for the various temperature ranges at the different initial level categories. For that reason, the data do not permit establishment of end point levels at any given combination of initial MPN level and mean temperature.

Fecal coliforms. Data collected for fecal coliform MPN at the different temperature-initial level groupings followed more regular patterns and were more consistent than those for total coliforms (Figs. 8 through 10; Table 3). With a single exception, at all combinations of temperature and initial MPN level, MPN values were under 50 after 24 hours of depuration and remained below that level through the subsequent 48 hours of depuration.

The only exception was the initial MPN level of 1,001-5,000 at temperatures between 14-19°C (Fig. 10). In that case, the 24 hour MPN was 68. However, after 48 hours, it was 45. These values represent a single sample each. The mean MPN, based on five samples, after 72 hours 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 temperature = 14.7°C) and initial level of 79,000, the MPN value was reduced to <18 in 24 hours and stayed at that level for the next 48 hours of depuration (Fig. 10).

Initial mean MPN levels in the mean temperature range of 9-14°C were all under 500 (Fig. 8). MPN values in the five samples included in that range were: <18, 68, 230, 230 and 230. This was due to an absence of higher fecal coliform counts in oysters collected in nature from waters in that temperature range. MPN values for fecal coliforms ranged from <18 to 390 in samples of oysters from four locations at water temperatures under 15°C (Table 4). It appears, therefore, that at temperatures under 14°C fecal coliform MPN levels greater than 500 occur infrequently in oysters from tributaries of the Chesapeake Bay.

The temperature range 9-14°C actually involved a range of mean temperatures between 10.4-13.5°C. Combined data showed that oysters were depurated of fecal coliforms from a mean MPN level of 110 down to a level of <20 in 24 hours and remained at approximately the same level for the next 48 hours (Fig. 8). Eight of the nine (89%) samples analyzed after 24 hours of depuration had an MPN value of <18 (Table 3). The exception was a sample from a single experiment conducted at the lowest mean temperature studied (10.4°C). In that experiment (Expt. 11) the initial level was 68. Although after 24 hours the MPN level was 78, it had decreased to <18 after 48 hours.

The next lowest mean temperature was 11.8°C and it also involved a single experiment with replications in two separate trays (Expt. 46-2). In that experiment, with an initial MPN of 230, duplicate samples from each of the two trays showed levels of <18 after 24, 48 and 72 hours in every case.

In summary, at a temperature range of 14° to 29°C oysters were depurated of fecal coliforms from MPN levels as high as 10^4 down to less than 50 in 24 hours (Fig. 10). The same was found at temperatures as low as 12°C when initial MPN levels were 230 or less.

Discussion

This discussion will be concerned exclusively with fecal coliform data. Results for total coliforms will not be discussed any further since the wide variations associated with the means and the erratic decay curves do not permit concrete conclusions.

At temperatures higher than 14°C oysters were able to depurate themselves of fecal coliforms to an MPN of <50 in 24 hours from initial levels of contamination as high as 79,000. After 48 hours there were many instances in which the MPN level had decreased to less than 20. However, because a few samples still showed counts of <50, we can not say that levels <20 can be attained in 48 hours in all instances.

At temperatures between 12° and 14°C oysters also depurated themselves to a level of less than 50 in 24 hours when mean initial levels were 230 or less. No conclusive data for depuration at temperatures under 12°C were collected. One experiment was conducted at such temperatures but the initial level was too low (68) to provide a satisfactory test. There was a reduction in MPN level to <18 in 48 hours and this suggests that depuration may be possible at temperatures close to 10°C.

SECTION 2
Dissolved Oxygen

Introduction

An adequate supply of dissolved oxygen (DO) is a basic necessity for all molluscan life and if supplies are not adequate, oysters may fail to depurate, or may even die. A DO level lower than 0.8 ml/l (1.1 mg/l) may result in a 50% decline in the production of feces and pseudofeces and at 0.3 ml/l (0.4 mg/l) production of these biodeposits virtually stops (Haven and Bendl, 1975). As oxygen level approaches zero, hydrogen sulfide is produced and it can be lethal to oysters in a day or less (Chen and Morris, 1971; Haven and Bendl, 1975).

In Chesapeake Bay and its tributaries, DO values may fall to nearly zero during the summer in restricted coves or at depths greater than 20 feet in open water. However, surface water DO in open estuaries seldom falls below 3 ppm.

During feeding and respiration, oysters pump large volumes of water through their gills. A 3 to 4 inch oyster may pump up to 34 l/hr (Loosanoff and Nomejko, 1946). Over a 24 hour period, total volumes pumped may range from 9 to 239 liters (Galtsoff, 1964). From this water, oysters remove suspended solids to be used as food and extract oxygen for respiration. Oxygen uptake by <u>Crassostrea virginica</u> has been measured at 2.8 ml/hr/oyster at 24-25°C for 4 inch oysters (Galtsoff, 1964). This uptake is influenced by both temperature and oyster size.

Since volume flow rate of water past oysters held in tanks is a factor influencing the quantity of oxygen available to the oysters it must be taken into consideration along with oxygen in depuration of oysters. It is possible for volume flow of water to be so low in relation to the volume of oysters in the tank that DO levels could be reduced by oyster respiration to levels detrimental to the oysters and, consequently, to their depuration.

However, an excess of oxygen in solution can also interfere, under special circumstances, with the activity of oysters and their depuration. There is an upper limit to oxygen solubility in sea water at different combinations of water temperature and salinity. Solubility decreases with a rise in temperature and with an increase in salinity. When cold sea water is warmed up (as would have to be done if using a flow-through system during winter) it won't be able to hold as much oxygen and other gases in solution as when it was cold. The excess gases are then released as very small bubbles which may cause the death of oysters by embolism. In such a case, provisions have to be made for allowing the excess gas bubbles to escape before the water flows over the oysters. Monitoring of dissolved oxygen in the water is, therefore, an essential part of operation of a depuration plant.

Materials and Methods

A full description of the apparatus and techniques used in these experiments is given in the progress report for the period 15 February to 15 June 1974.

Ambient DO concentrations were lowered to the desired levels by bubbling nitrogen through river water in a three-inch diameter cylinder before if flowed into the experimental trays, following the method of Silver, Warren and Doudoroff (1963). Oxygen tension of the water in the oyster trays was measured with a YSI oxygen polarographic probe and meter.

Daily measurements were made of the quantity of feces and pseudofeces produced by oysters held at various dissolved oxygen levels.

Results

Fecal coliform levels in oysters were reduced to less than 50 in 24 hours at DO concentrations of 1.8 mg/l and higher in experiments conducted at water temperatures of 26°C with initial levels as high as 2100 (Table 5, Fig. 11). At oxygen concentrations higher than 0.8 mg/l fecal coliforms were reduced to less than 50 in 48 hours. When oxygen concentration was reduced to 0.6 mg/l the MPN was reduced from 2100 to less than 50 in 72 hours.

At temperatures between 14-19°C the oxygen concentrations tested ranged from 7.5 to 9.5 mg/l. In twelve experiments with a mean initial MPN of 750, the MPN of fecal coliforms was lowered to less than 50 in 24 hours (Table 5, Fig. 12). In the temperature range of 9-14°C (oxygen concentration ranging from 9.0 to 10.2 mg/l) the mean initial MPN of fecal coliforms in four experiments was very low (<34). MPN after 24, 48 and 72 hours was under 23 (Table 5, Fig. 13).

Depuration of total coliforms was erratic in relation to dissolved oxygen concentrations. In the temperature range of 19-29°C the MPN was lowered to less than 230 in 48 hours at oxygen concentrations of 0.8 mg/l and higher (Table 6, Fig. 14). Between 14-19°C the MPN was slightly higher than 230 after 24 and 48 hours and under that figure at 72 hours (Table 6, Fig. 12). At temperatures between 9-14°C the MPN was higher after 48 hours than at 0 hour and higher at 72 hours than at 48 (Table 6, Fig. 13).

Oysters held at oxygen concentrations under 1.1 mg/l did not produce any feces or pseudofeces.

Mean water flow rates in shallow tray experiments ranged from 1.7 to 8.0 GPM/Bu. Within these ranges there was no indication that flow rates affected the results obtained (Figs. 15 and 16).

Discussion

The quantity of oxygen available to oysters affects their physiological activity. Most marine bivalves, including oysters, are able to maintain their oxygen consumption constant over a wide range of oxygen concentrations (Walsh, 1974). The effectiveness with which such regulation is achieved is subject to time limitations at different oxygen levels. Therefore, it is essential that oxygen supply available to oysters in depuration tanks be sufficiently high to prevent stresses thay may affect adversely the rates at which they depurate.

Experiments at VIMS with small oysters have shown that at temperatures between 22° and 24°C and oxygen concentrations between 0.8 and 1.2 mg/l (0.6-0.9 ml/l) mortality was 14% in 13 days and there was a marked reduction of biodeposition (Haven and Bendl, 1975). At 1.1 mg/l (0.8 ml/l) fecal production was about half of that shown by controls.

In our experiments, at mean DO levels of 1.8 mg/l and higher, oysters depurated fecal coliform bacteria to MPN levels of 50 or lower in 24 hours. Below 1.8 mg/l depuration took a longer time. At a mean DO level of 1.1 and 0.8 mg/l, depuration to values less than 50 MPN took 48 hours and at a mean of 0.6 mg/l, 72 hours were required to reach the same MPN level.

The results of these studies and those of the other investigations cited above suggest that dissolved oxygen concentrations in depuration tanks should be maintained above 2 mg/l to ensure satisfactory depuration.

A major factor contributing to maintenance of adequate oxygen levels in depuration tanks is the rate of water flow. Water flow must be high enough to avoid reduction of dissolved oxygen to a level that could be detrimental to depuration as a result of respiration by a large number of oysters. Furfari (1966) calculated that the required volume flow for a bushel of 500 oysters was 1.04 GPM/Bu under the following conditions: a saturated volume of dissolved oxygen of 8.1 ml/l at 15°C and a salinity of 36°/oo. Our experiments in shallow trays were conducted at flows higher than 1.04 GPM/Bu to ensure an adequate supply of dissolved oxygen and food to the experimental animals so that the effect of other factors could be assessed without concern for food and oxygen levels, except when the latter was intentionally lowered.

SECTION 3
Turbidity

Introduction

Levels of suspended solids (turbidity) in water may, if they are too high, have an adverse effect on oyster activity and, therefore, on depuration. Consequently, the effect of varying turbidity levels on the depuration process was studied in the laboratory to determine if suspended solids (within the range normally encountered in Chesapeake Bay) inhibited depuration.

In Chesapeake Bay, over productive oyster beds, natural turbidity near the bottom ranges from less than 5 to about 65 mg/l with an average range from about 31 to 36 mg/l (Brehmer and Haltwanger, 1966; Nichols, 1972; Haven and Morales-Alamo, 1972). Most of these solids are inorganics in the clay-silt size group.

In relation to these sediment loads, Loosanoff (1961) showed that levels over 100 mg/l of clay inhibited oyster activity; no negative effect was found below this level. In contrast, Mackin (1962) found that oysters continued their pumping activity in waters with a suspended solids concentration as high as 700 mg/l of silt.

Materials and Methods

Turbidity of the water in most of these experiments was that occurring naturally. In some experiments, however, various levels of finely-ground suspensions of the clay mineral kaolinite were added to the water to obtain higher turbidity levels. Controls were maintained which received only river water without additives. A full description of the techniques utilized appears in the progress report for the period 15 February 1974 to 15 June 1974.

Results

In our laboratory studies, oysters with initial fecal coliform MPN levels between 18 to 7100, and subjected to mean solid levels ranging up to 77 mg/l over a temperature range from 12° to 27°C, depurated to an MPN of less than 50 in 24 hours or less (Table 7; Figs. 17-19). At the end of 24 hours at least 93% of the samples showed MPN fecal coliform levels under 50.

While oysters depurated fecal coliform bacteria over wide limits, the same oysters subjected to the same range of solids and temperatures did not depurate total coliforms with any degree of regularity (Table 8; Figs. 20-22). Some depurated to levels below 230 in 24 to 48 hours, but others did not. In some instances, levels increased. The variations in results showed no relation to turbidity. We concluded that results obtained with total coliforms were the result of factors other than those associated with turbidity.

Discussion

The levels of suspended solids normally present in Chesapeake Bay will not inhibit depuration of fecal coliform bacteria in a 48 hour period. Local areas, however, may exist where waves or currents may increase turbidities to levels in excess of those included in our studies. These areas should be avoided when locating a depuration plant.

Two aspects associated with turbidity levels need further comment. During their feeding process, oysters in depuration tanks may remove up to about 80 to 90% of the suspended solids in the inflowing water and deposit them as biodeposits on the bottom of the tank (Haven and Morales-Alamo, 1970). Although the quantity of the solids deposited does not influence the depuration process, biodeposits contain high levels of fecal coliform bacteria and should be removed daily to avoid recontamination of water (See Section 2 of Part III).

High levels of suspended solids in the water interfere with proper operation of ultraviolet treatment units by absorbing the radiation and shielding bacteria from it. Therefore, the treated water must be monitored to make sure that turbidity does not interfere with effective radiation of the water.

SECTION 4
Salinity

Introduction

Oysters live and reproduce successfully over a wide range of salinities. In Chesapeake Bay, they occur over a mean salinity range from 5^{o}/oo to slightly over 32^{o}/oo. Because of this tolerance, it was expected that they would depurate over a wide range of salinities and this proved to be the situation.

Materials and Methods

The data presented here are based on the same experiments used in other sections except that they have been arranged according to salinity.

Results

Total coliforms. Mean total coliform MPN levels were reduced to below or close to 230 in 48 hours at all salinity ranges in the temperature range of 19.1-29.0°C (Table 9). Actual salinity range included was 14.0-21.4°/oo. In the temperature range of 9.1-19.0°C (salinity range included was also 14.0-21.4) results were erratic. In several cases mean MPN increases at 48 and/or 72 hours even though significant decreases had been seen earlier. No trend is evident that would indicate that some salinity levels in the range of 14.0-21.4°/oo would affect oyster depuration differently from others.

Total coliform data presented in Table 9 are also characterized by wide variations around the means. Those wide variations further reduce the utility of the data for prediction of depuration with time at different salinity levels.

Fecal coliforms. In the range included in these experiments (14.0-21.4°/oo), salinity showed no differential effect on depuration of oysters. At all combinations or ranges of temperature, salinity and initial levels, the mean MPN of fecal coliforms was reduced to <50 in 24 hours in 36 of 38 experiments (Table 10). The two exceptions had values of <61 and <68. In all experiments the mean MPN was <50 in 48 hours.

Discussion

Oysters depurated fecal coliforms over a salinity range from 14.0°/oo to 21.4°/oo to an MPN less than 50 in 24 to 48 hours. In all probability, depuration above 21.4°/oo would be equally practical since there is no evidence in the literature that salinities from 25°/oo to 32°/oo inhibit oyster activity (Galtsoff, 1964). The lower limit for depurating oysters has not been determined since ambient salinities at Gloucester Point did not go below 14°/oo at the time our studies were made. However, 10°/oo is the suggested lower limit for depuration of oysters in the Gulf of Mexico region (Huntley and Hammerstorm, 1971). There is no reason to believe that oysters in Chesapeake Bay will fail to depurate above that salinity.

Depuration of oysters when there is a difference in salinity between the growing area and that the depuration plant was not investigated. Galtsoff (1964) states, "when the salinity change is about $10^{o}/oo$ and continues for several hours, both the rate of water transport (through the gills) and the time the oyster remains open are decreased". Therefore, a difference of $10^{o}/oo$ or greater between the salinity at the site of harvest and that at the depuration plant site is not advisable. In the Chesapeake Bay region, a depuration plant should be located where mean salinities average about $15\text{-}20^{o}/oo$, so that oysters from all regions of the bay might be depurated without adversely influencing the process. If salinity differences become greater than $10^{o}/oo$, then bacteriological tests should be made during depuration to monitor the process.

SECTION 5

Effect of the oyster pathogens *Dermocystidium marinum* and *Minchinia nelsoni* (MSX) on depuration rates.

Introduction

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).

Materials and Methods

In a series of studies, two populations of oysters, infected and uninfected, were placed in shallow trays and processed separately for total and fecal coliform levels per 100 g of tissues at 0, 24, 48 and 72 hours after placement in the depuration system. Experiments were conducted using oysters which were contaminated by holding them in a polluted estuary or which had been exposed to sewage in the laboratory.

Intensity of infections was evaluated for *D. marinum* using the Ray method (Ray, 1954) except that his categories 1 and 2 are considered herein as heavy, categories 3 and 4 are called moderate, and categories 5 and 6 are called light. Infections were induced by adding zoospore suspensions to oysters held at 25-30°C in aquaria with York River water. Incubation times were 2 to 4 weeks. MSX infections were determined by examination of histological slides of oysters fixed in formalin-alcohol-acetic acid, sectioned, and stained in hematoxylin-eosin. Estimates of numbers of plasmodia in each oyster were semi-quantitative evaluations; however, by this method approximate judgments of the extent of stress by the pathogen could be made. Heavy infections were those in which at least one plasmodium could be seen in 76-100% of the fields of view at 400X magnification using a 40X objective (excludes view of adductor muscle and heart). Moderate infections were those in which 25-75% of the fields of view at 400X contained one or more plasmodia. Light infections ranged from those in which only one plasmodium was seen in a cross-section of an oyster, up to those in which 24% of the fields of view at 400X contained one or more plasmodia.

Results and Discussion

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, the infected oysters (including heavily infected ones) depurated as rapidly as the uninfected ones (Figs. 23-26 and Tables 11-14). Gaping oysters were not included in the study. *M. nelsoni* does not cause necrosis of tissues, but nevertheless, causes a lowered physiological condition which is reflected in lowered wet weights of oyster meats and ultimately may result in death. As with *D. marinum*, depuration rates were not different from uninfected oysters. Table 15 shows the number of infected oysters and levels of infection involved in the experiments.

SECTION 6

Pumping Rate and Biodeposition Activity

Introduction

Oysters pump large volumes of water through their gills to extract particulate food material and oxygen for respiration. Previous investigations have shown that volumes pumped by 3 to 4 inch oysters ranged from 9 to 239 l/day (Galtsoff, 1964) and up to 34 l/hour (Lossanoff and Nomejko, 1946).

From this water, a single oyster may remove up to 3.9 g/week of solids, dry weight (Haven and Morales-Alamo, 1966). These solids are voided as feces or pseudofeces. Feces are strings about 1 mm wide and variable in length after having been compacted in the oyster's gut. Pseudofeces are loosely aggregated clumps of material rejected at the oyster's mouth and ejected by action of the valves.

Feces and pseudofeces are termed biodeposits and the quantity produced is an index of the biological activity of oysters since it is a reflection of the pumping activity of the oyster. Quantities of biodeposits produced (primarily pseudofeces) are also a reflection of the turbidity in the water (Haven and Morales-Alamo, 1966).

Materials and Methods

Pumping rate and biodeposition activity were measured separately in two series of experiments. In our experiments, pumping rates could not be correlated with production of biodeposits because the apparatus used for measurement of pumping rate did not permit collection of biodeposits.

In pumping rate experiments oysters were held in specially designed aquaria and each oyster was fitted with a cone-shaped plastic apron that directed water passing through the gills to an overflow tube in an adjoining chamber (Figs. 27 and 28). Volume of overflowing water was measured for a 30 second period every two hours during the day time and the corresponding pumping rate computed. Figure 29 shows the experimental apparatus. This technique is an accepted method used by other investigators (Moore, 1910; Nelson, 1936; Nelson, 1938 and Galtsoff, 1964). Flow of water past the oysters in the aquaria ranged from 26.4 to 74.4 l/hour.

The following measurements were recorded for environmental parameters in the course of the pumping rate experiments: mean temperature = 19.1-19.5^{o}C, mean salinity = 20.4-20.8^{o}/oo, mean DO = 6.8-7.0 mg/l, and mean turbidity = 15.6-17.5 mg/l.

Biodeposits were collected in five experiments. In two, oysters had been infected in the laboratory with _Dermocystidium marinum_ and in another they were infected with _Minchinia nelsoni_; in the other two, oysters were not infected. Every 24 hours oysters were carefully removed from the trays after water flow had been stopped and feces and pseudofeces for each oyster collected for measurement into separate test tubes.

Classification of oysters into activity categories (see progress reports for periods 16 June-15 October 1974, p. 24 and 16 October 1974 to 15 February 1975, p. 3) was determined by the quantity of biodeposits produced by each oyster on a per day basis, i.e., quantities produced on every day the oyster was held in the tray were added together and divided by the number of days. However, the activity of an oyster during each individual 24 hour period was considered before it was selected for analysis. Thus, an oyster in a 72 hour sample was classified as inactive only if it had been inactive in each of the two other preceding 24 hour periods.

Results

Pumping rates. The mean pumping rate of the 19 oysters studied ranged from 1.41 to 10.50 l/hour (Table 16). However, the variation of individual measurements was very great. In several oysters (those with the highest mean values) it ranged from less than 5 to greater than 15 l/hour. Figures 4 and 5 of our progress report for the period 15 June to 15 October 1974 illustrate the variations found. Within that range, all but one of the oysters depurated themselves of fecal coliform bacteria below an MPN of 50 in 24 hours. The single exception had a mean pumping rate of 5.97 l/hour and showed a count of 340 at 72 hours.

Seventeen of the 19 oysters depurated themselves of total coliform bacteria to an MPN of 230 or below in 24 hours. The two above that level had pumping rates of 5.97 and 6.50 l/hour.

Biodeposition. There appeared to be no relation between quantities of biodeposits produced and reduction of total and fecal coliform bacteria. Oysters with very low or zero production of biodeposits depurated themselves of fecal coliforms as well as those with high to moderate production at any of the initial levels and depuration time groupings (Table 17). The percentage of oysters with very low or zero biodeposition showing an MPN equal to or less than 20 ranged from 67 to 100 at all depuration times (24, 48 and 72 hours).

Likewise, oysters which produced very low quantities of biodeposits, or none at all, were able to reduce the MPN of total coliform bacteria to 230 or less in 24, 48 or 72 hours (Table 18). The number of inactive oysters analyzed was small (14 out of 214). However, 10 of them had reduced the MPN to 230 or less in either 24 or 48 hours. There was no evidence that more active oysters depurated significantly better than the inactive ones.

Discussion

Oysters with mean pumping rates of around 1.5 l/hour depurated as well as those with mean pumping rates of 5 or 6 l/hour (the latter often showed instantaneous rates as high as 15 l/hour). These pumping rates are significantly lower than those found by other investigators as summarized by Jörgensen (1955). It may be said that a high pumping rate is not necessary for an oyster to clean itself of fecal coliform bacteria.

The pumping rate of oysters is extremely variable. It is affected by changes in natural environmental factors but it may also show variability in the absence of such changes (Galtsoff, 1964). There was considerable variation in the pumping rate of oysters in our experiments both among the individual animals as well as among individual measurements for each oyster. However, variations were very small in oysters with low pumping rates (less than 2 l/hour) and for those, the mean is a fairly precise measurement of their pumping activity.

The results obtained when production of biodeposits was compared with depuration rates agree with those obtained in pumping rate studies. Fecal coliform levels were reduced to MPN levels which may be considered acceptable (less than 50) in oysters which produced no biodeposits while held in depuration trays. Since biodeposition is directly related to the pumping and filtration activity of the oysters, reduced biodeposition implies reduced filtration and very probably reduced pumping. It appears that oysters do not have to be active in terms of pumping rate and biodeposition to depurate themselves of fecal coliform bacteria. Therefore, pumping rates and biodeposition rates are not good indices of effective depuration.

SECTION 7

Effects of Oyster Size, Meat Quality, Source of Oysters, and Amount of Food in the Water on Rates of Depuration

The effects on depuration rates of oyster size, meat quality, source of oysters and amount of food were also investigated. As reported in our previous progress reports, 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 MPN by 48 hours.

There follows a brief summary of the ranges investigated:

1. Oyster size - Oysters ranged from 2 to 5 inches in length during these studies. No difference in depuration rate related to size was noted.

2. Meat quality - In Virginia, meat quality is measured as condition index (Haven, 1960). The mean range of indices investigated was from 7.2 to 10.8. No difference in depuration was evident in that range.

3. Source of oysters - Oysters came from four different river systems during this study. In no instance was there a difference in depuration which could be attributed to this factor.

4. Food - Chlorophyll levels were measured as an index of algae in the water. Levels ranged from 2.7 to 23.6 mg/l. In no instance was there a difference in depuration rates for fecal coliform bacteria which could be attributed to differences in chlorophyll levels.

PART II. EFFECT OF ENVIRONMENTAL FACTORS AND TANK DESIGN ON DEPURATION OF OYSTERS IN COMMERCIAL-SIZE TANKS

Introduction

Laboratory 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 recirculation of the water. Therefore, they provided data on the reaction of the oyster as an individual organism to the different environmental conditions in terms of depuration. These results are covered in Part I of this volume.

Having established those optimal ranges in shallow trays, we conducted experiments in large commercial-size tanks for two reasons: 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 in large tanks of different design.

Introductory information pertaining to specific environmental factors such as temperature, salinity and dissolved oxygen has been presented in the corresponding sections of Part I of this volume. Therefore, it is not repeated here and the reader is referred to those sections for such information.

General information which will aid in understanding of the material presented in this part is given in the following paragraphs.

Separation of Oysters into Batches

In some experiments, groups of oysters which had been exposed to contamination in nature for different lengths of time or at different locations were subjected to depuration simultaneously in the same tank. Never were more than two such groups used in any one experiment. Each such group was held in separate trays and identified and processed as a batch. Batches from different experiments were grouped together according to the selected ranges of initial MPN level (0 hour) and of environmental factors without regard to the experiment number they were a part of. For example, in Table 28, Expt. 48 included two batches of oysters. The batch in trays 1, 4, 5 and 8 was grouped separately in Table 19 from the batch in trays 3 and 7 because their initial MPN level fell in different ranges.

Description of Tanks

Associated experimental procedures used in these studies have been described in the progress reports for the periods 16 February to 15 May 1975, 16 May to 15 September 1975 and 16 October 1975 to 15 February 1976. Parts of them are summarized here briefly. The nomenclature used in reference to the four types of commercial-size tanks is based on the measurements of the horizontal planes of three of them, i.e., 4 x 8 tank is 4 feet wide by 8 feet long. The name of the fourth, the flume, is based on its design.

4 x 8 Tank. This tank was constructed of 3/4 inch plywood coated with fiberglass resin. Its inside dimensions were 7 feet, 9 inches in length, 4 feet in width and 18 inches in depth. Water depth was held at 14½ inches. It was originally constructed with three overflow pipes at the drain end (Figure 31A). Later it was modified by replacement of the overflow pipes with a baffle slanted at a 27° angle (Figure 31B). The baffle had eight holes (5/16 inch diameter) which allowed some of the water to flow out near the bottom and at mid-depth.

The tank had a capacity of 253 gallons at a water depth of 14¼ inches. With eight trays of oysters, each holding 0.6 bushel, the volume of water to oyster load ratio was 46½ gallons/bushel.

The wire trays holding oysters were arranged in two layers of four trays each with the long axis of the trays perpendicular to the long axis of the tank. The trays were suspended on L-shaped steel bars hung from the tank sides (Figures 32A and B). Trays in this tank were numbered as follows: trays in the top layer were numbered 1 to 4 from inflow end to drain end; trays in the bottom layer were numbered 5 to 8 in the same direction.

Water flowed into the tank through holes in a horizontal 1½ inch PVC pipe located across the head of the tank at a level midway between the two layers of trays (Figure 32B).

2 x 8 Tank. This tank was built out of specially laminated plywood that did not require additional waterproofing. Its inside dimensions were 8 feet in length, 26 inches in width and 41 3/4 inches in depth at the drain end and 42½ inches in depth at the opposite end. Figures 33A through E show construction details of the tank. Figure 33F shows the arrangement of inflow water pipes along one side wall of the tank and a small pump with pipe arms

at the bottom on the opposite side wall used to push bottom water upward. In all experiments with this tank, water overflowed evenly over the sides. In one experiment a set of three siphons was used to drain water from the bottom of the tank instead of allowing it to overflow.

Figure 33G shows the arrangement and numbering of trays in the 2 x 8 tank in relation to the side wall inflow pipes. Table 29 gives the results for each tray sampled in depuration studies in this tank. The numbering system used to identify the trays is given in Figure 33G. Figure 33H shows one of the wire trays used for holding oysters in the tank and the hoist used to lift them into and out of the 2 x 8 tank.

Capacity of the 2 x 8 tank was 457 gallons. With eight trays in the tank, each holding 0.6 bushel of oysters, the water volume to oyster ratio was 88.7 gallons/ bushel. Figures 35A, B and C show the layout in our depuration laboratory of the 2 x 8 tank when operated simultaneously with two 2 x 4 tanks.

2 x 4 Tank. The 2 x 4 tank was constructed of 3/4 inch plywood coated with fiberglass resin. It measured 4 feet in length, 22¼ inches in width and 33 inches in depth at the inflow end. The floor had a grade (slope) of 3 inches in 41 inches between the inflow end and a trough at the drain end (Figure 34A-C). The bottom surface of the trough was inclined towards the drain outlets. A siphon made of 2 inch PVC pipe drained water out of the tank at the bottom of the trough and maintained the water level at 1½ inches below the top edge of the tank sides.

Tray supports and inflow pipes in this type of tank were arranged so that in some experiments the tank held five trays and in others three trays. Trays were numbered 1 to 5 from top to bottom in the first case and similarly, 1 to 3 in the second instance. Tank capacity at the siphon overflow level was 155 gallons. With five trays in the tank, each holding 0.6 bushel, the ratio of water volume to oysters was 51.5 gallons/bushel, close to the ratio for a load of 4.8 bushels in the 4 x 8 tank. With three trays in the 2 x 4 tank the water/oyster ratio is 85.9 gallons/bushel which is fairly close to that for the 2 x 8 tank with an oyster load is 4.8 bushels.

Figures 35A to C show the layout in our depuration laboratory when two 2 x 4 tanks were operated simultaneously with the 2 x 8 tank.

Flume. The fiberglass flume used in these depuration studies was 13 feet long and 26 inches wide. Inside dimensions for the space filled with water were 12 feet in length, 24 3/4 inches in width and 6½ inches in depth. At the perimeter on the bottom there was a ledge 1½ inches wide by 2 inches high used to support trays off the bottom. Water depth was 4½ inches (Figure 36B). Two baffles built in at each end of the flume maintained water depth constant. The volume capacity of the flume was 97 gallons. The maximum oyster load used in our experiments was 2.6 bushels. This resulted in a water volume to oyster load ratio of 37.3 gallons/bushel. Figure 36A shows the arrangement used in operating two flumes simultaneously in association with the 12-lamp Kelly-Purdy-type of ultraviolet unit.

During depuration experiments, oysters in the flume were sampled from the head, the middle and the drain end of the flume. Samples from each of these positions were labelled as H, M and E, respectively.

Description of Wire Trays Used in Large Tanks

In the large tanks described above oysters were held in trays made of one-inch mesh heavy-gauge wire with a durable PVC coating (Fig. 33H). They are custom-made by the Marlboro Wire Goods Co., Marlboro, Massachusetts. The tray sides flare slightly outward and its dimensions were 40½ inches long and 19 inches wide at the top, 38½ inches long and 17¼ inches wide at the bottom, and 4 inches deep.

Description of Ultraviolet Treatment Units

Two types of ultraviolet lamp units were used in experiments with these tanks. One was manufactured commercially by AquaNomics, Inc., of California as Model 4L-368-P-50, a four-lamp unit constructed of PVC (Fig. 30A). The lamps were 34 inches 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. AquaNomics, Inc. is now out of business.

The second unit was constructed by us following the Kelly-Purdy design (Fig. 30B). It was constructed of 3/4 inch plywood coated inside with fiberglass resin. It measured 68½ inches in length, 32½ inches in width and the lower part holding the water was 3 inches deep. Twelve lamps identical to those used in the commercial unit were used in the wooden unit. The distance from the center of the lamps to the bottom of the unit was five inches. Water depth was usually maintained at a 1/2 to 3/4 inch depth.

Analysis of depuration in large tanks in relation to temperature and other environmental parameters is presented in the two sections that follow.

SECTION 1

Temperature

Introduction

The introductory remarks appearing under the same heading for shallow trays are also applicable here and will not be repeated. The reader is referred to that section.

Materials and Methods

All but one of the experiments in commercial-size tanks were conducted at ambient water temperatures. These ranged within limits similar to those in the shallow trays.

Results

Thirty-seven experiments were conducted using commercial-size tanks to depurate oysters contaminated in nature. The 4 x 8 tank was used in six experiments, the 2 x 8 tank in 12, the 2 x 4 tank in 10 and the flume in nine.

The actual mean temperature ranges included in these experiments were as follows: 4 x 8 (20.2-28.8^{o}C), 2 x 8 (10.1-26.5^{o}C), 2 x 4 (10.1-18.4^{o}C) and flume (24.3-29.0^{o}C).

Total coliforms. As was the case in the experiments in shallow trays, these data for all four tanks are characterized by the frequent occurrence of wide confidence interval estimates around the means (Figs. 37-43; Tables 19-22). Sometimes oysters depurated to less than 230 MPN by 48 hours but more often such a level was not obtained and sometimes there was an increase. Depuration of total coliforms at all initial levels in the temperature range 24.1-29.0^{o}C progressed downward uniformly reaching an MPN under 230 after 72 hours in all large tanks except the flume. In the flume, total coliforms were reduced to 230 after 72 hours at the initial MPN range of 10,001-25,000 (Fig. 43). At the two other initial MPN levels they were reduced only to 380 and 480 in the same time period. The means in the latter two cases were accompanied by extremely wide confidence intervals.

In the temperature range of 19.1-24.0°C, the mean MPN was reduced to under 500 in 72 hours in five of the six decay curves for the 4 x 8, 2 x 8 and 2 x 4 tanks (Figs. 39-42). The one instance when an MPN of 500 was not reached in 72 hours was the one with the highest initial level (81,000, Fig. 40). Nevertheless, a reduction of 97% (down to 2,200) was still accomplished in that case.

The only experiments in the temperature range 14.1-19.0°C were conducted in the 2 x 8 tank (Figs. 39 and 41). When the mean initial MPN was 3,300 a reduction to 460 was accomplished in 48 hours but this was followed by a bounce up to 2,500 after 72 hours. At an initial MPN of $\geq$30,000 the mean total coliform level was greater than 1,000 after each of the three time periods.

Experiments at a temperature range of 9.1-14.0°C were conducted only in the 2 x 8 and 2 x 4 tanks. In the 2 x 8 tank, depuration was slow during the first 48 hours when the initial MPN was 2,800 (Fig. 39). After 72 hours the mean MPN was down to 230 but there was a large variation around the mean. At a similar initial MPN in the 2 x 4 tank results paralleled those found in the 2 x 8 tank (Fig. 42). However, the mean MPN after 72 hours was higher in the 2 x 4 tank (560) with an extremely wide associated variation.

At an initial MPN of 49,000 in the 2 x 8 tank there was a reduction of 98% (down to 880) in 24 hours (Fig. 41). However, there was very little further reduction in the subsequent 48 hours. After 72 hours the mean MPN was 400 and the associated variation was no greater than that attributable to the MPN technique.

Fecal coliforms. Results of analyses for fecal coliforms in commercial-size tank experiments were more uniform and showed less variation than the total coliform analyses. Differences between depuration at different initial MPN and temperature groups stand out more clearly with the fecal coliform data than they did with the total coliform data. Therefore, they will be examined in more detail.

Temperature range 24.1-29.0°C - In the temperature range of 24.1-29.0°C, results with the 4 x 8 tank appeared somewhat better than those with the 2 x 8 tank and the flume. No experiments were conducted in this range in the 2 x 4 tank.

Within the above temperature range and in the initial MPN range of 501-1,000, experiments were completed only in the 4 x 8 tank and the flume. In the 4 x 8 tank the mean initial MPN was 790. This was reduced to <18 in 24 hours and remained at that same level through the final 48 hours of depuration (Fig. 44, Table 23). The corresponding initial level in the flume was 820. This was lowered to <40 in 24 hours and to <19 in 48 hours (Fig. 50, Table 26). The MPN was <34 at the end of 72 hours.

At the initial MPN range of 1,001-5,000 the reduction of fecal coliforms was also faster during the first 24 hours in the 4 x 8 tank than in the 2 x 8 and flume. In the 4 x 8 tank an initial MPN of 2,900 was lowered to <18 in 24 hours and remained between <18 and <21 from there on (Fig. 45). In the 2 x 8 tank the MPN was <57 after 24 hours, <28 after 48 hours and <29 after 72 hours (Fig. 47). In the flume, the MPN was reduced to <36 in 24 hours and stayed close to that (<40) after 48 hours (Fig. 50). The 72 hour samples, however, showed an increase to <86.

In the 2 x 8 tank it took 48 hours of depuration to reduce the MPN to <50 and 72 hours to bring it down to <21 in two pairs of experiments with respective initial levels of 10,000 and 52,000 (Fig. 48, Table 24).

<u>Temperature range 19.1-24.0°C</u> - Experiments in this temperature range were conducted only in the 4 x 8 and 2 x 8 tanks. Results were similar in both tanks at an initial MPN <500, the MPN was lowered to <25 in the 4 x 8 tank and to <23 in the 2 x 8 tank after 24 hours (Figs. 44 and 46, Tables 23 and 24). In both cases the 48- and 72-hour levels were <18.

In the MPN initial range of 501-1,000 the only data came from the 4 x 8 tank. From an initial level of 510 the level was reduced to <18 in 24 hours and remained at that level subsequently (Fig. 44, Table 23).

At an initial level between 1,001 and 5,000 the MPN was between <40 and <50 after 24 hours in the 4 x 8 and 2 x 8 tanks, respectively, (Figs. 45 and 47, Tables 23 and 24). It went down to <18 after 48 hours in the 4 x 8 tank and remained there after 72 hours. In the 2 x 8 tank, the level was also <18 after 24 hours but went up to <26 after 72 hours.

In a single experiment in the 4 x 8 tank at an initial level of 6,300 the MPN was reduced to <22 in 24 hours and stayed at that level or lower for the subsequent two days (Fig. 45, Table 23).

Temperature range 14.1-19.0°C - This temperature range only included experiments in the 2 x 8 and 2 x 4 tanks. At an initial MPN of <500 the MPN after 24 hours was <22 in the 2 x 8 tank (Fig. 46, Table 24). The 48 and 72 hour levels were <29 and <18, respectively. In the 2 x 4 tank the 24 hour MPN was 42 and <37 after 48 hours (Fig. 49, Table 25). After 72 hours it was <63.

In the initial MPN range of 1,001-5,000 the MPN after 24 hours was 110 in the 2 x 8 tank and <70 in the 2 x 4 (Figs. 47 and 49, Tables 24 and 25). It went down further, to <31, after 48 hours in the 2 x 4 tank and stayed at that level until the end of 72 hours. In the 2 x 8 tank the level after 48 hours was slightly higher than at 24 hours (134) but after 72 hours it had decreased to <28.

Temperature range 9.1-14.0°C - The range of temperature actually recorded within this category was 10.1-11.5°C. Experiments included in this range were conducted in the 2 x 8 and 2 x 4 tanks only. The initial MPN was low (<200) in both tanks (Figs. 46 and 49, Tables 24 and 25).

Depuration rate was slower during the first 24 hours in the 2 x 4 tank than in the 2 x 8. At the end of that period the level was <47 in the 2 x 8 tank and 104 in the 2 x 4. After 48 hours the MPN in samples from both tanks was similar: <43 in the 2 x 4 and <31 in the 2 x 8. At the end of 72 hours the MPN had decreased further to <21 in the 2 x 4 tank and to <25 in the 2 x 8.

Experiments Using Artificial Contamination with E. coli. All results presented so far in this section are based on experiments with oysters contaminated in nature. Five additional experiments were conducted at temperatures under 13°C using oysters contaminated with *Escherichia coli* in the laboratory. Samples were analyzed for fecal coliform bacteria only using the A-1 technique described in Section 4 of Part III. Results from those experiments appear in Fig. 51 and Table 27.

A single experiment was conducted in the 4 x 8 tank at a mean water temperature of 5.6°C. The mean initial MPN of fecal coliforms was low (130). The mean MPN for oyster samples collected at 24, 48 and 72 hours after depuration started were all higher than the initial level. After 72 hours it was 225.

Two other experiments were conducted in the 4 x 8 tank at mean temperatures of 10.4 and 10.5°C. The initial MPN in one (temperature = 10.4°C) was 54,000. After 24 hours of depuration it had decreased to a mean of <19 with all oyster samples showing levels under 20. In the subsequent two days the mean MPN was <25 after 48 hours and <30 after 72 hours. In the second experiment (temperature = 10.5°C) the initial MPN was much lower (180). After 24 hours the MPN had decreased to <23. After 48 hours it was <18 and remained at that level after 72 hours.

Two experiments were conducted in the 2 x 8 tank at mean temperatures of 11.8 and 12.3°C. The initial MPN in the first was 710. In 24 hours it decreased to <25 and after 48 and 72 hours the MPN was <19 and <18, respectively.

The initial MPN in the second experiment (temperature = 12.3°C) was 5,100. It was reduced by 99%, down to 45, after 24 hours of depuration. There was a slight increase to 67 after 48 hours but after 72 hours the MPN level had decreased to 18.

<u>Summary: Fecal Coliforms</u>

The data on fecal coliform levels in oysters contaminated in nature and depurated in the commercial-size tanks used in these studies may be summarized as follows:

1. Based on these temperature studies, there was no evidence that one tank was better than another.

2. At the lower temperatures tested (10.1-11.5°C), oysters with mean initial MPN ≤ 200 were depurated to a level of <50 fecal coliforms in 24 hours and <25 in 72 hours in the 2 x 8 and 2 x 4 tanks.

3. At temperatures higher than 14°C and mean initial MPN <500 oysters were depurated to a level of 50 fecal coliforms in the same time period in the 2 x 4 tank. It took 72 hours of depuration to bring the level down to <25 in the 2 x 4 tank.

4. At temperatures between 14 and 29°C and a mean initial MPN of 3,000, fecal coliforms were reduced to <50 in 48 hours in both the 2 x 4 and 2 x 8 tanks. No further improvement was noted after 72 hours.

5. At a temperature of 26°C and mean initial MPN of 10,000 and 52,000, fecal coliform levels in oysters were reduced to <50 in 48 hours and <25 in 72 hours in the 2 x 8 tank.

6. Data available for the 4 x 8 tank are limited to a mean temperature range between 20 and 29°C and a mean initial MPN range between 500 and 6,300. Under those conditions, oysters were depurated to levels of <25 fecal coliforms in 24 hours and <18 in 48 hours when the initial MPN was under 1,000. At initial MPN between 1,000 and 6,300, fecal coliforms in oysters were reduced to <50 in 24 hours and <18 in 48 hours.

Discussion

At temperatures higher than 14°C, oysters depurated fecal coliforms to an MPN level of less than 50 in 48 hours regardless of the initial level up to a maximum MPN of 3,000 in the larger tanks tested (2 x 8, 2 x 4 and 4 x 8).

In commercial-size tanks and at temperatures between 10 and 14°C, oysters with initial MPN of 200 or less will depurate themselves of fecal coliforms to a level of <50 in 48 hours and to levels of 25 or less in 72 hours. No data were collected at this temperature range for initial levels greater than 200. The absence of such data is associated with the fact that in the localities sampled by us in nature fecal coliform levels in oysters were consistently low at temperatures under 14°C. Because of this, the conclusions presented here are limited to a maximum initial level of 200 at the temperature range of 10 to 14°C.

However, it is possible that oysters may be able to depurate themselves of higher numbers of fecal coliforms. This is suggested by data collected in experiments with oysters contaminated in the laboratory with suspensions of _E. coli_. Oysters with initial contamination levels of _E. coli_ as high as 500,000 can be depurated to <50 in 24 hours at temperatures between 10.4 and 12.3°C (Fig. 51). Applicability of data based on artificial contamination with _E. coli_ has been questioned by Heffernan and Cabelli (1971) in studies done with the hard clam _Mercenaria mercenaria_. They found that the hard clam eliminated _E. coli_ at a faster rate than it cleansed itself of naturally occurring fecal coliforms. Therefore, these results will not be related at this time to depuration of oysters contaminated in nature. They, nevertheless, point out a relatively high degree of activity in oysters at those temperatures.

The only data available for initial levels above 3,000 were collected at a temperature of 26°C in the 2 x 8 tank. As will be shown in Section 1, Part III, they indicate that oysters depurated to an MPN below 50 fecal coliforms in 48 hours when the initial MPN was as high as 39,000 (mean MPN for three batches of oysters: Expt. 53, trays 1 and 8; Expt. 54, trays 1 and 2; and Expt. 54, trays 3, 6, 7 and 8. See Table 29). However, when one batch of oysters (Expt. 54, trays 1 and 2) is separated from the others, the mean initial level is higher (52,000). Oysters still depurated themselves to an MPN below 50 in 48 hours (Fig. 48).

SECTION 2

Effect of Environmental Factors Other than Water Temperature and Tank Design on Depuration of Oysters in Commercial-Size Tanks

Introduction

Experiments in shallow trays indicated that in the ranges in which environmental factors such as temperature, salinity, dissolved oxygen and turbidity occur normally within the geographical distribution of *Crassostrea virginica*, only the range of temperature is wide enough to be of major significance.

Preliminary examination of the data collected during depuration experiments in commercial-size tanks appeared to substantiate those findings. Therefore, it was decided that the data for experiments in commercial-size tanks would be broken down and analyzed in detail only in reference to temperature. This was followed by examination of the combined data for each of the four tanks. The rationale for combination of the data in that manner was that if any of the environmental factors included affected depuration adversely, anomalies would be evident in the results which would point out such an effect.

A detailed discussion of temperature appears in Section 1 above. Results are presented below for each tank type when the data for all experiments are combined and averaged together at each of the 24 hour depuration periods.

Examination of the data for individual trays shows that tray position was not a factor in the results obtained. Therefore, it was possible to combine the data for all trays.

Results

4 x 8 Tank. Six experiments were conducted in this tank. Data for individual experiments appear in Table 28.

The decay curve for total coliforms shows a significant decline in the mean MPN level through the first 24 hours (Fig. 52). The variation around the means does not permit distinction between the mean for 24 hours and those for 48 and 72 hours. The mean level was 410 after 24 hours (representing a decrease of 93%) with an upper interval limit of 1,100.

The mean MPN level of fecal coliforms decreased sharply during the first 24 hours from 1,400 to <24, a reduction of 98 percent (Fig. 53). The 48 and 72 hour means were both <18. Variation around these means was small. The percentage of samples with an MPN equal to or less than 20 was 83 after 24 hours, 94 after 48 hours and 100 after 72 hours.

2 x 8 Tank. Twelve experiments were conducted in this tank. A summary of the data for individual experiments appears in Table 29.

The decay curve for total coliforms shows a significant decline of 94% in mean MPN during the first 24 hours (Fig. 52). The mean and upper interval limits were 740 and 2,000, respectively. The variation around the means for the 48 and 72 hour periods does not allow us to detect any further change in the mean MPN level after 24 hours.

Mean MPN level of fecal coliforms appeared to decrease at a slower rate in this tank than in the 4 x 8 tank (Fig. 53). After 72 hours, the mean level was the same (<24) attained after only 24 hours in the 4 x 8 tank. Nevertheless, the level attained in 24 hours, <41, represented a reduction of at least 98% from the initial level. The level after 48 hours was <28. The percentage of samples with an MPN equal to or less than 20 in the 2 x 8 tank was 50 after 24 hours, 74 after 48 hours and 84 after 72 hours.

2 x 4 Tank. Ten experiments were conducted in this tank. A summary of the data for individual experiments appears in Table 30.

Results obtained for total coliforms were similar to those found in the 4 x 8 and 2 x 8 tanks in that after a sharp decline in MPN level during the first 24 hours no difference could be detected at subsequent time intervals (Fig. 52). The decrease in mean MPN during the first 24 hours represented 92% of the initial level; the mean MPN was 410 with an upper interval estimate of 1,200.

Decrease of the mean MPN level of fecal coliforms appeared to proceed at a slower rate in the 2 x 4 tank than in the 4 x 8 and 2 x 8 (Fig. 53). From an initial mean level of 834, fecal coliforms were reduced by 91%, to <75, in the first 24 hours. The levels after 48 and 72 hours were <35 and $\lesseqgtr$41, respectively.

The percentage of samples with a mean fecal coliform level equal to or less than 20 was 20 after 24 hours, 57 after 48 hours and 68 after 72 hours.

<u>Flume</u>. Nine experiments were conducted in the fiberglass flume. One of the nine was not carried beyond 24 hours. A summary of the data for individual experiments appears in Table 31.

The data for total coliforms showed a pattern similar to that found in the other large tanks. After a sharp decrease of 94% in the first 24 hours of depuration, the variation around the means for the 24, 48 and 72 hour samples did not allow detection of differences between them (Fig. 52). After 24 hours the mean MPN was <730 with an upper interval estimate of 3,200. The associated variation for the 48 and 72 hour means (especially the latter) in the flume experiments was greater in each case than the corresponding ones for the other large tanks.

Results of depuration in the flume in terms of fecal coliforms were comparable to those found in experiments in the 2 x 8 tank and appeared slightly better than those in the 2 x 4 tank, except at the 72 hour sampling time (Fig. 53). After 72 hours there was an increase in the mean MPN level from the <30 observed after 48 hours to <56. This increase is a reflection of a similar increase in four of the eight experiments with a 72 hour duration in the flume (Table 31). The 72 hour level in the other four experiments was <18.

The percentage of samples with an MPN equal to or less than 20 was 55 after 24 hours and 70 after both 48 and 72 hours.

Summary

The data on total coliform levels during depuration experiments in all four large tanks were characterized by wide variations around the means. These precluded any interpretation of the depuration beyond 24 hours.

In all four tanks there was a sharp decrease in MPN level of between 93 and 94% in the first 24 hours. These reductions represent a substantial degree of depuration. However, the fact that the mean MPN after 24 hours ranged between 400 and 750 with very wide confidence intervals creates the probability that some of the samples could have a mean MPN as high as between 1,100 to 3,200 depending on which tank is being referred to. These results point out a need for further studies directed toward determination of the reasons for the large variations in total coliform levels during the process of depuration.

The MPN levels for fecal coliforms during depuration in the large tanks showed very little variation around the means. This was due to the reduction of fecal coliforms to levels between <18 and 20 in most of the samples. Thus, these data permit the formulation of definitive statements about the process of depuration in the large tanks tested.

The decay curves for mean MPN levels of fecal coliforms show that oysters depurated themselves to low levels (<40) in 48 hours in all tanks. The fact that mean levels recorded for samples from the 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^{o}C. In the 2 x 4 tank they ranged from 10.1 to 18.4^{o}C. Those in the 4 x 8 tank only ranged from 20.2 to 28.8^{o}C.

Mean temperatures in the flume ranged between 24.4 and 29.0^{o}C, but results obtained were not as good as those in the 4 x 8 tank. In this case, temperature cannot be considered the factor associated with the difference.

The mean MPN remained below 40 after 72 hours of depuration in three of the tanks (4 x 8, 2 x 8 and 2 x 4). An unexplainable jump in the MPN level between the 48 and 72 hour periods in some of the experiments in the flume caused the 72 hour mean level to increase to <56. The cause for this jump is not known. Ignoring the 72 hour data, it can be stated that the flume is satisfactory for depuration of oysters in 48 hours if a level of fecal coliforms of <40 is considered acceptable.

A single sample among those analyzed after 72 hours in the 2 x 4 tank showed an MPN of $\geq$ 24,000 (this unknown upper limit is indicated by the arrow pointing up above the point plotted on the decay curve). That single value should not be overemphasized, however. Sixty-eight percent of those samples were <20 and 83 percent were <45.

Discussion

The consistency with which oysters depurated fecal coliforms in all experiments in commercial-size tanks indicates that for the ranges included in these studies, none of the environmental factors monitored had an adverse effect on oyster depuration.

The results presented above and illustrated in Figs. 52 and 53 show only two major anomalies. One is the wide variation of MPN values for total coliforms. These variations, however, are found throughout all the data and cannot be attributed to effects of specific environmental factors. The other anomaly is the bounce in fecal coliform MPN at 72 hours in the flume. This bounce cannot be attributed to specific environmental factors either since it was not found in the other three types of tanks. It is more than likely associated with tank design.

Ability to make a decision on which of the four types of tanks resulted in better depuration is handicapped by the fact that all were not tested within the same temperature ranges. Nevertheless, comparison of the results available suggests that all tanks performed with relatively equal efficiency. The bounce in MPN level at 72 hours found in the flume renders its performance questionable.

The short table inserted below shows the percentage of the individual pooled samples of 6-8 oysters that showed an MPN level of <50 after each of three days of depuration.

Depuration Time (Hrs)	Percentage of samples with MPN <50			
	4 x 8 Tank	2 x 8 Tank	2 x 4 Tank	Flume
24	89	68	49	75
48	100	84	71	80
72	100	95	83	70

The simplicity of the 4 x 8 tank in terms of construction details and in operation provide it with an advantage over the 2 x 8 and 2 x 4. It would be the more desirable of the three for use in a depuration plant.

PART III. BACTERIOLOGY

SECTION 1

Anticipated Coliform Levels after Various Time Periods of Depuration with Respect to Initial Levels in Oysters Vs. Associated Estuarine Growing Areas

Experimental Depuration Runs

The results of depuration experiments in commercial-size tanks were studied to determine if there was a relationship between rate of depuration and initial coliform level. Total and fecal coliforms in these experiments generally decreased relatively rapidly over the first 24 hour period of depuration after which coliform reduction occurred at a much slower rate (Figs. 37-42 and 44-49 and Tables 19-21 and 23-25). Decay curves for total coliforms were characterized by large and frequently overlapping confidence limits around the mean values. In contrast, confidence intervals around the mean fecal coliform levels during depuration generally could not be determined using the graphical approach (Velz, 1951) because of the preponderance of samples with indeterminate values, i.e., <18 FC/100 g.

These studies show that the initial level may be a significant factor in the resulting total and fecal coliform levels after various time periods. This was most apparent in the 2 x 8 tank where the greatest variation occurred in the initial total and fecal coliform levels (Figs. 39-41, 46-48 and Tables 20 and 24). In order to further analyze the data, results are tabulated and grouped according to tank design, initial levels, and temperature of depuration, separating those experiments conducted at temperatures of <12°C (Tables 32 and 33). Percent coliform levels remaining after various time periods were calculated based on mean levels, but it should be realized that these percent values are somewhat unreliable in the case of total coliforms due to the wide confidence limits around the mean values. Tables 34 and 35 present the data in a summarized form. Information relating to flume experiments is not included since the results obtained in this tank showed wide variations and increases in fecal coliform levels at 72 hours as discussed previously (Part II). Neither is analysis of data with respect to initial levels in tray experiments included since oysters were depurated under conditions which avoided crowding and provided optimal accessibility to treated water and thus would not illustrate the effects of initial levels under commercial conditions.

In only one instance was an experiment conducted in a commercial tank where the initial level was <1,000 TC/100 g. This experiment was carried out in the 4 x 8 tank, and although the mean water temperature was 20.2°C, no reduction in total coliforms was observed at 24 hours, and 30-32 percent of the coliforms still remained at 48 and 72 hours. However, the initial level is based on the analysis of only one sample of oysters and it may have been an underestimate of the original degree of contamination.

Depuration runs were conducted in the 2 x 4 and 2 x 8 tanks at both low and high temperatures. With moderately contaminated oysters, i.e., 2,000-4,000 TC/100 g, 10.5 percent or fewer coliforms remained after 24 hours when depurated at 15.6-26.1°C in contrast to 50-53.6 percent coliforms remaining after the same time period when depuration was conducted at 10.1-11.5°C. Coliform levels were not significantly reduced until 72 hours depuration at the cooler temperatures in contrast to the attainment of levels ranging around 230 TC/100 g by 48 hours in all three tanks at warmer temperatures.

When depuration experiments were conducted at temperatures of 17.4°C or greater, higher initial coliform levels could still be depurated to mean values close to 230 TC/100 g. In the 2 x 4 and 4 x 8 tanks, coliform levels were reduced from 12,000 and 15,000 respectively by 24 hours, whereas in the 2 x 8 tank, 72 hours was necessary to depurate coliforms from 19,000/100 g to a similar endpoint. The slower depuration rate of total coliforms in the 2 x 8 tank should not be overemphasized since depuration rates of fecal coliforms as discussed below did not appear to be influenced by tank design.

One experiment was conducted each in the 2 x 4 and 2 x 8 tanks using very heavily contaminated oysters, i.e., 30,000-49,000 TC/100 g at temperatures of 11.5°C and 14.6°C respectively. Although the calculated percent coliforms remaining shows a sharp reduction, the mean values after 72 hours were greater than that obtained in experiments employing even more heavily contaminated oysters, i.e., 75,000 TC/100 g at warmer temperatures where a mean value of 250 TC/100 g was reached after 72 hours.

Fecal coliform results from experiments conducted at warm and cool temperatures were more consistent than those for total coliforms, but were similar in that more rapid reduction was observed at warmer tmeperatures. When initial levels were 180-190 FC/100 g, depuration at 10.1-11.5°C resulted in approximately <47-100 FC/100 g remaining at 24 hours although by 48 hours fecal coliforms had been reduced to <50/100 g. In contrast, at 15.6-23.7°C, fecal coliform levels at slightly higher levels of 210-32/100 g were reduced to <23-58/100 g by 24 hours.

Experiments involving higher fecal coliform levels were conducted only at warmer temperatures. Analysis of the results shows that initial levels of 700-3,300 FC/100 g were reduced to <50 FC/100 g in the 2 x 8 and 4 x 8 tanks and to <68 FC/100 g in the 2 x 4 tank by 24 hours. Fecal coliforms were depurated from very high levels only in the 2 x 8 tank where mean values of 7,000 and 39,000 FC/100 g were reduced to levels <50/100 g in 48 hours.

Thus, depuration of both total and fecal coliforms occurs at a slower rate at cooler temperatures. The initial numbers of total and fecal coliforms present are determining factors in the time required to reduce coliform numbers to a specified level.

In summary, oysters may be depurated to levels below 50 FC/100 g in 48 hours when initial levels are as high as 39,000 FC/100 g and the water temperature is around 26°C. Fifty FC/100 g can be attained in 48 hours when initial levels are 180-190 and temperatures are 10.1-11.5°C. Oysters with higher initial levels were not available for the lower temperature studies.

Relationship of Coliform Concentration in Oysters and Shellfish Growing Waters

Data pertaining to coliform levels in oysters and the surrounding waters were analyzed to determine if a relationship existed between the two and if it was influenced by temperature. A positive correlation with respect to total coliforms was noted only at temperatures less than 15.0°C (Fig. 54, Table 36). Although there was a lack of correlation at warmer temperatures, the oyster-water index, i.e., ratio of geometric mean MPN's in oysters and water, was greatest at temperatures above 15.0°C. Positive fecal coliform relationships were noted at all temperature ranges although the correlation at temperatures above 20°C was very small (Fig. 55, Table 37). When temperatures increased above 15.0°C, oysters concentrated fecal coliforms over levels present in the surrounding water. Concentration of total and fecal coliforms has been reported to be greater at warmer temperatures during the months of July and August as opposed to the month of January (Vasconcelos, Jakubowski, and Ericksen, 1969). In the case of both total and fecal coliforms, the greatest concentration occurred between 15.1-20.0°C at which time the oyster-water index was 16.25 and 5.98 respectively. Using artificially contaminated oysters, Kelley, Ascisz, and Presnell (1960) likewise reported that the greatest mean ratios of coliform MPN in oysters to that in water occurred in late fall when the temperature was 15-20°C.

Although the data showed trends in coliform relationships in oysters and water as influenced by temperature, the influence of other environmental factors such as salinity, run-off, tidal changes and physiological factors of the oysters which were analyzed was not discernable. If more information had been available for these parameters, a better correlation between coliform levels in the oyster and water may have been obtained. Previous studies have noted the variation in oyster-water relationships and have reported that the variations are closely associated with run-off as reflected by fluctuations in salinity (Presnell and Kelley, 1961). These workers noted that under conditions of low salinity the increase in coliform content of oysters was not proportionate to water coliform increases and attributed this finding to the decreased and erratic feeding by the oysters. It has also been observed that a lag occurs in the shellfish response when the coliform levels in the surrounding water vary with changes in tide (Vasconcelos, Jakubowski, and Ericksen, 1969).

Thus, the degree of uptake of coliform bacteria is a dynamic interplay of various environmental and physiological factors. The data obtained in the present study, as well as that presented in the literature, illustrate that coliform levels in shellfish may not necessarily be predicted from a single grab sample of the surrounding water. Information concerning these influencing factors as well as replicate sampling would be required to establish the relationship between coliform levels in oysters and shellfish growing waters.

Expected Levels of Total and Fecal Coliforms in Depurated Oysters Harvested from Waters of Varying Sanitary Quality

Due to the lack of consistent positive correlations for both total and fecal coliforms at the various temperature ranges, our data did not allow us to calculate, using linear regression analysis, what the theoretical coliform levels in the water would have been from the levels obtained in the oysters. In order to perform such calculations for comparison with the actual coliform levels obtained in the water, it was necessary to use data compiled by Hope and Wiley (1961) for the coliform levels in oysters collected from Virginia waters of varying sanitary quality during the months of April-November and December-March. Although there were positive correlations at the two different temperature ranges, the degree of correlation was generally low, ranging as low as 0.18 for total coliforms to 0.37 for fecal coliforms during the winter months. For both total and fecal coliforms, the actual levels in the water could not be predicted when the levels in the oysters were known (Tables 34 and 35). This would mean, conversely, that if the coliform levels in the water are known, the coliform content in oysters could not be predicted based on both the data of Hope and Wiley (1961) and those collected during the course of the depuration study.

At temperatures less than 12^{o}C an increase in total coliform levels in the growing waters was not accompanied by an increased concentration of total coliforms in oysters, thus making it difficult to set an upper limit for coliform levels in the water. Oysters containing 49,000 TC/100 g were harvested from waters containing 23 TC/100 ml while another group of oysters having 2,000-2,800 TC/100 g were collected from waters with a total coliform concentration of 280/100 ml. At temperatures above 14^{o}C, total coliform contents of oysters increased as coliform levels in the surrounding waters increased. Oysters from waters with a mean of 700 TC/100 ml depurated in six out of eight experiments to levels near 230 TC/100 g by 24 hours. Depuration of oysters from water with a mean of 5,000 TC/100 ml required 72 hours to reach a similar endpoint which was obtained in five out of seven experiments.

As in the case of total coliforms, time of depuration of fecal coliforms to values <50/100 g was related to the fecal coliform level of the harvest water. At warmer temperatures oysters collected from waters containing 350 FC/100 ml could be depurated by 24 hours to levels near 50 FC/

100 g, whereas oysters from waters with a fecal coliform concentration of 700-1,300/100 ml required 48 hours depuration to reduce the fecal coliform content to <50/100 g. At cooler temperatures, 48 hours was required to depurate oysters to the same level when harvested from waters having 170 FC/100 ml. Thus, maximum fecal coliform levels in waters from which oysters failed to depurate to <50 FC/100 g by 48 or 72 hours was not reached.

SECTION 2

Effects of Oyster Biodeposit Accumulation on the Depuration Process and Characteristics of Oyster Wastes

Introduction

It has been suggested that depuration tanks be drained at 24 hour intervals and the shellfish and tanks be hosed down using potable water or treated seawater in order to remove shellfish wastes (Furfari, 1966). The procedure was considered critical to successful depuration because bacteria present in biodeposits could recontaminate the shellfish. In order to ascertain the significance of biodeposits on depuration, the effects of flushing were examined. Biodeposits were characterized with respect to coliform types and levels. In addition, the viability of coliforms from oyster wastes was determined.

Materials and Methods

Eight experiments in the 2 x 8 tank were conducted to examine the effects of 6 hour flushings, alternating 6 and 12 hour flushings and 24 hour flushings. After 6 or 12 hours, the tanks were drained and the tank bottom flushed free of oyster wastes. Twenty-four hour flushings were performed by removing the oysters and hosing down both the tank and shellfish. Three experiments were carried out in the 2 x 4 tank to compare results obtained when the tank was flushed every 24 hours with those obtained when oyster wastes were allowed to accumulate without flushing for 72 hours. Potable water was used for flushing and tanks were filled with UV-treated seawater within one hour. Experiments were conducted at temperatures above 15.0°C.

Biodeposits from the bottom of the 2 x 4 tank receiving 24 hour flushings were also collected with a wide bore pipet at 4, 24, 48 and 72 hours. After biodeposits had settled for 5-10 minutes, excess water was decanted and the sample centrifuged for 5 minutes at 1500 x g. The supernatant was discarded and the packed volume decimally diluted for coliform MPN analysis. In two of the experiments positive brilliant green tubes from four samples each of oysters and biodeposits were streaked on eosin methylene blue (EMB) agar plates and representative colonies isolated. Isolates were identified using the Minitek (BBL) and APO-20 Enterobacteriaceae System (Analytab Products, Inc.).

Shallow plexiglass trays were also used in two experiments conducted at mean temperatures of 19.9 and 21.0°C to determine coliform levels present in biodeposits and oysters. Biodeposits were examined for coliform content as described above except that feces and pseudofeces were collected separately from individual oysters which were subsequently analyzed. After biodeposits had been collected, oysters were removed and the trays cleaned and refilled with UV-treated water. In additional tray experiments conducted at 17.6 and 17.8°C, coliform isolates from oyster samples were identified.

The viability of coliforms in biodeposits was examined in flask experiments using water collected from a 2 x 4 tank after 48 hours depuration. Feces and pseudofeces were collected separately from oysters depurated 48 hours in small plexiglass trays. Biodeposits were centrifuged as described above and added to drain water at ratios of 1:100 and 1:1000. Flasks containing 750 ml of drain water with and without biodeposits were stoppered with cotton plugs and placed in a shaker at 100-150 RPM. After various time intervals, flask contents were sampled for total and fecal coliform content. Wet mounts were prepared from each flask to determine if ciliated protozoa were present.

Results and Discussion

On the basis of total and fecal coliforms, it would appear that flushing tanks more frequently than at 24 hour intervals did not enhance depuration. The effects of flushing frequency in the 2 x 8 tank are illustrated in Figs. 56-59 and Tables 38-39. Confidence limits about mean total coliform levels frequently overlapped during the first 48 hours of depuration. If comparisons are made of 72 hour samples, depuration of moderate initial total coliform levels would appear to be better when 6 hour flushings were used while depuration of higher initial levels was best when tanks were flushed every 24 hours as opposed to 6 hours or alternating 6 hour and 12 hour flushings. Analysis of fecal coliform results showed that although all mean values were <50 FC/100 g at 48 hours, oysters had depurated somewhat better in tanks receiving more frequent flushings. However, at 24 and 72 hour depuration, no difference existed between samples from tanks flushed at different intervals.

Experiments conducted to determine the effects of 24 hour flushings versus no flushings were performed in the 2 x 4 tank holding three oyster trays stacked one above the other. A siphon at the bottom resulted in a net downward water flow such that a portion of the biodeposits from one tray would be expected to fall on the tray(s) immediately below. In tanks which were not flushed, 24 and 48 hour samples of oysters were collected from the top trays, while at 72 hours it was possible to collect oyster samples at all three tray depths after draining the tanks. It can be seen in Figs. 60-61 and Tables 40-41 that all mean values were <230 TC/100 g by 72 hours and <50 FC/100 g by 24 hours, regardless of whether the tank was flushed or not. While both total and fecal coliform levels declined in biodeposits during the experiments, total coliforms remained at relatively high levels at 72 hours, i.e., 25,000 TC/100 g, with the potential for contaminating tank waters. Drain water samples from both tanks, however, revealed that total coliforms were <10, <5 and <1.8/100 ml at 24, 48 and 72 hours respectively. Fecal coliforms at all sampling times were <1.8/100 ml in both tanks.

In view of the fact that the results are based on only three experiments, elimination of flushing at 24 hours cannot be recommended at this time. Although our studies indicate that 24 hour flushings may or may not be associated with increased depuration rates, it has been reported elsewhere that a higher degree of cleansing may be obtained if the 24 hour washdown is eliminated (SNYCD, 1969). Devlin and Eng (1973) reported that draining and flushing tanks periodically may resuspend detritus during refilling which leads to a temporary increase in the bacterial level in tank seawater and oysters.

Examination of feces and pseudofeces collected separately in plexiglass tray experiments showed that greater coliform densities are found in feces (Figs. 62-63 and Tables 42-43). Extremely high coliform levels, i.e., 2,500,00 TC/100 g and 150,000 FC/100 g, were observed in feces collected four hours after oysters were placed in trays. It is interesting to note that total and fecal coliform counts in the oysters decreased dramatically from 27,000 TC/100 g and 750 FC/100 g to 1,200 TC/100 g and <49/100 g within four hrs. Coliform densities in both feces and pseudofeces declined at a rapid rate during the first 48 hours of the experiments. The absence of a continued rapid decline of coliform levels in the oysters may indicate that those coliforms remaining after the gut contents have been emptied are retained in anatomical locations which have a slower flushing rate.

Decreases in coliform levels in biodeposits in both plexiglass trays and 2 x 4 tank experiments would seem to indicate that coliforms do not multiply in biodeposits or if they do, that the rate of death is greater than that of multiplication. These observations would agree with those obtained in flasks where feces and pseudofeces were added to tank drain water at a ratio of 1:100. During the first 6 hours, total and fecal coliform densities fluctuated around the initial level but declined significantly from 24 to 72 hours (Table 44). Protozoa were seldom observed in wet mount preparations; thus grazing by protozoa probably was not responsible for the decline in bacteria.

During the course of two plexiglass tray experiments, 0 and 72 hour oyster samples were analyzed with respect to coliform types present. Samples at 0 hour contained predominantly Escherichia coli and Klebsiella pneumoniae, both of which decreased significantly by 72 hours (Table 45). However, the initial mean coliform levels of 11,000/100 g was reduced to only 2,100/100 g. The high total coliform levels at 72 hours were primarily attributable to Citrobacter freundii which was undetectable at 0 hour. The results prompted an investigation of coliform types in both oysters and biodeposits during depuration to determine if the above findings could be repeated and if Citrobacter likewise increased in biodeposits.

Species composition analysis was made of samples collected from the 2 x 4 tanks receiving 24 hour flushings. In both 0 hour oyster and biodeposit samples, E. coli, K. Pneumoniae, and Enterobacter cloacae were the predominant coliforms (Table 46). The detection of E. cloacae in these experiments in contrast to the preceding tray studies was evidenced by the inclusion of atypical coliform colonies (colonies without a distinctive nucleated center on EMB agar). During the course of depuration, E. coli and E. cloacae decreased significantly, whereas the percentages of both K. pneumoniae and C. freundii increased. Although a dramatic increase of C. freundii was not observed, it should be noted that these 72 hour samples successfully depurated to low levels in contract to the preceding experiments. All 72 hour oyster isolates of C. freundii were obtained from one sample which had the highest total coliform density of the four samples analyzed, 230/100 g.

The coliform species composition of oysters changes during the depuration process and these changes in oysters which successfully depurated were similar to those that occur in their biodeposits. The predominance of C. freundii in oysters which did not depurate and its infrequent occurrence in samples which reached low total coliform levels at 72 hours suggests that high coliform counts of oysters depurated under optimum environmental conditions may possibly be due to high levels of C. freundii. Additional studies are needed to determine if elevated coliform densities are always accompanied by high levels of C. freundii and to ascertain what factors promote the growth and/or survival of this particular coliform type.

In summary, biodeposits contain high levels of total and fecal coliforms but apparently do not have a detrimental effect on the depuration process as long as they are not resuspended. As evidenced in tank studies, coliform levels in biodeposits decrease over time but remain relatively high after 72 hours without accompanying tank water contamination. Even when oyster wastes accumulated during the course of several depuration runs, there was no adverse effect on efficiency of the depuration process. However, if biodeposits were agitated, their characteristic high coliform content could pose a threat to successful depuration.

SECTION 3

Frequency and Method of Sampling

A rigorous statistical examination of the size of oyster samples was not conducted during this study. The number of oysters within each sample was fewer than that recommended by APHA (1970) (6-8 oysters versus 12 oysters), but was logistically unavoidable due to our desire to process numerous samples from various tank locations during the course of each experiment.

Oyster samples were routinely examined prior to and after 24, 48 and 72 hours depuration. Coliform counts prior to depuration were determined by examining two or three pooled samples from a specific harvest area. Representative results of oyster samples collected for depuration in two commercial tanks are presented to illustrate the degree of variation among samples (Table 47). Table 48 illustrates the variation in total and fecal coliform densities in oysters examined individually as determined using the graphical method of Velz (1951). Coliform levels at the upper confidence limit of 95 percent are presented to illustrate the degree of variation present in the data versus that inherent in the technique for estimating coliform numbers. Comparison of the actual and theoretical coliform levels at the 95 percentile confidence limits shows that variation in the data obtained within a given lot of oysters could not be attributed solely to the variation inherent in the technique. Thus, oysters simultaneously collected from a specific area varied as to the number of total and fecal coliforms they contained.

During the course of depuration in commercial-size tanks, samples of pooled oysters from various locations were examined. Tables 28-31 show that although the majority of samples showed similar total coliform levels, one or two samples had levels far exceeding the mean values. Encountering erratic fecal coliform levels was less frequent than in the case of total coliforms. The data also showed that location in the tank did not influence depuration. Samples having erratic total coliform densities could not be attributed to a specific tray location, but rather such samples were obtained from various areas within the tanks.

We believe that our sample size was adequate to estimate coliform levels in the oyster populations at an adequate level of accuracy. This is evidenced by the predictability with which coliform levels dropped to levels below 230 TC/100 g and 50 FC/100 g when physicochemical conditions were suitable. It is not known whether the few exceptions were due to sample size not reflecting the true picture in the oyster population or the population actually was at higher levels. We favor the latter interpretation because of the predictability of the large majority of results under optimum physico-chemical conditions.

SECTION 4

Evaluation of Two Rapid Methods for the Enumeration of Fecal Coliforms in Oysters

Introduction

The sanitary quality of commercial shellfish has been routinely monitored using the APHA fecal coliform MPN 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 hours for completion and necessitates holding depurated shellfish under refrigeration for several days before release to the market. In response to the need for a rapid technique, Cabelli and Heffernan (1970a) developed a 24 hour test known as the elevated temperature coliform plate (ETCP) method which was successfully used in depuration studies involving the quahaug, Mercenaria mercenaria and the soft clam, Mya arenaria (Cabelli and Heffernan, 1970b). Recently, Medium A-1 has been developed for MPN enumeration of Escherichia coli in shellfish and seawater which eliminates the standard lactose broth enrichment step (Andrews, Diggs and Wilson, 1975 and Andrews and Presnell, 1972). Both the ETCP and Medium A-1 tests were evaluated as methods for monitoring oyster depuration.

Materials and Methods

Oysters collected from shellfish grounds in the Pagan, Poropotank and York Rivers of Virginia were pooled in lots containing 2-10 oysters and examined for fecal coliform content using the procedures described by APHA (1970). MPN determinations were based on 5-tube replicates. When parallel testing with the ETCP method, the MPN procedure was modified to include a gravimetric step, i.e., a 1:10 dilution by weight of oyster sample was used for inoculating and preparing the necessary dilutions.

Elevated temperature (ET) coliforms were enumerated in modified MacConkey Agar (MMA) (Cabelli and Heffernan, 1970a). Sixty ml of melted double strength MMA (50-55°C) was added to a sterile cup containing 6 g shellfish or decimal dilutions thereof and 54 ml phosphate buffered saline (PBS). Contents of the cup were gently mixed and equally distributed over 6 petri dishes such that each contained approximately 1 g shellfish or decimal portions thereof. Plates containing 0.5 g shellfish were prepared by adding twice the volume of MMA and PBS and distributing over 12 petri dishes. Numbers of ET coliforms/100 g shellfish were calculated using appropriate replication and dilution factor adjustments.

Artificially contaminated samples were prepared by adding 1 ml of an appropriate dilution of E. coli that would result in about 30 colonies/plate to cups containing various concentrations of oyster homogenate in PBS. Immediately after shaking the cup contents, melted MMA was added, gently mixed and pour plates prepared.

Samples enumerated in Medium A-1 (Andrews and Presnell, 1972) were incubated at 35 $\pm$ 0.3^{o}C in a water-jacketed air incubator for 3 hours prior to being transferred to a water bath at 44.5 $\pm$ 0.2^{o}C for an additional 21 hours unless otherwise specified.

All gassing EC and Medium A-1 tubes from 50 parallel examinations were examined for the coliform type present by streaking on eosin methylene blue agar plates. Different colony types on individual plates were isolated, transferred for gas production at elevated temperature, identified as to IMViC type, and tested for cytochrome oxidase using taxo N discs (BBL). Isolates which gave IMViC reactions other than that for E. coli were identified using the API-20 Enterobacteriaceae System (Analytab Products, Inc.).

Terminology of Fishbein, et al. (1967), is followed in the presentation and discussion of the results.

Results and Discussion

Comparisons of fecal coliforms (FC) and elevated temperature (ET) coliform densities in naturally polluted oysters prior to and after various periods of depuration in a flow-through seawater system are shown in Table 49. Analyses of the 0 hour sample in Trial 1 using the standard method revealed a fecal coliform content of 4,900/100 g. By the ETCP technique, 1 g quantities of the same homogenate were negative. In contrast, ET coliforms were observed on plates containing approximately 0.1 g shellfish each. We, therefore, speculated that oyster meat had a graded inhibitory effect on the growth of ET coliforms and inoculated plates with 0.5 g quantities in Trials 2 and 3. ETCP counts for these experiments were observed to be indirectly proportional to the quantity of shellfish inoculum.

Thus, it appeared that oyster brei exerted an inhibitory effect on the expression of ET coliforms which could be lowered by decreasing the quantity of oyster meat plated.

The influence of length of incubation period on ET coliform enumerations in the above experiments was also evaluated (Table 49). The results showed that the incubation period significantly affected ET coliform counts. While the colony counts increased with increasing incubation periods in plates containing 1 or 0.5 g oyster brei, little or no effect was observed on plates containing 0.1 g quantities.

Additional experiments were conducted using both relatively clean (fecal coliforms = <100) and polluted oysters (fecal coliforms = 110-1100) to further evaluate the standard FC and ETCP tests as functions of shellfish concentration and incubation period (Table 50).

In five out of the six experiments using moderately polluted oysters, the FC count exceeded the ETCP count when plates containing approximately 1 g shellfish were examined after 18 hours incubation. A similar comparison could not be made with the group of relatively clean oysters since the counts often were below detectability. However, when the incubation period was prolonged to 72 hours, ETCP counts, regardless of brei concentration, exceeded the corresponding FC count in all but one experiment. As noted in the previous experiments, plates containing 0.1 g shellfish yielded higher ETCP counts after adjusting for the dilution factor than did plates containing more concentrated brei after 18 hours incubation. Extending the incubation period resulted in enumeration of more ETCP colonies with the most dramatic increases occurring on plates containing 1 and 0.5 g shellfish.

As the preceeding experiments were conducted using naturally polluted oysters, the possibility that the increased ETCP counts obtained upon extended incubation were not due to typical ETCP coliforms was considered. An experiment was designed to determine if the effects of brei concentration and incubation time could be repeated using a pure culture of E. coli. Test oysters were initially depurated for 24 hours to yield homogenates free of FC and ET bacteria. A known concentration of E. coli was added to various concentrations of shellfish homogenate immediately before distributing in petri dishes for ETCP analysis. Results (Table 51) were similar to findings with naturally polluted oysters. Inhibition of E. coli was decreased by decreasing the amount of oyster homogenate plated and by prolonging the incubation period.

Comparison of the Medium A-1 test with the standard fecal coliform test through the parallel examination of 143 oyster samples indicated the two methods were not significantly different (calculated t value = 0.63; $t_{0.05}$ = 1.98; Null hypothesis not rejected).

All gassing EC and Medium A-1 tubes from fifty parallel examination experiments were examined for coliform type(s). Percentages of recovery for E. coli and/or coliforms other than E. coli; i.e., false positive (FP) group, are shown in Table 52. The data presented indicate both procedures gave high recoveries of E. coli. Percentages of E. coli detection for the standard fecal coliform and Medium A-1 tests were 99.2 and 100 percent respectively. While only one percent of the positive Medium A-1 tubes contained a coliform exclusive of E. coli type I and II which produced gas at 44.5°C, six percent of the EC tubes were discovered to do so. Our results for Medium A-1 are consistent with those of Andrews, Diggs, and Wilson (1975) who reported the recovery of E. coli from 99% of the gassing tubes inoculated with oyster brei. However, these investigators reported a lower specificity for the standard test, i.e., 23% false positive tubes, than we observed.

On a few occasions, turbid but nonaerogenic tubes were incubated longer than the usual 24 hr period and were subsequently observed to gas. Therefore, the Medium A-1 was examined to determine the effect of extending the incubation period of development of a positive reaction. Out of a total of 346 positive tubes, 94.5% were positive following the routine 24 hr incubation period. Prolonging incubation an additional 3 hrs resulted in the appearance of 0.6% more positive tubes while 4.9% of the tubes gassed after a 24 hr extension. One-half of the isolates from the delayed gassing tubes were E. coli, whereas the remaining were identified as Enterobacter and Klebsiella.

The overall performance of the Medium A-1 test was superior to the ETCP procedure for determination of fecal coliforms in oysters. The ETCP test necessitates incubation of oyster samples for up to 72 hrs to recover the maximum number of E. coli and thus did not serve as a rapid method for oysters, although it is reported to be a satisfactory 24 hr test for hard and soft shell clams (Cabelli and Heffernan, 1970a and 1970b). The inhibitory effect observed may be due to hydrolytic enzymes or toxic substance(s) released upon lysis or mechanical disruption of the cells by homogenization. Our data suggest that the viable count suppression is reversible since counts tended to increase upon prolonged incubation. Some degree of coliform suppression using the MPN procedure was suggested by higher estimates of organisms from ETCP enumerations following 72 hr incubation versus the standard FC quantitations.

The Medium A-1 test resulted in a 24 hr determination of fecal coliform content with the occurrence of fewer false positives compared to the 72 hr standard procedure. In addition to the reduction in time and labor, Medium A-1 is more economical, media supplies costing \$0.22/sample when using 5 tube replicates for 4 dilutions in contrast to \$0.64/sample for analysis using the standard fecal coliform method.

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APPENDIX

Tables and Figures

TABLES

Table 1. Range and mean of environmental factors recorded during all individual experiments on oyster depuration included in final report for period 15 June 1973-15 June 1976.

Expt. No.	Tank Type	Date[1]	Temp. (C) Range (Mean)	Salinity (°/oo) Range (Mean)	D.O. (mg/l) Range (Mean)	pH Range (Mean)	Tot. Sol. (mg/l) Range (Mean)	Flow Rate (GPM/Bu) Range (Mean)
2	ST(12)[2]	10 Nov 73	(13) 12.5-21.3[3] (18.2)	(5) 20.8-22.2[3] (21.2)	--	--	--	(3) 3.1- 3.1[3] (3.1)
9	ST(36)	4 Mar 74	(19) 12.0-18.5 (17.0)	(4) 16.7-18.1 (17.3)	(7) 6.6-10.1[3] (9.1)	(2) 6.6- 6.7[3] (6.7)	--	--
10	ST(36)	12 Mar 74	(17) 10.3-20.8 (17.3)	(6) 17.4-18.3 (17.8)	(6) 7.0- 9.1 (7.8)	(1) 6.2- 6.2 (6.2)	--	--
11	ST(36)	19 Mar 74	(15) 9.2-11.5 (10.4)	(4) 16.1-18.6 (17.2)	(4) 9.7-10.6 (10.2)	--	--	(3) 3.0- 3.0 (3.0)
12	ST(25)	20 Mar 74	(14) 13.3-17.8 (16.0)	(3) 17.0-18.6 (17.6)	(3) 9.3- 9.8 (9.5)	--	--	(3) 7.3- 7.3 (7.3)
13-1	ST(25)	1 Apr 74	(11) 12.2-15.4 (13.5)	(4) 16.5-16.7 (16.6)	(4) 8.8- 9.2 (9.0)	(1) 5.5- 5.5 (5.5)	(13) 4.0-37.2 (19.9)	--
13-2	ST(25)	1 Apr 74	(11) 12.2-15.4 (13.5)	(4) 16.5-16.7 (16.6)	(4) 8.8- 9.2 (9.0)	(1) 5.5- 5.5 (5.5)	(13) 21.3-146.0 (63.8)	--
13-3	ST(25)	1 Apr 74	(11) 12.2-15.4 (13.5)	(4) 16.5-16.7 (16.6)	(4) 8.8- 9.2 (9.0)	(1) 5.5- 5.5 (5.5)	(24) 16.0-19.2 (10.2)	--
15-2	ST(36)	15 Apr 74	(12) 13.2-15.8 (14.7)	(4) 14.7-17.4 (15.7)	(4) 9.3-10.0 (9.5)	(2) 7.8- 7.8 (7.8)	(12) 4.0-34.0 (16.7)	(3) 3.5- 3.5 (3.5)
15-3	ST(36)	15 Apr 74	(12) 13.2-15.8 (14.7)	(4) 14.7-17.4 (15.7)	(4) 9.3-10.0 (9.5)	(2) 7.8- 7.8 (7.8)	(12) 1.0-12.5 (5.0)	(3) 3.3- 3.3 (3.3)
16	ST(36)	22 Apr 74	(14) 13.9-17.6 (15.7)	(4) 15.8-18.1 (16.9)	(4) 7.7- 8.8 (8.3)	--	(13) 2.5-27.3 (11.1)	(1) 8.0- 8.0 (8.0)
17-1	ST(36)	29 Apr 74	(18) 16.8-19.8 (18.2)	(4) 8.6-18.1 (15.5)	(3) 8.0- 8.4 (8.2)	--	(14) 16.0-126.0 (44.4)	--
17-2	ST(36)	29 Apr 74	(18) 16.8-19.8 (18.2)	(4) 8.6-18.1 (15.5)	(3) 8.0- 8.4 (8.2)	--	(14) 25.0-148.2 (77.1)	--
17-3	ST(36)	29 Apr 74	(18) 16.8-19.8 (18.2)	(4) 8.6-18.1) (15.5)	(3) 8.0- 8.4 (8.2)	--	(26) 3.0-25.5 (13.4)	--
18-1	ST(36)	6 May 74	(10) 16.3-17.5 (17.2)	(3) 17.6-17.7 (17.6)	(3) 8.1- 8.3 (8.2)	--	(22) 16.5-38.0 (24.2)	(3) 3.4- 3.4 (3.4)
18-2	ST(36)	6 May 74	(10) 16.3-17.7 (17.2)	(3) 17.6-17.7 (17.6)	(3) 8.1- 8.3 (8.2)	--	(22) 16.5-38.0 (24.2)	(3) 6.1- 6.1 (6.1)
19-1	ST(36)	13 May 74	(10) 17.9-20.3 (19.1)	(4) 16.4-18.6 (17.8)	(4) 7.1- 8.1 (7.5)	--	(10) 11.5-20.0 (16.3)	(9) 4.2- 4.4 (4.3)

Table 1, Contd.)

Expt. No.	Tank Type	Date[1]	Temp. (C) Range (Mean)	Salinity (°/oo) Range (Mean)	D.O. (mg/l) Range (Mean)	pH Range (Mean)	Tot. Sol. (mg/l) Range (Mean)	Flow Rate (GPM/Bu) Range (Mean)
19-2	ST(36)[2]	13 May 74	(10) 17.9-20.3[3] (19.1)	(4) 16.4-18.6[3] (17.8)	(4) 7.1- 8.1[3] (7.5)	--	(10) 11.5-20.0[3] (16.3)	(3) 2.0- 2.1[3] (2.0)
20-1	ST(36)	20 May 74	(10) 20.8-22.5 (21.9)	(4) 16.9-17.3 (17.0)	(4) 6.7- 7.4 (7.2)	--	(13) 4.5-27.2 (9.8)	(3) 4.9- 4.9 (4.9)
20-2	ST(36)	20 May 74	(10) 20.8-22.5 (21.9)	(4) 16.9-17.3 (17.0)	(4) 6.7- 7.4 (7.2)	--	(13) 17.3-33.3 (26.2)	(3) 5.3- 5.3 (5.3)
20-3	ST(36)	20 May 74	(10) 20.8-22.5 (21.9)	(4) 16.9-17.3 (17.0)	(4) 6.7- 7.4 (7.2)	--	(13) 17.3-29.0 (26.2)	(3) 2.0- 2.0 (2.0)
21	ST(36)	10 Jun 74	(24) 24.0-26.4 (25.0)	(8) 16.4-16.8 (16.5)	(8) 6.6- 7.2 (6.9)	--	(30) 12.0-30.7 (18.9)	(12) 4.8- 6.4 (5.4)
22	ST(36)	8 Jul 74	(13) 25.0-28.0 (26.5)	(4) 19.5-19.8 (19.6)	(4) 6.6- 7.6 (7.0)	(3) 7.3- 7.4[3] (7.3)	(13) 2.7-38.7 (12.8)	(6) 5.0- 5.4 (5.2)
23	ST(36)	15 Jul 74	(8) 26.2-27.4 (26.7)	(6) 19.4-20.7 (19.8)	(12) 4.7- 6.7 (5.5)	(3) 7.2- 7.6 (7.4)	(12) 8.7-39.3 (16.8)	(6) 5.6- 6.0 (5.8)
26	ST(36)	22 Jul 74	(6) 25.0-26.6 (25.8)	(5) 20.3-20.7 (20.5)	(5) 4.6- 5.9 (5.4)	--	(12) 10.7-34.7 (20.4)	(3) 5.8- 5.8 (5.8)
27	ST(36)	29 Jul 74	(9) 26.9-28.5 (27.6)	(3) 19.9-20.1 (20.0)	(3) 5.7- 5.7 (5.7)	--	(13) 10.0-30.7 (16.0)	(12) 4.8- 5.4 (5.2)
28-1	ST(36)	6 Aug 74	(7) 25.3-26.5 (25.8)	(4) 20.1-20.4 (20.2)	(21) 3.2- 6.2 (4.9)	--	(10) 7.2-23.3 (12.7)	(3) 1.7- 1.7 (1.7)
28-2	ST(36)	6 Aug 74	(7) 25.3-26.5 (25.8)	(4) 20.1-20.4 (20.2)	(21) 3.9- 6.2 (4.8)	--	(10) 7.2-23.3 (12.7)	(3) 1.7- 1.7 (1.7)
28-3	ST(36)	6 Aug 74	(7) 25.3-26.5 (25.8)	(4) 20.1-20.4 (20.2)	(19) 0.1- 0.9 (0.6)	--	(10) 7.2-23.3 (12.7)	(3) 1.7- 1.7 (1.7)
28-4	ST(36)	6 Aug 74	(7) 25.3-26.5 (25.8)	(4) 20.1-20.4 (20.2)	(18) 0.6- 2.4 (1.8)	--	(10) 7.2-23.3 (12.7)	(3) 1.7- 1.7 (1.7)
30-1	ST(36)	19 Aug 74	(18) 24.9-27.4 (26.0)	(4) 20.0-20.2 (20.1)	(19) 4.1- 6.5 (5.2)	(1) 7.4- 7.4 (7.4)	(12) 10.5-64.7 (26.8)	(3) 1.8- 1.8 (1.8)
30-2	ST(36)	19 Aug 74	(18) 24.9-27.4 (26.0)	(4) 20.0-20.2 (20.1)	(19) 3.6- 6.5 (5.3)	(1) 7.4- 7.4 (7.4)	(12) 10.5-64.7 (26.8)	(3) 1.8- 1.8 (1.8)
30-3	ST(36)	19 Aug 74	(18) 24.9-27.4 (26.0)	(4) 20.0-20.2 (20.1)	(22) 0.4- 1.9 (0.8)	(1) 7.4- 7.4 (7.4)	(12) 10.5-64.7 (26.8)	(3) 1.8- 1.8 (1.8)
30-4	ST(36)	19 Aug 74	(18) 24.9-27.4 (26.0)	(4) 20.0-20.2 (20.1)	(22) 0.5- 2.1 (1.1)	(1) 7.4- 7.4 (7.4)	(12) 10.5-64.7 (26.8)	(3) 1.8- 1.8 (1.8)
31	ST(25)	3 Sep 74	(10) 24.2-28.9 (25.8)	(4) 21.1-21.8 (21.4)	(4) 4.8- 6.4 (5.5)	(3) 7.1- 7.4 (7.3)	(10) 10.0-35.3 (22.4)	(3) 2.5- 2.5 (2.5)

Table 1, Contd.

Expt. No.	Tank Type	Date[1]	Temp. (C) Range (Mean)	Salinity (°/oo) Range (Mean)	D.O. (mg/l) Range (Mean)	pH Range (Mean)	Tot. Sol. (mg/l) Range (Mean)	Flow Rate (GPM/Bu) Range (Mean)
32	ST(25)[2]	9 Sep 74	(8) 23.8-26.5[3] (24.8)	(4) 19.0-19.8[3] (19.4)	(4) 5.7- 6.4[3] (6.0)	(4) 7.5- 7.7[3] (7.6)	(12) 6.8-26.0[3] (16.4)	(3) 2.5- 2.5[3] (2.5)
35	ST(25)	21 Oct 74	(4) 14.1-15.3 (14.8)	(2) 20.6-20.6 (20.6)	(2) 8.4- 8.5 (8.4)	(2) 7.5- 7.6 (7.6)	(5) 6.4-17.6 (11.1)	(1) 6.5- 6.5 (6.5)
36	ST(25)	4 Nov 74	(9) 16.6-19.1 (17.9)	(5) 13.7-22.0 (20.1)	(5) 6.6- 7.9 (7.4)	(4) 7.2- 7.5 (7.4)	(10) 5.6-24.0 (12.9)	(2) 6.5- 6.5 (6.5)
40[4]	ST(25)	7 Dec 74	(15) 11.6-14.5 (13.6)	(4) 22.2-23.4 (22.8)	(4) 9.0- 9.3 (9.1)	--	(10) 8.0-32.4 (14.9)	(3) 6.3- 6.2 (6.2)
46-1	ST(25)	24 Mar 75	(35) 14.8-22.0 (19.6)	(9) 12.2-13.6 (14.0)	(12) 8.0- 9.9 (8.8)	(2) 7.9- 8.0 (7.9)	(11) 6.0-36.0 (14.1)	(12) 3.4- 3.4 (3.4)
46-2	ST(25)	24 Mar 75	(35) 9.4-14.8 (11.8)	(9) 12.2-14.5 (14.0)	(12) 7.9- 9.9 (9.2)	(2) 7.9- 8.0 (7.9)	(11) 6.0-36.0 (14.1)	(12) 3.4- 3.4 (3.4)
48	4 x 8	12 May 75	(14) 18.0-24.8 (20.2)	(5) 15.7-20.4 (16.9)	(8) 5.3- 6.4 (6.0)	(3) 7.8- 8.2 (8.0)	(11) 13.0-47.5 (24.8)	(3) 1.1- 1.1 (1.1)
49	Flume	2 Jun 75	(6) 23.3-25.0 (24.4)	(3) 15.8-17.3 (16.4)	(4) 3.5- 6.5 (4.8)	(2) 7.4- 7.9 (7.7)	(6) 11.5-23.0 (17.7)	(1) 1.5- 1.5 (1.5)
50	4 x 8	9 Jun 75	(9) 22.4-24.0 (23.3)	(4) 17.7-18.0 (17.8)	(12) 3.9- 6.4 (5.0)	(4) 7.8- 8.0 (7.9)	(12) 13.0-26.5 (19.7)	(3) 1.0- 1.0 (1.0)
51	4 x 8	16 Jun 75	(14) 24.8-27.9 (25.8)	(4) 17.9-18.4 (18.2)	(14) 5.5- 6.3 (5.8)	(3) 7.9- 8.1 (8.0)	(12) 12.5-24.0 (16.1)	(3) 1.9- 1.9 (1.9)
53	2 x 8	7 Jul 75	(19) 25.2-28.0 (26.5)	(11) 15.5-18.1 (17.3)	(41) 3.0- 4.5 (3.5)	(4) 7.6- 7.7 (7.7)	(9) 8.0-43.0 (17.7)	(9) 1.4- 2.3 (2.1)
54	2 x 8	14 Jul 75	(19) 25.8-27.0 (26.2)	(5) 16.4-18.1 (17.5)	(24) 3.0- 3.6 (3.3)	(1) 7.5- 7.5 (7.5)	(12) 11.0-27.2 (19.8)	(12) 3.1- 4.0 (3.4)
55	Flume	14 Jul 75	(14) 26.5-27.9 (27.2)	(5) 13.9-17.2 (15.9)	(10) 3.7- 6.2 (4.8)	(4) 7.7- 7.9 (7.8)	(12) 3.5-19.2 (10.6)	(6) 2.1- 2.3 (2.2)
56	Flume	4 Aug 75	(18) 16.8-29.9 (27.4)	(5) 16.1-18.4 (17.0)	(14) 1.0- 4.1 (2.8)	(1) 7.9- 7.9 (7.9)	(12) 2.0-27.2 (12.6)	(5) 0.9- 1.2 (1.1)
57	4 x 8	11 Aug 75	(16) 26.8-28.7 (27.4)	(4) 18.7-19.1 (18.9)	(24) 4.0- 5.5 (4.6)	--	(12) 9.6-25.6 (21.5)	(3) 2.2- 2.2 (2.2)
58	4 x 8	18 Aug 75	(13) 28.2-30.5 (28.8)	(2) 16.5-18.3 (17.4)	(23) 4.1- 4.8 (4.4)	(1) 8.6- 8.6 (8.6)	(11) 11.5-28.1 (17.7)	(4) 1.9- 2.2 (2.0)
59	Flume	25 Aug 75	(13) 28.1-30.1 (28.9)	(3) 18.7-19.7 (19.1)	(7) 2.7- 5.8 (4.2)	--	(10) 9.6-18.4 (15.8)	(3) 1.5- 1.7 (1.6)

Table 1, Contd.

Expt. No.	Tank Type	Date[1]	Temp. (C) Range (Mean)	Salinity (°/oo) Range (Mean)	D.O. (mg/l) Range (Mean)	pH Range (Mean)	Tot. Sol. (mg/l) Range (Mean)	Flow Rate (GPM/Bu) Range (Mean)
60	2 x 8	2 Sep 75	(17) 25.3-27.0[3] (26.1)	(3) 18.0-19.1[3] (18.5)	(24) 2.5- 3.5[3] (3.1)	--	(11) 12.7-22.8[3] (17.9)	(8) 1.8- 2.1[3] (2.0)
61	4 x 8	8 Sep 75	(8) 25.0-26.6 (25.9)	(3) 17.6-17.8 (17.7)	(18) 2.3- 3.6 (3.0)	(3) 7.8- 7.9[3] (7.9)	(8) 4.0-27.5 (17.0)	(8) 1.8- 2.4 (2.2)
62	2 x 8	22 Sep 75	(15) 22.6-24.1 (23.1)	(3) 17.6-18.7 (18.1)	(18) 5.4- 6.1 (5.6)	(2) 7.7- 7.7 (7.7)	(12) 11.5-32.0 (18.5)	(6) 2.0- 2.3 (2.1)
63	2 x 8	29 Sep 75	(13) 23.0-24.5 (23.7)	(4) 16.1-16.9 (16.5)	(18) 3.9- 5.2 (4.4)	(3) 7.8- 7.9 (7.9)	(12) 5.4-33.0 (14.7)	(6) 2.0- 2.1 (2.0)
65	2 x 8	14 Oct 75	(19) 20.3-22.0 (21.0)	(4) 15.1-17.0 (16.0)	(21) 4.3- 6.5 (5.0)	(4) 7.7- 8.0 (7.8)	(12) 8.0-17.0 (12.0)	(8) 1.9- 2.2 (2.1)
67	2 x 8	20 Oct 75	(16) 19.0-21.0 (19.9)	(7) 17.4-19.1 (18.0)	(21) 5.0- 6.8 (5.6)	(3) 7.7- 7.9 (7.7)	(10) 9.0-13.5 (11.5)	(4) 2.1- 2.2 (2.2)
68	2 x 8	27 Oct 75	(13) 19.0-20.2 (19.5)	(9) 16.1-17.1 (16.5)	(21) 6.0- 7.0 (6.5)	(4) 7.6- 7.9 (7.8)	(11) 4.5-18.0 (11.5)	(9) 1.9- 2.2 (2.1)
69	2 x 8	3 Nov 75	(17) 16.8-18.2 (17.6)	(7) 16.5-16.8 (16.7)	(14) 6.2- 7.4 (6.7)	(4) 7.8- 7.9 (7.8)	(12) 9.0-35.0 (13.7)	(6) 2.0- 2.2 (2.1)
70	2 x 4	10 Nov 75	(12) 17.1-19.2 (18.0)	(4) 16.0-17.8 (16.6)	(17) 6.4- 7.8 (6.8)	(4) 7.7- 7.8 (7.8)	(12) 4.4-12.4 (8.2)	(13) 1.7- 2.3 (2.0)
71	2 x 8	17 Nov 75	(18) 13.8-15.7 (14.6)	(6) 16.1-17.2 (16.8)	(21) 6.9- 8.3 (7.3)	(4) 7.8- 8.0 (7.9)	(11) 2.8-11.2 (6.7)	(9) 2.0- 2.2 (2.1)
72	2 x 8	1 Dec 75	(16) 10.0-12.6 (11.5)	(4) 17.6-17.9 (17.7)	(21) 8.1- 8.7 (8.3)	(4) 7.9- 8.0 (7.9)	(12) 6.0-11.6 (8.5)	(8) 2.0- 2.5 (2.2)
73	2 x 8	8 Dec 75	(13) 9.3-10.7 (10.1)	(3) 17.6-18.1 (17.8)	(13) 8.4- 9.2 (8.8)	(4) 7.9- 8.0 (7.9)	(11) 3.2- 9.2 (5.8)	(9) 1.8- 2.2 (2.1)
74	4 x 8	13 Jan 76	(16) 4.8- 6.5 (5.6)	(5) 14.4-19.8 (16.5)	(6) 11.0-11.1 (11.0)	--	(12) 9.2-17.5 (13.4)	(2) 2.0- 2.0 (2.0)
78	2 x 8	1 Mar 76	(27) 11.0-12.5 (11.8)	(4) 15.6-16.4 (15.9)	(11) 8.5-16.0 (9.4)	--	(11) 2.5-15.0 (7.6)	(9) 1.8- 2.0 (2.0)
79	4 x 8	9 Mar 76	(19) 10.0-11.8 (10.5)	(5) 15.1-16.0 (15.7)	(9) 9.3-10.0 (9.6)	(3) 7.9- 8.1 (8.0)	(13) 8.0-52.7 (16.2)	(5) 1.9- 2.1 (2.0)
80	4 x 8	16 Mar 76	(25) 9.0-11.3 (10.4)	(3) 15.5-16.6 (16.1)	(12) 9.4- 9.9 (9.6)	--	(12) 8.5-17.5 (14.1)	(16) 0.9- 1.3 (1.0)
81	2 x 8	23 Mar 76	(25) 11.1-13.9 (12.3)	(4) 15.1-15.2 (15.1)	(18) 8.4- 9.5 (9.0)	(3) 8.3- 8.4 (8.3)	(12) 13.0-23.2 (18.3)	(17) 0.8- 1.0 (1.0)

Table 1, Contd.

Expt. No.	Tank Type	Date[1]	Temp. (C) Range (Mean)	Salinity (o/oo) Range (Mean)	D.O. (mg/l) Range (Mean)	pH Range (Mean)	Tot. Sol. (mg/l) Range (Mean)	Flow Rate (GPM/Bu) Range (Mean)
82	2 x 4	26 Apr 76	(16) 14.8-16.5[3] (15.6)	(2) 17.9-18.2[3] (18.0)	(8) 5.2- 5.6[3] (5.6)	--	(8) 20.0-33.5[3] (25.1)	(10) 0.9- 1.0[3] (1.0)
83	2 x 4	3 May 76	(18) 16.2-18.1 (17.4)	(4) 17.9-18.5 (18.3)	(12) 5.2- 5.7 (5.4)	--	(12) 13.0-30.5 (20.3)	(16) 0.8- 1.0 (1.0)
84	2 x 4	10 May 76	(21) 17.6-19.8 (18.4)	(4) 18.1-18.6 (18.4)	(12) 4.6- 5.5 (4.9)	--	(13) 8.5-70.0 (24.1)	(17) 0.9- 1.1 (1.0)
149	Flume	2 Jun 75	(10) 23.4-25.7 (24.4)	(4) 15.5-17.3 (16.4)	(6) 3.0- 6.7 (4.9)	(1) 7.9- 7.9[3] (7.9)	(12) 11.5-24.0 (18.8)	(4) 1.5- 1.6 (1.5)
155	Flume	28 Jul 75	(14) 26.5-27.9 (27.2)	(5) 13.9-17.2 (15.9)	(10) 3.1- 6.0 (4.9)	(4) 7.7- 7.9 (7.8)	(12) 3.5-19.2 (10.6)	(6) 1.8- 2.2 (2.0)
156	Flume	4 Aug 75	(18) 16.8-29.9 (27.4)	(5) 16.1-18.4 (17.0)	(14) 2.8- 6.4 (4.9)	(1) 7.9- 7.9 (7.9)	(11) 3.5-27.2 (13.8)	(5) 1.9- 2.5 (2.3)
159	Flume	25 Aug 75	(12) 28.1-30.1 (29.0)	(2) 18.7-18.8 (18.7)	(9) 2.9- 6.3 (5.4)	--	(10) 10.4-22.4 (15.8)	(3) 1.6- 1.8 (1.7)
169	ST(25)[2]	3 Nov 75	(11) 16.8-18.2 (17.6)	(4) 16.5-16.8 (16.7)	--	(2) 7.8- 7.8 (7.8)	(8) 9.0-19.0 (11.8)	(3) 4.3- 4.3 (4.3)
170	2 x 4	10 Nov 75	(12) 17.1-19.2 (18.0)	(4) 16.0-17.8 (16.6)	(13) 6.2- 7.8 (6.7)	(4) 7.8- 7.9 (7.8)	(12) 4.4-12.4 (8.2)	(13) 1.5- 2.1 (2.0)
171	2 x 4	17 Nov 75	(18) 13.8-15.7 (14.6)	(6) 16.1-17.2 (16.8)	(15) 6.8- 8.3 (7.3)	(4) 7.7- 7.9 (7.8)	(11) 2.8-10.0 (6.7)	(9) 1.9- 2.1 (2.0)
172	2 x 4	1 Dec 75	(16) 10.0-12.6 (11.5)	(4) 17.6-17.9 (17.7)	(16) 8.0- 8.7 (8.3)	(4) 7.9- 8.0 (7.9)	(12) 5.2-11.6 (8.5)	(7) 1.8- 2.1 (2.0)
173	2 x 4	8 Dec 75	(14) 9.3-10.7 (10.1)	(3) 17.6-18.1 (17.8)	(9) 8.4- 8.9 (8.7)	(4) 7.9- 8.0 (7.9)	(11) 3.2- 9.2 (5.8)	(9) 1.8- 2.1 (2.0)
182	2 x 4	26 Apr 76	(23) 14.8-16.5 (15.6)	(2) 17.9-18.2 (18.0)	(12) 4.3- 5.6 (4.9)	--	(12) 20.0-29.0 (25.1)	(15) 1.0- 1.1 (1.0)
183	2 x 4	3 May 76	(18) 16.2-18.1 (17.4)	(4) 17.9-18.5 (18.3)	(12) 4.6- 5.3 (4.8)	--	(12) 13.0-30.5 (20.3)	(15) 0.9- 1.1 (1.0)
184	2 x 4	10 May 76	(21) 17.6-19.8 (18.4)	(4) 18.1-18.6 (18.4)	(12) 4.2- 4.8 (4.5)	--	(13) 8.5-24.5 (24.1)	(17) 0.9- 1.1 (1.0)
259	Flume	25 Aug 75	(11) 28.1-30.1 (28.9)	(2) 18.7-18.8 (18.7)	(9) 2.2- 6.5 (5.5)	--	(10) 10.4-20.0 (14.8)	(3) 1.6- 1.7 (1.7)
270	ST(25)	10 Nov 75	(11) 17.3-19.0 (17.8)	(4) 16.0-17.8 (16.6)	--	(4) 7.7- 7.8 (7.8)	(12) 4.4-12.4 (8.2)	--

Table 1, Contd.

Expt. No.	Tank Type	Date[1]	Temp. (C) Range (Mean)	Salinity (°/oo) Range (Mean)	D.O. (mg/l) Range (Mean)	pH Range (Mean)	Tot. Sol. (mg/l) Range (Mean)	Flow Rate (GPM/Bu) Range (Mean)
271	2 x 4	17 Nov 75	(18) 13.8-15.7[3] (14.6)	(6) 16.1-17.2[3] (16.8)	(15) 6.7- 8.3[3] (7.1)	(4) 7.7- 7.9[3] (7.7)	(11) 2.8-11.2[3] (6.7)	(9) 1.9- 2.0[3] (2.0)
272	2 x 4	1 Dec 75	(16) 10.0-12.6 (11.5)	(4) 17.6-17.9 (17.7)	(14) 8.0- 8.7 (8.1)	(4) 7.8- 8.0 (7.9)	(12) 5.2-11.6 (8.5)	(8) 2.1- 2.2 (2.1)
273	2 x 4	8 Dec 75	(14) 9.3-10.7 (10.1)	(3) 17.6-18.1 (17.8)	(9) 8.3- 8.8 (8.6)	(4) 7.8- 7.9 (7.8)	(11) 3.2- 9.2 (5.8)	(9) 1.9-2.2 (2.1)

[1] Date given is that of first day of experiment. Experiment duration was usually three days and data given cover the whole period.

[2] ST = Shallow tray; figure in parentheses gives number of oysters in tray.

[3] Figure in parentheses preceding range values gives number of observations included.

[4] Experiment using oysters contaminated with sewage in the laboratory.

Table 2. Mean MPN level, 90% confidence interval and cumulative percentages for samples at selected levels of total coliforms in oysters subjected to depuration in shallow trays. Grouped according to initial oyster MPN level and water temperature. Oysters contaminated in nature.

Initial level in oysters (MPN/100g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Expts.	No. Samples	MPN/100 g Mean	MPN/100 g 90% Confidence Interval		Percent of samples with MPN levels ≤stated values 500	230	100	% Samples with MPN >1000
<1000	9.1-14.0 (11.8-13.5)[1]	0	4	4	710	1900	260	50	0	0	25
		24		8	< 100	860	11	88	75	50	13
		48		8	< 140	1100	18	63	63	63	25
		72		8	< 270	11000	7	75	50	50	25
	14.1-19.0 (14.8-17.9)	0	2	29	< 190	1100	31	60	52	38	24
		24		29	< 19	--[2]	--[2]	100	100	100	0
		48		12	< 41	130	12	92	92	92	0
		72		12	< 18	--	--	100	100	100	0
	19.1-24.0 (19.6)	0	1	3	960	2100	440	33	0	0	33
		24		4	< 97	940	10	75	75	75	25
		48		4	44	150	13	100	100	75	0
		72		4	< 31	48	21	100	100	75	0
	24.1-29.0 (25.8-27.6)	0	2	14	710	2300	210	50	29	0	24
		24		17	270	1900	39	65	65	41	35
		48		15	< 250	1200	52	73	53	40	20
		72		23	< 50	--	--	91	87	83	9
1001- 10000	9.1-14.0 (10.4)	0	1	1	1700	--	--				100
		24		1	490	--	--				0
		48		1	1200	--	--				100
		72		1	2300	--	--				100
	14.1-19.0 (15.7-19.1)	0	9	8	3100	9600	1000	13	0	0	88
		24		14	270	2600	27	64	57	50	36
		48		13	< 240	2200	25	69	62	38	31
		72		16	< 180	4700	7	56	56	50	25
	19.1-24.0 (21.9)	0	3	1	4600	--	--	0	0	0	100
		24		4	64	150	26	100	100	50	0
		48		4	72	440	12	100	75	75	0
		72		5	< 29	--	--	100	100	80	0
	24.1-29.0 (25.0-26.7)	0	7	52	1800	4700	690	12	6	6	79
		24		53	< 63	--	--	10	74	65	8
		48		56	< 24	--	--	100	94	87	0
		72		32	< 24	--	--	100	94	94	0

Table 2, contd.

Initial level in oysters (MPN/100g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Expts.	No. Samples	MPN/100 g Mean	90% Confidence Intervals		Percent of samples with MPN levels $\leq$ stated values: 500	230	100	% Samples with MPN $>$1000
$>$10000	14.1-19.0										
	(17.0)	0	1	1	920000	--	--	0	0	0	100
		24		1	1100	--	--	0	0	0	100
		48		1	4900	--	--	0	0	0	100
		72		1	950	--	--	0	0	0	0
	(14.7)	0	2	1	130000	--	--	0	0	0	100
		24		2	63	--	--	100	100	50	0
		48		2	$<$ 49	--	--	100	100	50	0
		72		2	290	--	--	100	50	0	0
	(17.8)	0	1	3	11000	--	--	0	0	0	100
		24		--	--	--	--	--	--	--	--
		48		--	--	--	--	--	--	--	--
		72		3	210	--	--	100	67	0	0
	24.1-29.0	0	5	23	19000	42000	8200	0	0	0	100
	(24.8-25.8)	24		25	$<$ 72	170	30	96	84	68	4
		48		25	$<$ 44	--	--	96	96	92	4
		72		26	$<$ 46	--	--	88	88	85	4

Footnotes:

[1] Actual temperatures included appear in parentheses.

[2] Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 3. Mean MPN level, 90% confidence interval and cumulative percentages for samples at selected levels of fecal coliforms in oysters subjected to depuration in shallow trays. Grouped according to initial oyster MPN level and water temperature. Oysters contaminated in nature.

Initial level in oysters (MPN/100 g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Expts.	No. Samples	MPN/100 g Mean	90% Confidence Interval		Percent of samples with MPN levels ≤ stated values 100	50	20	% Samples with MPN > 200
< 500	9.1-14.0 (10.4-13.5)[1]	0	5	5	≤ 110	590	19	40	20	20	60
		24		9	≤ 22	--[2]	--[2]	100	89	89	0
		48		9	≤ 19	--	--	100	100	89	0
		72		9	≤ 18	--	--	100	100	100	0
	14.1-19.0 (14.8-17.9)	0	6	38	≤ 43	--	--	76	61	53	13
		24		33	≤ 18	--	--	100	100	97	0
		48		16	≤ 18	--	--	100	100	94	0
		72		15	< 18	--	--	100	100	100	0
	19.1-24.0 (19.6-21.9)	0	2	4	250	530	130	0	0	0	100
		24		6	≤ 29	--	--	83	83	83	17
		48		6	≤ 27	--	--	83	83	83	17
		72		6	< 18	--	--	100	100	100	0
	24.1-29.0 (25.0-26.7)	0	6	61	≤ 210	1100	37	30	22	18	57
		24		65	≤ 19	--	--	97	95	89	0
		48		64	≤ 19	--	--	95	95	95	0
		72		30	< 18	--	--	100	100	100	0
501- 1000	14.1-19.0 (18.1-19.1)	0	5	5	600	2200	160	0	0	0	60
		24		9	< 37	75	16	78	78	56	11
		48		9	≤ 29	--	--	89	89	78	11
		72		12	< 28	--	--	92	92	67	8
	24.1-29.0 (25.0-27.6)	0	2	3	790	--	--	0	0	0	100
		24		3	< 40	--	--	67	67	67	33
		48		3	≤ 18	--	--	100	100	100	0
		72		4	≤ 18	--	--	100	100	100	0
1001- 5000	14.1-19.0 (15.7-17.9)	0	2	4	3600	14000	830	0	0	0	100
		24		1	68	--	--	100	0	0	0
		48		1	45	--	--	100	100	0	0
		72		5	< 40	210	7	80	80	60	20
	19.1-24.0 (21.9)	0	2	1	1100	--	--	0	0	0	100
		24		2	< 18	--	--	100	100	100	0
		48		2	≤ 26	--	--	100	100	50	0
		72		3	≤ 18	--	--	100	100	100	0

Table 3, contd.

Initial level in oysters (MPN/100g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Expts.	No. Samples	MPN/100 g Mean	90% Confidence Interval		Percent of samples with MPN levels ≤ stated values 100	50	20	% Samples with MPN ≥ 200
1001- 5000	24.1-29.0	0	6	25	1700	8000	350	4	0	0	92
	(24.8-25.8)	24		27	≤ 21	--	--	96	96	85	0
		48		29	≤ 20	--	--	97	97	97	3
		72		27	< 18	--	--	100	96	93	0
> 5000	14.1-19.0	0	2	1	79000	--	--	0	0	0	100
	(14.7)	24		2	≤ 18	--	--	100	100	100	0
		48		2	≤ 18	--	--	100	100	100	0
		72		2	< 18	--	--	100	100	100	0

Footnotes:

1 Actual temperatures included appear in parentheses.

2 Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 4. Coliform MPN levels in oysters collected at several field stations in lower Chesapeake Bay at water temperatures under 14.5 C.

Date	Location	Temperature (C)	MPN/100 g	
			Total Coliforms	Fecal Coliforms
13 Nov 1973	Wilson Creek	8.6	100	41
3 Dec 1973	Wilson Creek	8.5	120	30
3 Dec 1973	Poropotank River	9.5	320	68
18 Dec 1973	Poropotank River	1.5	97	32
13 Feb 1974	Pagan River	5.0	28000	<18
19 Mar 1974	Pagan River	8.8	1700	68
6 May 1974	Wormley Creek	14.2	13000	390
26 Nov 1974	Poropotank River	14.5	700	130
11 Mar 1975	Poropotank River	11.0	580	<18
24 Mar 1975	Wormley Creek	13.2	960	230
14 Apr 1975	Wormley Creek	12.8	440	150
1 Dec 1975	Wormley Creek	14.0	4300	190
8 Dec 1975	Wormley Creek	7.7	2800	180

Table 5. Mean MPN levels and cumulative percentages of fecal coliforms in oysters subjected to depuration in shallow trays. Grouped according to water temperature and dissolved oxygen concentrations. Oysters contaminated in nature.

Range Mean Water Temperature (C)	Range Mean D.O. (mg/l)	Depuration Time (Hrs)	No. Expts.	No. Samples	MPN/100g Mean	MPN/100g 90% Confidence Interval		Percent of Samples With MPN Levels ≤ Stated Values 100	50	20	% Samples With MPN >200
9.1-14.0 (10.4-13.5)[1]	6.1-12.0 (9.0-10.2)[1]	0	4	2	<34	--[2]	--[2]	100	50	50	--
		24		5	<23	--	--	100	80	80	--
		48		5	<21	--	--	100	100	80	--
		72		5	<18	--	--	100	100	100	--
14.1-19.0 (14.7-19.0)	6.1-12.0 (7.5- 9.5)	0	12	10	750	220	2500	10	10	10	70
		24		16	<34	39	30	88	75	56	6
		48		16	<25	--	--	94	94	81	6
		72		20	<24	--	--	95	95	75	5
19.1-29.0 (26.0)	0.9 (0.6)	0	1	2	2100	--	--	--	--	--	100
		24		1	270	--	--	--	--	--	100
		48		1	78	--	--	100	--	--	--
		72		1	45	--	--	100	100	--	--
(25.8)	0.9 (0.8)	0	1	1	330	--	--	--	--	--	100
		24		1	20	--	--	100	100	100	--
		48		1	20	--	--	100	100	100	--
		72		1	20	--	--	100	100	100	--
(26.0)	1.0-2.0 (1.1)	0	1	1	330	--	--	--	--	--	100
		24		1	140	--	--	--	--	--	--
		48		1	45	--	--	100	100	--	--
		72		1	45	--	--	100	100	--	--
(25.8)	1.0-2.0 (1.8)	0	1	2	2100	--	--	--	--	--	100
		24		1	20	--	--	100	100	100	--
		48		1	ID	--	--	100	100	100	--
		72		1	ID	--	--	100	100	100	--
(24.8-26.0)	4.1-6.0 (4.8-6.0)	0	9	62	470	110	2000	23	18	15	73
		24		68	<21	--	--	96	96	85	1
		48		67	<19	--	--	97	97	97	1
		72		77	<19	--	--	97	96	95	1
(19.6-26.5)	6.1-12.0 (6.9- 8.8)	0	6	31	240	168	260	19	10	6	65
		24		34	<20	--	--	94	94	94	3
		48		33	<22	--	--	91	91	88	3
		72		13	<18	--	--	100	100	100	--

Footnotes:

1 Actual temperatures included appear in parentheses.

2 Absence of confidence intervals through table due to eigher a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 6. Mean MPN levels and cumulative percentages of total coliforms in oysters subjected to depuration in shallow trays. Grouped according to water temperature and dissolved oxygen concentrations. Oysters contaminated in nature.

Range Mean Water Temperature (C)	Range Mean D. O. (mg/1)	Depuration Time (Hrs)	No. Expts.	No. Samples	MPN/100g Mean	MPN/100g 90% Confidence Interval		Percent of Samples With MPN Levels ≤ Stated Values 500	230	100	% Samples With MPN >1000
9.1-14.0 (10.4-13.5)[1]	6.1-12.0 (9.0-10.2)[1]	0	4	2	690	--	--	--	0	0	50
		24		5	490	190	1200	--	40	0	20
		48		5	840	130	5100	--	20	20	60
		72		5	2100	200	16000	--	20	20	60
14.1-19.0 (14.7-19.0)	6.1-12.0 (7.5- 9.5)	0	12	10	9900	2000	47500	--	0	0	90
		24		16	340	8	16000	--	56	44	38
		48		16	<310	11	8700	--	63	38	31
		72		19	<180	19	1700	--	58	47	37
19.1-29.0 (26.0)	0.9 (0.6)	0	1	2	17000	--[2]	--[2]	0	0	0	100
		24		1	2300	--	--	0	0	0	100
		48		1	1300	--	--	0	0	0	100
		72		1	3300	--	--	0	0	0	100
(25.8)	0.9 (0.8)	0	1	1	1300	--	--	0	0	0	100
		24		1	110	--	--	100	100	0	0
		48		1	45	--	--	100	100	100	0
		72		1	45	--	--	100	100	100	0
(26.0)	1.0- 2.0 (1.1)	0	1	1	1300	--	--	0	0	0	100
		24		1	490	--	--	100	0	0	0
		48		1	45	--	--	100	100	100	0
		72		1	170	--	--	100	100	0	0
(25.8)	1.0- 2.0 (1.8)	0	1	2	17000	--	--	0	0	0	100
		24		1	45	--	--	100	100	100	0
		48		1	ID	--	--	100	100	100	0
		72		2	220	--	--	50	50	50	0
(24.8-26.0)	4.1- 6.0 (4.8- 6.0)	0	9	63	3200	640	16000	16	11	5	83
		24		68	<73	--	--	88	84	76	9
		48		68	<52	--	--	93	87	81	6
		72		75	<36	--	--	95	91	88	4
(19.6-26.5)	6.1-12.0 (6.9- 8.8)	0	6	30	2000	1500	2800	13	0	0	73
		24		33	<110	--	--	88	64	45	12
		48		34	<29	--	--	100	97	83	0
		72		13	<25	--	--	100	100	85	0

Footnotes:

[1] Actual temperatures included appear in parentheses.

[2] Absence of confidence intervals through table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 8. Contd.

Range Mean Water Temperature (C)	Range Mean Total Solids (mg/l)	Depuration Time (Hrs)	No. Expts.	No. Samples	MPN/100g Mean	MPN/100g 90% Confidence Interval		Percent of Samples With MPN Levels ≤ Stated Values 100	50	20	% Samples With MPN >200
	20.1-30.0	0	7	16	<82	--	--	63	50	38	31
(21.9-26.0)	(20.4-26.8)	24		23	<22	--	--	96	96	78	--
		48		24	<19	--	--	96	96	96	--
		72		28	<21	--	--	93	93	93	4

Footnotes:

[1] Actual temperatures included appear in parentheses.

[2] Absence of confidence intervals through table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 9. Mean MPN levels and cumulative percentages of total coliforms in oysters subjected to depuration in shallow trays. Grouped according to initial MPN level in oysters, water temperature and salinity. Oysters contaminated in nature.

Range Mean Water Temperature (C)	Range Mean Water Salinities (°/oo)	Range Initial Level in Oysters (MPN/100 g)	Depuration Time (Hrs)	No. Expts.	No. Samples	Mean MPN/100 g	Percent of Samples With MPN Levels ≤Stated Values: 500	230	100	% Samples With MPN > 1000
9.1-14.0 (11.8)[1]	13.1-16.0 (14.0)[1]	< 1000	0	1	3	960	0	0	0	100
			24		2	< 36	100	100	100	0
			48		4	< 27	100	100	100	0
			72		2	< 75	100	50	50	0
(10.4-13.5)	16.1-19.0 (16.6-17.2)	< 1000	0	4	2	690	50	0	0	50
			24		5	430	80	40	0	20
			48		5	840	20	20	20	80
			72		5	2100	40	20	20	60
14.1-19.0 (18.2)	13.1-16.0 (15.5)	1001- 10000	0	3	2	2000	50	0	0	50
			24		4	63	100	75	75	0
			48		5	< 290	60	60	40	40
			72		4	240	50	50	50	50
(14.7)	(15.7)	> 100000	0	2	1	130000	0	0	0	100
			24		2	63	100	100	50	0
			48		2	< 49	100	100	50	0
			72		2	290	100	0	0	0
(15.7-19.1)	16.1-19.0 (16.9-17.8)	1001- 10000	0	4	5	4500	0	0	0	100
			24		6	920	33	17	17	67
			48		6	380	67	50	17	33
			72		6	79	83	83	33	17
(17.2-17.8)	(16.6-17.6)	10001- 25000	0	3	3	11000	0	0	0	100
			24		--	--	--	--	--	--
			48		--	--	--	--	--	--
			72		3	< 78	100	80	40	0
(17.0)	(17.3)	> 100000	0	1	1	920000	0	0	0	100
			24		1	1100	0	0	0	100
			48		1	4900	0	0	0	100
			72		1	950	0	0	0	0
(14.8-17.9)	19.1-22.0 (20.1-20.6)	< 1000	0	2	35	< 120	77	60	48	14
			24		29	< 19	100	100	93	0
			48		12	< 41	92	92	92	0
			72		11	< 870	82	82	82	9

Table 9, Contd.

Range Mean Water Temperature (C)	Range Mean Water Salinities (‰)	Range Initial Level in Oysters (MPN/100 g)	Depuration Time (Hrs)	No. Expts.	No. Samples	Mean MPN/100 g	Percent of Samples With MPN Levels ≤ Stated Values 500	230	100	% Samples With MPN > 1000
19.1-29.0 (19.6)	13.1-16.0 (14.0)	< 1000	0	1	3	960	33	0	0	33
			24		4	< 97	75	75	75	0
			48		4	44	100	100	75	0
			72		4	< 31	100	100	75	0
(21.9-25.0)	16.1-19.0 (16.5-17.0)	1001- 10000	0	5	3	3600	0	0	0	100
			24		6	92	100	83	33	0
			48		6	64	100	83	67	0
			72		8	< 24	100	100	87	0
(25.0)	(16.5)	10001- 25000	0	1	1	17000	0	0	0	100
			24		1	330	100	0	0	0
			48		1	78	100	100	100	0
			72		1	20	100	100	100	0
(25.8-27.6)	19.1-22.0 (20.0-21.4)	< 1000	0	2	14	710	50	29	0	50
			24		17	270	65	65	41	23
			48		15	250	73	53	40	20
			72		23	< 50	91	87	83	9
(25.8-26.7)	(19.6-20.5)	1001- 10000	0	5	50	1790	10	6	6	76
			24		51	< 61	85	76	59	8
			48		54	< 20	100	100	94	0
			72		6	86	100	67	67	0
(24.8-25.8)	(19.4-20.2)	10001- 25000	0	4	22	19000	0	0	0	100
			24		24	67	96	87	71	4
			48		24	< 46	96	96	92	4
			72		24	< 42	92	92	87	4

[1] Figures in parentheses give range of means actually recorded.

Table 10. Mean MPN levels and cumulative percentages of fecal coliforms in oysters subjected to depuration in shallow trays. Grouped according to initial MPN level in oysters, water temperature and salinity. Oysters contaminated in nature.

Range Mean Water Temperatures (C)	Range Mean Water Temperatures (o/oo)	Initial Level in Oysters (MPN/100 g)	Depuration Time (Hrs)	No. Expts.	No. Samples	Mean MPN/100 g	Percent of Samples With MPN Levels ≤Stated Values 100	50	20	% Samples With MPN >200
9.1-14.0 (11.8)[1]	13.1-16.0 (14.0)[1]	< 500	0	1	3	230	0	0	0	100
			24		4	ID	100	100	100	0
			48		4	ID	100	100	100	0
			72		4	< 36	75	75	75	25
(10.4-13.5)	16.1-19.0 (16.6-17.2)		0	4	2	≤ 34	100	50	50	0
			24		5	≤ 23	100	80	80	0
			48		5	≤ 21	100	100	80	0
			72		5	ID	100	100	100	0
14.1-19.0 (18.2)	13.1-16.0 (15.5)	501- 1000	0	3	2	40	0	0	0	100
			24		5	≤ 37	80	80	40	0
			48		5	≤ 37	80	80	80	20
			72		5	≤ 21	100	100	80	0
(14.7)	(15.7)	> 5000	0	2	1	79000	0	0	0	100
			24		2	ID	100	100	100	0
			48		2	ID	100	100	100	0
			72		2	ID	100	100	100	0
(16.0-17.2)	16.1-19.0 (17.3-17.6)	< 500	0	4	3	160	33	33	0	67
			24		4	< 22	100	100	75	0
			48		4	ID	100	100	100	0
			72		4	ID	100	100	100	0
(19.1)	(17.8)	501- 1000	0	2	3	650	0	0	0	67
			24		4	≤ 37	75	75	75	25
			48		4	≤ 22	100	100	75	0
			72		5	≤ 31	80	80	80	20
(15.7-17.8)	(16.8-16.9)	1001- 5000	0	2	4	3600	0	0	0	100
			24		1	68	100	0	0	0
			48		1	45	100	100	0	0
			72		5	< 40	80	80	60	20
(14.8-17.9)	19.1-22.0 (20.1-20.6)	< 500	0	2	35	< 40	78	61	56	11
			24		29	ID	100	100	100	0
			48		12	ID	100	100	92	0
			72		13	ID	100	100	100	0

Table 10 Contd.

Range Mean Water Temperatures (C)	Range Mean Water Temperatures (‰)	Initial Level in Oysters (MPN/100 g)	Depuration Time (Hrs)	No. Expts.	No. Samples	Mean MPN/100 g	Percent of Samples With MPN Levels ≤ Stated Values: 100	50	20	% Samples With MPN >200
19.1-29.0 (19.6)	13.1-16.0 (14.0)	<500	0	1	3	230	0	0	0	100
			24		4	<37	75	75	75	25
			48		4	<33	75	75	75	25
			72		4	ID	100	100	100	0
(21.9-25.0)	16.1-19.0 (16.5-17.0)	<500	0	2	2	303	0	0	0	100
			24		2	ID	100	100	100	0
			48		2	ID	100	100	100	0
			72		2	ID	100	100	100	0
(25.0)	(16.5)	501- 1000	0	1	1	640	0	0	0	100
			24		1	ID	100	100	100	0
			48		1	ID	100	100	100	0
			72		3	ID	100	100	100	0
(21.9-25.0)	(16.5-17.0)	1001- 500	0	3	2	1600	0	0	0	100
			24		4	ID	100	100	100	0
			48		4	<21	100	100	75	0
			72		6	ID	100	100	100	0
(25.8-26.7)	19.1-22.0 (19.6-21.4)	<500	0	5	60	<190	32	23	18	57
			24		64	<19	97	97	91	0
			48		54	<19	94	94	94	0
			72		48	<19	96	96	96	2
(27.6)	(20.0)	501- 1000	0	1	2	870	0	0	0	100
			24		2	<61	50	50	50	50
			48		2	<18	100	100	100	0
			72		2	<18	100	100	100	0
(24.8-25.8)	(19.4-20.5)	1001- 5000	0	5	24	1700	0	0	0	100
			24		22	<20	100	100	96	0
			48		24	<21	96	96	96	4
			72		21	ID	100	95	95	0

[1] Figures in parentheses give range of means actually recorded.

Table 11. Mean MPN levels, 90% confidence interval and cumulative percentages for total coliforms in healthy and MSX and/or Dermo infected oysters depurated in shallow (plexiglass) trays. Oysters contaminated in nature.

Type Sample	Depuration Time (Hrs.)	No. Samples	MPN / 100 g Mean	MPN / 100 g 90% Confidence Interval[3]		Percent of samples with MPN levels ≤stated values 500	230	100	Percent of samples with MPN levels >1000
Healthy	0	87	<1,800	150	22,000	26	16	8	67
	24	76	<66	---	---	88	78	68	8
	48	81	<42	---	---	93	89	84	5
	72	63	<44	---	---	92	89	86	6
MSX	0	3	4,300	1,500	22,000	---	---	---	100
	24	9	<37	18	73	100	100	89	---
	48	7	<41	15	110	100	100	86	---
	72	3	<18	---	---	100	100	100	---
Dermo (Nat)[1]	0	4	300	40	2,200	75	75	25	25
	24	8	<28	---	---	100	88	88	---
	48	6	<18	---	---	100	100	100	---
	72	3	<31	8	110	100	100	100	---
Dermo (Lab)[2]	0	7	410	50	3,300	57	14	14	43
	24	6	<21	---	---	100	100	100	---
	48	6	<32	---	---	100	100	100	---
	72	6	<20	---	---	100	100	100	---

Footnotes:

[1]Nat = Disease acquired in estuary
[2]Lab = Laboratory infected
[3]Note: Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 12. Mean MPN levels, 90% confidence interval and cumulative percentages for fecal coliforms in healthy and MSX and/or Dermo infected oysters depurated in shallow (plexiglass) trays. Oysters contaminated in nature.

Type Sample	Depuration Time (Hrs.)	No. Samples	MPN / 100 g			Percent of samples with MPN levels ≤stated values			Percent of samples with MPN levels >200
			Mean	90% Confidence Interval[3]		100	50	20	
Healthy	0	87	<250	22	2,800	32	24	20	56
	24	76	<19	---	---	97	97	92	---
	48	81	<19	---	---	96	96	95	1
	72	63	<19	---	---	97	95	95	2
MSX	0	3	590	100	3,600	---	---	---	67
	24	9	<19	---	---	100	100	89	---
	48	7	<24	---	---	87	87	87	---
	72	3	<18	---	---	100	100	100	---
Dermo (Nat)[1]	0	4	123	8	1,900	75	50	25	25
	24	8	<18	---	---	100	100	100	---
	48	6	<18	---	---	100	100	100	---
	72	3	<18	---	---	100	100	100	---
Dermo (Lab)[2]	0	7	<43	13	140	71	57	57	14
	24	6	<18	---	---	100	100	100	---
	48	6	<18	---	---	100	100	100	---
	72	6	<18	---	---	100	100	100	---

Footnotes:

[1]Nat = Disease acquired in estuary
[2]Lab = Laboratory infected
[3]Note: Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 13 . Mean MPN levels, 90% confidence interval and cumulative percentages for total coliforms in healthy and MSX and/or Dermo infected oysters depurated in shallow (plexiglass) trays. Oysters contaminated with raw sewage.

Type Sample	Depuration Time (Hrs.)	No. Samples	MPN / 100 g Mean	MPN / 100 g 90% Confidence Interval[4]		Percent of samples with MPN levels ≤stated values 500	230	100	Percent of samples with MPN levels >1000
Healthy	0	4	92,000	31,000	270,000	---	---	---	100
	24	5	<112	20	600	100	80	40	---
	48	5	72	13	390	100	80	60	---
	72	1	230	---	---	100	100	---	---
Dermo (Nat)[1]	0	10	41,000	15,000	110,000	---	---	---	100
	24	9	<118	36	370	100	78	44	---
	48	11	56	21	140	91	91	91	9
	72	11	<55	---	---	82	82	73	---
MSX + Dermo[2] (Lab)[3]	0	1	130,000	---	---	---	---	---	100
	24	2	470	---	---	50	50	---	50
	48	1	40	---	---	100	100	100	---
	72	1	170	---	---	100	100	---	---

Footnotes:

[1]Nat = Disease acquired in estuary
[2]MSX + Dermo = Dual infection with both pathogens
[3]Lab = Laboratory infected
[4]Note: Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 14 . Mean MPN levels, 90% confidence interval and cumulative percentages for fecal coliforms in healthy and MSX and/or Dermo infected oysters depurated in shallow (plexiglass) trays. Oysters contaminated with raw sewage.

Type Sample	Depuration Time (Hrs.)	No. Samples	MPN / 100 g Mean	MPN / 100 g 90% Confidence Interval[4]		Percent of samples with MPN levels <stated values 100	50	20	Percent of samples with MPN levels >200
Healthy	0	4	4,300	630	28,000	---	---	---	100
	24	5	<21	---	---	100	100	80	---
	48	5	<20	---	---	100	100	80	---
	72	1	20	---	---	100	100	100	---
Dermo (Nat)[1]	0	10	1,000	180	5,500	---	---	---	90
	24	9	<18	---	---	100	100	100	---
	48	11	<18	---	---	100	100	100	---
	72	11	<18	---	---	100	100	100	---
MSX + Dermo[2] (Lab)[3]	0	1	4,900	---	---	---	---	---	100
	24	2	30	---	---	100	100	50	---
	48	1	<18	---	---	100	100	100	---
	72	1	<18	---	---	100	100	100	---

Footnotes:

[1]Nat = Disease acquired in estuary
[2]MSX + Dermo = Dual infection with both pathogens
[3]Lab = Laboratory infected
[4]Note: Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 15. Data used in construction of Figs. 23-26.

Depuration Elapsed Time (hrs)	Origin of Coliform Contamination	#Oysters & Disease State*				Mean Total Coliform Levels (MPN/100g)				Mean Fecal Coliform Levels (MPN/100g)			
		Healthy	MSX	D. marinum Lab	D. marinum Nat.	Healthy	MSX	D. marinum Lab.	D. marinum Nat.	Healthy	MSX	D. marinum Lab.	D. marinum Nat.
0	Natural	87	3 (3H)	7 (1H,4M,2L)	4 (1M,3L)	<1,753	4,329	405	298	<250	590	<43	123
24	"	76	9 (5H,3M,1L)	6 (3M,3L)	8 (1H,3M,4L)	<66	<37	<21	<28	<19	<19	<18	<18
48	"	81	7 (2H,5M)	6 (3M,3L)	6 (2H,2M,2L)	<42	<41	<32	<18	<19	<24	<18	<18
72	"	63	3 (2M,1L)	6 (1H,4M,1L)	3 (1M,2L)	<44	<18	<20	<31	<19	<18	<18	<18
0	Raw Sewage	4	1+ (M=MSX H= Dermo)	10 (4H,2M,4L)	--	92,139	130,000	41,119	--	4,316	4,900	1,005	--
24	"	5	2+ (1M,1H=MSX) (2H =Dermo)	9 (2H,4M,3L)	--	<112	470	<118	--	<21	30	<18	--
48	"	5	1+ (M=Dermo L =MSX)	11 (1H,5M,5L)	--	72	40	56	--	<20	<18	<18	--
72	"	1	1+ (M=Dermo H=MSX)	11 (3H,7M,1L)	--	230	170	<55	--	20	<18	<18	--

+ = Dual infection with both pathogens; *L= light infection; M= moderate infection; H= heavy infection; "Lab"= laboratory infected; "Nat"= disease acquired in estuary.

Table 16. Cumulative percentages of samples of oysters with fecal coliform MPN values equal to or less than given levels. Oysters grouped according to initial MPN levels and biodeposition activity. Oysters contaminated in nature.

Range of Initial MPN	Expt. Nos.	Mean Initial Level (MPN/100 g)	Depuration Time (Hrs)	Bio-deposition Activity[1]	No. Oysters	% Samples ≤ MPN Value Given MPN/100 g: ID	20	50	100
< 500	31	< 51	24	H-M	20	80	85	95	95
	36	< 40		L	4	100	100	100	100
				VL	2	100	100	100	100
				I	1	100	100	100	100
			48	H-M	17	82	94	94	94
				L	4	75	100	100	100
				VL	3	67	67	100	100
				I	1	100	100	100	100
			72	H-M	13	92	92	92	92
				L	15	93	100	100	100
				VL	4	75	75	75	75
501-1000	23-1	790	24	H-M	21	100	100	100	100
	23-2	700		VL	1	100	100	100	100
1001-5000	32	1700	24	H-M	9	56	89	100	100
	40	1900		L	5	100	100	100	100
				VL	11	55	73	100	100
				I	7	86	86	100	100
			48	H-M	10	100	100	100	100
				L	15	87	93	100	100
				VL	12	67	92	92	92
				I	1	100	100	100	100
			72	H-M	13	83	92	92	100
				L	11	91	100	100	100
				VL	10	90	100	100	100
				I	4	75	100	100	100

[1] H-M = High to Moderate; L = Low; VL = Very Low; I = Inactive.

Table 17. Cumulative percentages of samples of oysters with total coliforms MPN values equal to or less than given levels. Oysters grouped according to initial MPN levels and biodeposition activity. Oysters contaminated in nature.

Range of Initial MPN	Expt. Nos.	Mean Initial Level (MPN/100 g)	Depuration Time (Hrs)	Biodeposition Activity[1]	No. Oysters	% Samples ≤ Value Given 100	230	500
<500	36	<340	24	H-M	7	100	100	100
				L	3	100	100	100
				VL	2	100	100	100
			48	H-M	8	100	100	100
				L	2	100	100	100
				VL	2	50	100	100
			72	H-M	4	100	100	100
				L	6	83	83	100
				VL	2	50	50	50
501- 1000	31	690	24	H-M	13	46	62	62
				L	1	0	100	100
				I	1	0	100	100
			48	H-M	9	33	56	78
				L	2	0	0	50
				VL	1	0	0	0
				I	1	100	100	100
			72	H-M	9	78	78	89
				L	9	89	89	100
				VL	2	50	50	50
1001- 5000	23-1 23-2	1600 2600	24	H-M	21	100	100	100
				VL	1	100	100	100
5001-100000	32 40	19000 61000	24	H-M	9	89	100	100
				L	5	60	80	100
				VL	11	36	64	91
				I	7	71	100	100

Table 17, Contd.

Range of Initial MPN	Expt. Nos.	Mean Initial Level (MPN/100 g)	Depuration Time (Hrs)	Bio-deposition Activity	No. Oysters	% Samples ≤ Value Given 100	230	500
5001-100000 (Continued)			48	H-M	10	100	100	100
				L	15	80	93	100
				VL	12	75	83	83
				I	1	100	100	100
			72	H-M	13	83	83	92
				L	11	87	93	93
				VL	10	70	80	80
				I	4	75	100	100

[1] H-M = High to Moderate
L = Low
VL = Very Low
I = Inactive

Table 18. Most probable number (MPN) of coliform bacteria in individual oysters subjected to depuration under experimental conditions designed to measure their pumping rate in laboratory aquaria.

Depuration Time (Hrs)		Mean Pumping Rate (l/hr)	MPN TC	MPN FC	Mean Water Flow (l/hr)
A. Experiments of 1-3 October 1974					
0	Pooled (8 oyst.)	--	984	224	
24		3.98	ID	ID	26.4
		5.32	ID	ID	72.0
		6.50	1700	ID	74.4
		9.97	20	20	69.6
48		2.30	45	ID	52.8
		5.15	ID	ID	51.2
		5.97	ID	ID	59.1
		10.50	20	ID	33.7
B. Experiments of 15-18 October 1974					
0	Pooled (8 oyst.)	--	3055	303	
24		3.01	20	20	69.6
		4.65	110	40	73.2
		1.50	120	ID	72.0
		1.54	ID	ID	67.2
48		3.13	ID	ID	71.7
		2.49	ID	ID	55.2
		4.28	20	ID	48.0
		1.56	ID	ID	64.8
72		2.71	ID	ID	57.6
		1.41	ID	ID	48.6
		5.97	340	340	51.0

Table 19. Mean MPN level, 90% confidence interval and cumulative percentages for samples at selected levels of total coliforms in oysters subjected to depuration in 4 x 8 tank. Grouped according to initial oyster MPN level and water temperature. Oysters contaminated in nature.

Initial level in oysters (MPN/100g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Oyster Batches[3]	No. Samples	MPN/100 g Mean	90% Confidence Interval		Percent of samples with MPN levels ≤stated values 500	230	100	% Samples with MPN >1000
<1000	19.1-24.0	0	1	1	310	--[2]	--[2]	100	0	0	0
	(20.2)[1]	24		4	540	2496	113	50	25	0	25
		48		4	94	156	54	100	100	50	0
		79		4	99	546	18	100	75	50	0
1001- 10000	19.1-24.0	0	2	5	2900	--	--	0	0	0	100
	(20.2-23.3)	24		8	780	1716	365	38	13	0	50
		48		8	700	1498	328	63	13	0	38
		72		8	530	1154	94	75	50	25	13
	24.1-29.0	0	2	6	6300	--	--	0	0	0	100
	(25.9-27.4)	24		12	500	2340	101	67	33	8	25
		48		12	150	484	49	92	67	25	0
		72		12	63	281	14	92	92	75	8
10001- 25000	19.1-24.0	0	1	3	19000	--	--	0	0	0	100
	(23.1)	24		6	200	1030	39	83	67	33	17
		48		6	64	250	17	100	83	67	0
		72		6	36	150	8	100	83	83	0
	24.1-29.0	0	1	3	12000	--	--	0	0	0	100
	(28.8)	24		4	150	--	--	100	75	25	0
		48		4	< 71	265	19	100	75	50	0
		72		4	130	--	--	100	100	25	0

Footnotes:

1 Actual temperatures included appear in parentheses.

2 Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

3 Some experiments included batches of oysters with different 0-Hr coliform levels. Batches having 0-Hr levels within a given range were grouped together regardless of the experiment they were a part of.

Table 20. Mean MPN level, 90% confidence interval and cumulative percentages for samples at selected levels of total coliforms in oysters subjected to depuration in 2 x 8 tank. Grouped according to initial oyster MPN level and water temperature. Oysters contaminated in nature.

Initial level in oysters (MPN/100g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Oyster Batches[3]	No. Samples	MPN/100g Mean	MPN/100g 90% Confidence Interval		Percent of samples with MPN levels ≤stated values 500	230	100	% Samples with MPN >1000
1001- 10000	9.1-14.0 (10.1)[1]	0	1	3	2800	7000	1100	0	0	0	100
		24		3	1500	10000	230	33	0	0	67
		48		3	1600	--	--	0	0	0	100
		72		3	230	1900	29	67	67	33	33
	14.1-19.0 (17.6)	0	1	3	3300	8400	1300	0	0	0	100
		24		6	950	3100	280	33	17	0	67
		48		6	460	2000	100	67	33	0	17
		72		6	2500	14000	430	17	0	0	67
	19.1-24.0 (21.0-23.7)	0	2	5	3500	--	--	0	0	0	100
		24		12	191	1000	35	75	58	42	8
		48		12	199	7500	5	67	67	58	25
		72		12	101	960	11	83	67	50	8
	24.1-29.0 (26.1)	0	1	3	6100	11000	3400	0	0	0	100
		24		5	1100	11000	100	40	20	0	60
		48		5	98	190	50	100	100	40	0
		72		5	71	170	29	100	100	60	0
10001-25000	19.1-24.0 (19.9)	0	1	3	17000	--	--	0	0	0	100
		24		6	610	--	--	33	0	0	0
		48		6	350	--	--	100	17	0	0
		72		6	540	1300	200	50	17	0	33
	24.1-29.0 (26.5)	0	1	3	21000	230000	2000	0	0	0	100
		24		4	1200	17000	76	50	25	0	50
		48		4	813	1700	360	50	0	0	25
		72		4	217	--	--	100	75	0	0
25000-100000	9.1-14.0 (11.5)	0	1	1	49000	--	--	0	0	0	100
		24		3	800	8600	69	33	0	33	33
		48		3	690	19000	26	33	33	0	67
		72		3	430	--	--	100	33	0	0
	14.1-19.0 (14.6)	0	1	3	≥30000	3.7×10^6	26	0	0	0	100
		24		2	1900	--	--	0	0	0	100
		48		2	1100	--	--	0	0	0	50
		72		2	2800	--	--	0	0	0	100
	19.1-24.0 (19.6)	0	1	4	81000	280000	23000	0	0	0	100
		24		6	9600	--	--	0	0	0	100
		48		6	3600	56000	200	0	0	0	67
		72		6	2200	--	--	0	0	0	83

Table 20, contd.

Initial level in oysters (MPN/100g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Oyster Batches[3]	No. Samples	MPN/100 g Mean	90% Confidence Interval		Percent of samples with MPN levels ≤stated values 500	230	100	% Samples with MPN >1000
25000-100000	24.1-29.0	0	2	4	34000	59000	20000	0	0	0	100
	(25.9-26.2)	24		8	1300	34000	47	38	25	13	50
		48		8	280	2000	40	63	63	25	37
		72		8	72	250	21	100	88	63	0
>100000	24.1-29.0	0	2	4	171000	--	--	0	0	0	100
	(26.2-26.5)	24		6	2500	87000	69	33	17	0	50
		48		6	440	1300	130	33	33	0	33
		72		6	65	150	28	100	100	50	0

Footnotes:

[1] Actual temperatures included appear in parentheses.

[2] Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

[3] Some experiments included batches of oysters with different 0-Hr coliform levels. Batches having 0-Hr levels within a given range were grouped together regardless of the experiment they were a part of.

Table 21. Mean MPN level, 90% confidence interval and cumulative percentages for samples at selected levels of total coliforms in oysters subjected to depuration in 2 x 4 tank. Grouped according to initial oyster MPN level and water temperature. Oysters contaminated in nature.

Initial level in oysters (MPN/100g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Oyster Batches[4]	No. Samples	MPN/100 g Mean	MPN/100 g 90% Confidence Interval		Percent of samples with MPN levels ≤ stated values 500	230	100	% Samples with MPN > 1000
1000- 10000	9.1-14.0	0	4	5	2000	5500	760	0	0	0	80
	(10.1-11.5)[1]	24		10	1000	2700	370	30	10	0	70
		48		10	1300	7600	190	20	10	0	80
		72		10	560	7500	40	60	30	10	20
	14.1-19.0	0	4	6	1700	5600	520	17	0	0	83
	(15.6-18.4)	24		8	81	330	21	88	88	63	0
		48		8	$\leq$ 180	4700	6	63	50	38	25
		72		12	$\geq$ 120	440	28	92	92	67	8
10001- 25000	14.1-19.0	0	4	6	8800	25000	300	0	0	0	100
	(17.4-18.0)	24		9	280	640	120	56	44	11	11
		48		9	$<$ 170	780	35	78	44	33	0
		72		12	230	1300	38	83	67	17	8
$>$ 25000	14.1-19.0	0	2	3	30000[3]	470000	180	0	0	0	100
	(14.6)	24		7	1500	--[2]	--[2]	0	0	0	71
		48		7	1500	3000	690	14	0	0	71
		72		7	1400	3000	620	29	0	0	57

Footnotes:

[1] Actual temperatures included appear in parentheses.

[2] Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

[3] Individual values: 3300, 3300, 2.4 X 10^6.

[4] Some experiments included batches of oysters with different 0-Hr coliform levels. Batches having 0-Hr levels with a given range were grouped together regardless of the experiment they were a part of.

Table 22. Mean MPN level, 90% confidence interval and cumulative percentages for samples at selected levels of total coliforms in oysters subjected to depuration in flume. Grouped according to initial oyster MPN level and water temperature. Oysters contaminated in nature.

Initial level in oysters (MPN/100g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Oyster Batches[3]	No. Samples	MPN/100 g Mean	90% Confidence Interval		Percent of samples with MPN levels $\leq$ stated values 500	230	100	% Samples with MPN $>$1000
1001- 10000	24.1-29.0	0	4	6	7200	12000	4300	0	0	0	100
	(24.4-29.0)[1]	24		12	1100	3700	310	33	8	0	42
		48		12	520	13000	21	58	42	25	42
		72		12	480	17000	13	50	42	33	42
10001- 25000	24.1-29.0	0	3	3	10000	34000	3100	0	0	0	100
	(24.4-29.0)	24		6	$\leq$ 76	--[2]	--[2]	83	83	67	17
		48		4	$\leq$ 26	--	--	100	100	100	0
		72		4	$\leq$ 42	--	--	75	75	50	25
$>$25000	24.1-29.0	0	2	4	29000	53000	15000	0	0	0	100
	(24.4-29.0)	24		6	3200	76000	140	50	17	0	50
		48		6	2200	56000	160	33	17	0	33
		72		6	380	20000	8	50	50	33	50

Footnotes:

[1] Actual temperatures included appear in parentheses.

[2] Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

[3] Some experiments included batches of oysters with different 0-Hr coliform levels. Batches having 0-Hr levels within a given range were grouped together regardless of the experiment they were a part of.

Table 23. Mean MPN level, 90% confidence interval and cumulative percentages for samples at selected levels of fecal coliforms in oysters subjected to depuration in 4 x 8 tank. Grouped according to initial oyster MPN level and water temperature. Oysters contaminated in nature.

Initial level in oysters (MPN/100g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Oyster Batches[3]	No. Samples	MPN/100 g Mean	MPN/100 g 90% Confidence Interval		Percent of samples with MPN levels ≤stated values 100	50	20	% Samples with MPN > 200
0- 500	19.1-24.0	0	2	2	380	--[2]	--[2]	0	0	0	100
	(20.2-23.3)[1]	24		5	< 25	--	--	100	80	80	0
		48		5	< 18	--	--	100	100	100	0
		72		5	< 18	--	--	100	100	100	0
501- 1000	19.1-24.0	0	1	2	500	1000	250	0	0	0	100
	(23.3)	24		5	< 18	--	--	100	100	100	0
		48		5	< 18	--	--	100	100	100	0
		72		5	< 18	--	--	100	100	100	0
	24.1-29.0	0	2	5	790	1900	340	0	0	0	100
	(27.4-28.8)	24		9	< 18	--	--	100	100	100	0
		48		9	< 18	--	--	100	100	100	0
		72		9	< 18	--	--	100	100	100	0
1001- 5000	19.1-24.0	0	1	2	1300	--	--	0	0	0	100
	(20.2)	24		2	< 42	--	--	100	100	0	0
		48		2	< 18	--	--	100	100	100	0
		72		2	< 18	--	--	100	100	100	0
	24.1-29.0	0	1	4	2900	6200	1300	0	0	0	100
	(25.8)	24		6	< 18	--	--	100	100	100	0
		48		6	< 21	--	--	100	100	83	0
		72		6	< 18	--	--	100	100	100	0
> 5000	19.1-24.0	0	1	3	6300	45000	940	0	0	0	100
	(23.1)	24		6	< 22	--	--	100	83	83	0
		48		6	< 20	--	--	100	100	83	0
		72		6	< 18	--	--	100	100	100	0

Footnotes:

[1] Actual temperatures included appear in parentheses.

[2] Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

[3] Some experiments included batches of oysters with different 0-Hr coliform levels. Batches having 0-Hr levels within a given range were grouped together regardless of the experiment they were a part of.

Table 24. Mean MPN level, 90% confidence interval and cumulative percentages for samples at selected levels of fecal coliforms in oysters subjected to depuration in 2 x 8 tank. Grouped according to initial oyster MPN level and water temperature. Oysters contaminated in nature.

Initial level in oysters (MPN/100g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Oyster Batches[3]	No. Samples	MPN/100 g Mean	MPN/100 g 90% Confidence Interval		Percent of samples with MPN levels ≤ stated values 100	50	20	% Samples with MPN ≥ 200
0- 500	9.1-14.0 (10.1-11.5)[1]	0	2	4	180	440	69	0	0	0	50
		24		6	≤ 47	--[2]	--[2]	83	50	50	17
		48		6	≤ 31	--	--	100	83	50	0
		72		6	≤ 25	--	--	100	83	67	0
	14.1-19.0 (17.6)	0	1	3	410	3600	45	0	0	0	67
		24		6	≤ 22	--	--	100	100	83	0
		48		6	≤ 29	--	--	100	67	67	0
		72		6	≤ 18	--	--	100	100	100	0
	19.1-24.0 (21.0-23.7)	0	2	5	140	--	--	20	0	0	20
		24		12	≤ 23	--	--	100	92	75	0
		48		12	≤ 18	--	--	100	100	100	0
		72		12	≤ 18	--	--	100	100	100	0
1001- 5000	14.1-19.0 (14.6)	0	1	3	3300	$1.4x10^6$	8	0	0	0	67
		24		2	120	200	63	50	0	0	0
		48		2	130	390	45	50	0	0	50
		72		2	≤ 28			100	100	50	0
	19.1-24.0 (19.6-19.9)	0	2	7	2900	--	--	0	0	0	0
		24		12	≤ 49	--	--	83	75	50	8
		48		12	≤ 18	--	--	100	100	100	0
		72		12	≤ 26	--	--	92	92	83	8
	24.1-29.0 (26.1-26.5)	0	2	6	3000	8700	950	0	0	0	83
		24		9	≤ 57	--	--	89	67	33	11
		48		9	≤ 28	--	--	78	78	78	0
		72		9	≤ 29	--	--	89	89	67	0
5001- 15000	24.1-29.0 (25.9-26.2)	0	2	4	10000	39000	2700	0	0	0	100
		24		8	≤ 90	--	--	63	50	38	13
		48		8	≤ 34	--	--	88	88	88	13
		72		8	≤ 21	--	--	100	88	88	0
>15000	24.1-29.0 (26.2-26.5)	0	2	4	52000	--	--	0	0	0	100
		24		6	190	--	--	83	33	17	17
		48		6	≤ 45	--	--	67	67	50	17
		72		6	≤ 21	--	--	100	100	83	0

Footnotes:

[1] Actual temperatures included appear in parentheses.

[2] Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

[3] Some experiments included batches of oysters with different 0-Hr coliform levels. Batches having 0-Hr levels within a given range were grouped together regardless of the experiment they were a part of.

Table 25. Mean MPN level, 90% confidence interval and cumulative percentages for samples at selected levels of fecal coliforms in oysters subjected to depuration in 2 x 4 tank. Grouped according to initial oyster MPN level and water temperature. Oysters contaminated in nature.

Initial level in oysters (MPN/100g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Oyster Batches[3]	No. Samples	MPN/100 g Mean	90% Confidence Interval		Percent of samples with MPN levels ≤ stated values: 100	50	20	% Samples with MPN > 200
0- 500	9.1-14.[illegible] (10.1-11.5)[1]	0	4	5	190	--[2]	--[2]	0	0	0	60
		24		10	104	250	42	50	40	0	20
		48		10	≤ 43	--	--	80	70	40	10
		72		10	≤ 21	--	--	100	90	90	0
	14.1-19.0 (15.6)	0	2	3	330	--	--	0	0	0	100
		24		4	41	--	--	100	75	25	0
		48		4	< 37	--	--	75	75	75	25
		72		3	< 63	--	--	83	83	83	17
1001- 5000	14.1-19.0 (14.6-18.4)	0	8	12	3300	15000	690	0	0	0	92
		24		20	≤ 70	220	23	60	50	25	15
		48		20	≤ 31	--	--	85	75	70	5
		72		25	≤ 32	--	--	92	76	56	0

Footnotes:

1 Actual temperatures included appear in parentheses.

2 Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

3 Some experiments included batches of oysters with different 0-Hr coliform levels. Batches having 0-Hr levels within a given range were grouped together regardless of the experiment they were a part of.

Table 26. Mean MPN level, 90% confidence interval and cumulative percentages for samples at selected levels of fecal coliforms in oysters subjected to depuration in flume. Grouped according to initial oyster MPN level and water temperature. Oysters contaminated in nature.

Initial level in oysters (MPN/100g)	Range Mean Water Temperatures (C)	Depuration Time (Hrs)	No. Oyster Batches[3]	No. Samples	MPN/100 g Mean	90% Confidence Interval		Percent of samples with MPN levels $\leq$ stated values: 100	50	20	% Samples with MPN $>$ 200
501- 1000	24.1-29.0	0	5	6	820	11000	320	0	0	0	50
	(24.4-29.0)[1]	24		12	$\leq$ 40	--[2]	--[2]	75	75	58	17
		48		10	$<$ 19	--	--	100	100	90	0
		72		10	$<$ 34	--	--	[illegible]	80	80	10
1001- 5000	24.1-29.0	0	4	7	1900	21000	280	0	0	0	57
	(24.4-29.0)	24		12	$<$ 36	--	--	83	75	58	8
		48		[illegible]	$<$ 4[illegible]	--	--	75	67	50	17
		72		12	$<$ 86	--	--	58	58	58	25

Footnotes:

[1] Actual temperatures included appear in parentheses.

[2] [illegible]sence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique [illegible]r to skewness of the data as a result of depuration.

[3] Some experiments included batches of oysters with different 0-Hr coliform levels. Batches having 0-Hr levels within a given range were grouped together regardless of the experiment they were a part of.

Table 27. Mean MPN levels and cumulative percentage of fecal coliforms in oysters contaminated with cultures of *E. coli* in the laboratory and subsequently depurated in commercial-size tanks. Mean temperature range = 5.6-12.3 C.

Expt. No.	Mean Temp. (C)	Depuration Time (Hrs)	No. Samples	Mean MPN/100g	Percent of Samples With MPN Levels ≤ Stated Values			% Samples With MPN > 200
					100	50	20	
4x8 tank								
74	5.6	0	1	130	0	0	0	100
		24	3	190	0	0	0	67
		48	2	170	0	0	0	50
		72	2	230	0	0	0	100
79	10.5	0	3	180	0	0	0	67
		24	3	23	100	100	67	0
		48	3	18	100	100	100	0
		72	3	18	100	100	100	0
80	10.4	0	3	54000	0	0	0	100
		24	3	19	100	100	100	0
		48	3	25	100	100	67	0
		72	3	30	100	67	67	0
2x8 tank								
78	11.8	0	3	710	0	0	0	100
		24	3	25	100	100	67	0
		48	3	19	100	100	100	0
		72	3	18	100	100	100	0
81	12.3	0	3	5100	0	0	0	100
		24	3	45	100	100	0	0
		48	3	67	67	33	33	33
		72	3	18	100	100	100	0

Table 28. Most probable number of total and fecal coliform bacteria in samples of oysters depurated in the 4 x 8 tank. Mean values for environmental factors given for each experiment.

Date	Expt. No.	Tray No.	Total Coliform MPN/100 g at Given Depuration Times in Hrs. 0	24	48	72	Fecal Coliforms MPN/100 g at Given Depuration Times in Hrs. 0	24	48	72	Mean Temp. (C)	Mean Sal. (°/oo)	Mean D.O. (mg/l)	Turbidity (mg/l) Range	Turbidity (mg/l) Mean	Mean Flow (GPM/Bu)
12-15 May 1975	48	1	310[1]	330	45	20	310	20	ID	ID	20.2	16.9	6.1	(13.0-47.5)	24.8	1.1
		4		640	170	61		78	ID	ID						
		5		170	130	460		20	ID	ID						
		8		2300	78	170		20	ID	20						
		3	1300	330	490	170	1300	40	ID	ID						
		7	4600	1700	1300	78	1300	45	20	20						
9-12 Jun 1975	50	1	4900	2300	330	790	460	ID	ID	20	23.3	17.8	5.0	(13.0-26.5)	19.7	1.0
		2	2300	1300	4900	78	330	ID	ID	ID						
		4	3300	790	340	490	790	20	ID	ID						
		5		1400	220	3300		ID	ID	ID						
		7		330	330	490		ID	ID	ID						
		8		230	2300	230		ID	ID	ID						
16-19 June 1975	51	2	4900	340	170	78	2300	20	ID	ID	25.8	18.2	5.8	(12.5-24.0)	16.1	1.9
		4	4900	2300	130	78	3300	ID	ID	ID						
		5	7900	4600	270	20	7900	20	ID	ID						
		7		220	490	20		ID	45	ID						
		8		460	170	170		20	ID	ID						
		1	7000	20	330	170	1100	ID	20	ID						
11-14 Aug 1975	57	1	7000	17000	110	2100	1100	340	ID	ID	27.4	18.9	4.6	(9.6-62.4)	21.5	2.2
		3	7000	110	840	20	700	ID	ID	ID						
		4		460	ID	45		20	ID	ID						
		5		170	45	20		ID	ID	ID						
		6		330	230	20		ID	ID	ID						
		8		700	68	78		ID	20	ID						
18-21 Aug 1975	58	1[2]	17000	330	260	130	260	ID	ID	ID	28.8	17.4	4.4	(11.5-28.1)	17.7	2.1
		2[2]	13000	78	ID	230	700	ID	ID	20						
		3[3]	7900	110	45	78	2200	ID	ID	ID						
		5[3]		170	130	110		ID	ID	ID						
		4[4]		790	45	700		490	ID	ID						
		6[4]		340	45	330		ID	ID	ID						
22-25 Sept 1975	62	2	33000	45	45	20	33000	ID	ID	ID	23.1	18.1	5.6	(11.5-32.0)	18.5	2.1
		4	17000	68	110	20	2300	68	40	ID						
		5	13000	2300	330	40	3300	20	20	ID						
		6		170	20	20		18	ID	ID						
		7		490	45	330		18	ID	ID						
		8		110	45	20		ID	ID	ID						

[1] When a group of trays had oysters from same batch, 0-hr values given apply to all trays enclosed within bracket. Values were used only once in computation of mean for combinations of more than one experiment.

[2] Mixed sample from top and bottom layers of oysters stacked three inches deep in tray (usual stacking).

[3] Sample from top layer of oysters stacked six inches deep in tray.

[4] Sample from bottom layer of oysters stacked six inches deep in tray.

Table 29. Most probable number of total and fecal coliform bacteria in samples of oysters depurated in the 2 x 8 tank. Mean values for environmental factors given for each experiment.

Date	Expt. No.	Tray No.	Total Coliforms MPN/100 g at Given Depuration Time in Hrs. 0	24	48	72	Fecal Coliforms MPN/100 g at Given Depuration Time in Hrs. 0	24	48	72	Mean Temp. (C)	Mean Sal. (°/oo)	Mean D.O. (mg/l)	Turbidity (mg/l) Range	Turbidity (mg/l) Mean	Mean Flow (GPM/Bu)
7-10 Jul 1975	53	2	22000[1]	330	2300	140	4600	78	170	45	26.5	17.3	3.6	(8.0-43.0)	17.7	2.1
		4	92000	3300	490	220	7900	45	18	20						
		5	4900	7900	790	330	2300	45	110	ID						
		7		230	490	93		78	ID	ID						
		1	160000	210	290	18	35000	ID	40	ID						
		8		270	330	18		45	220	ID						
14-17 Jul 1975	54	1	33000	3300	1100	220	13000	150	ID	61	26.2	17.5	3.3	(11.0-27.2)	19.9	3.4
		2		1300	170	330		78	20	20						
		3	240000	240000	140	170	33000	160000	ID	20						
		6	170000	4600	170	68	79000	68	20	ID						
		7	130000	4600	1300	120	79000	68	20	40						
		8		790	2300	170		78	130	20						
2- 5 Sept 1975	60	1	3300	140	45	68	790	18	20	20	26.1	18.5	3.1	(12.7-24.8)	17.9	2.0
		2	4900	7900	220	68	2200	2200	ID	ID						
		4	14000	2300	120	18	4600	40	ID	ID						
		5		490	170	130		ID	ID	48						
		7		1100	45	170		20	ID	20						
		8		460	170	78		45	20	20						
8-11 Sept 1975	61	1	160000	700	2300	45	3300	110	2300	20	25.9	17.7	3.0	(4.0-27.5)	17.0	2.2
		2	33000	490	45	68	7900	45	20	ID						
		4	79000	11000	170	110	13000	ID	20	18						
		5		170	1400	20		ID	20	ID						
		7		54000	110	78		13000	ID	ID						
		8		45	78	20		20	ID	ID						
29 Sept- 2 Oct 1975	63	1	3300	20	20	ID	170	20	ID	ID	23.7	16.5	4.4	(5.4-33.0)	14.7	2.0
		2	1100	78	ID	ID	78	45	ID	ID						
		4	4900	78	20	20	230	ID	ID	ID						
		5		45	78	68		20	ID	ID						
		7		170	78	1300		78	ID	ID						
		8		78	78	ID		ID	ID	ID						
14-17 Oct 1975	65	1	4900	790	1700	310	130	ID	20	ID	21.0	16.0	5.0	(8.0-17.0)	12.0	2.1
		2	5400	260	13000	130	140	ID	ID	ID						
		3	$\geq 2.4 \times 10^6$	230	110	790	230	ID	ID	20						
		6		790	790	490		45	ID	ID						
		7		490	78	78		ID	ID	ID						
		8		1800	7900	110		ID	ID	ID						
20-23 Oct 1975	67	1	13000	790	330	790	1100	20	ID	490	19.9	18.0	5.6	(9.0-13.5)	11.5	2.1
		3	17000	790	330	340	7900	40	ID	40						
		4	22000	790	490	1300	4900	18	ID	ID						
		5		270	310	130		45	ID	20						
		6		790	230	1700		45	ID	ID						
		8		490	490	330		20	20	20						

Table 29, Contd.

Date	Expt. No.	Tray No.	Total Coliforms MPN/100 g at Given Depuration Times in Hrs. 0	24	48	72	Fecal Coliforms MPN/100 g at Given Depuration Time in Hrs. 0	24	48	72	Mean Temp. (C)	Mean Sal. (°/oo)	Mean D.O. (mg/l)	Turbidity (mg/l) Range	Turbidity (mg/l) Mean	Mean Flow (GPM/Bu)
27-30 Oct 1975	68	5	79000	7900	700	1300	2300	4900	ID	20	19.6	16.5	6.5	(4.5-18.0)	11.5	2.1
		1	70000	13000	17000	3300	2200	ID	20	18						
		2	33000	7000	13000	4900	1700	ID	18	ID						
		3	240000	13000	1300	2300	4900	78	ID	ID						
		4		17000	13000	2800		170	20	ID						
		8		4900	790	790		20	20	20						
3- 6 Nov 1975	69	2	2200	230	110	490	790	ID	ID	ID	17.6	16.7	6.7	(9.0-35.0)	13.7	2.1
		3	7000	1100	700	1300	790	45	68	20						
		5	2300	2300	330	4900	110	20	20	20						
		6		2300	3300	790		20	68	ID						
		7		490	490	490		20	20	ID						
		8		1100	220	22000		ID	20	ID						
17-20 Nov 1975	71	PAT[2]	3300	2200	790	2300	1700	170	78	45	14.6	16.8	7.3	(2.8-11.2)	6.7	2.1
		PAT	3300	1700	1400	3300	170	78	230	ID						
		PAT	2.4×10^6				130000									
	72	PAT	49000	700	1300	330	170	ID	45	ID	11.5	17.7	8.4	(5.2-11.6)	8.5	2.2
		PAT		220	2300	490		20	ID	ID						
		PAT		3300	110	490		18	ID	ID						
	73	PAT	1300	3300	1700	220	220	330	45	37	10.1	17.8	8.8	(3.2- 9.2)	5.8	2.1
		PAT	3300	460	1300	1300	230	78	78	78						
		PAT	4900	2300	1700	45	110	68	20	ID						

[1] When a group of trays had oysters from same batch, 0-hr values given apply to all trays enclosed within brackets. Values were used only once in computation of mean for combinations of more than one experiment.

[2] PAT = Sample pooled from all trays.

Table 30. Most probable number of total and fecal coliform bacteria in samples of oysters depurated in the 2 x 4 tank. Mean values for environmental factors given for each experiment.

Date	Expt. No.	Tray No.	Total Coliforms MPN/100 g at Given Depuration Times in Hrs. 0	24	48	72	Fecal Coliforms MPN/100 g at Given Depuration Times in Hrs. 0	24	48	72	Mean Temp. (C)	Mean Sal. (°/oo)	Mean D.O. (mg/l)	Turbidity (mg/l) Range	Turbidity (mg/l) Mean	Mean Flow (GPM/Bu)
10-13 Nov 1975	70	1	3300[1]	490	110	230	1300	220	ID	ID	18.0	16.7	6.8	(4.4-12.4)	8.2	2.0
		3	7900	490	790	230	2300	45	ID	20						
		5	49000	1100	40	230	11000	490	ID	78						
		PAT[2]		170	45	330		20	20	20						
26-29 Apr 1976	82	1	460	45	700	78	210	45	78	20	15.6	18.1	5.6	(20.0-33.5)	25.1	1.0
		2	1100	950	3500	93	330	320	ID	20						
		3	3300	20	20	45	490	20	ID	20						
3- 6 May 1976	83	1	11000	68	790	220	700	45	20	45	17.4	18.3	5.4	(13.0-30.5)	20.3	1.0
		2	14000	130	ID	490	1700	20	ID	ID						
		3	13000	170	330	20	13000	45	330	ID						
10-13 May 1976	84	PAT	4900	45	1100	230	3300	ID	ID	45	18.4	18.4	4.9	(8.5-70.0)	24.1	1.0
		PAT	4900	68	170	40	4900	ID	ID	18						
		PAT	4900	110	330	170	4900	78	78	ID						
17-20 Nov 1976	171	1	3300	700	700	3300	170	78	78	110	14.6	16.8	7.1	(2.8-11.2)	6.7	2.0
		3	3300	1400	1700	3300	1700	130	170	45						
		5	2.4×10^{6}	1300	330	2300	130000	130	ID	130						
		PAT		3300	3300	2200		170	45	45						
17-20 Nov 1975	271	1	3300	3300	3300	330	170	1100	20	20	14.6	16.8	7.1	(2.8-11.2)	6.7	2.0
		3	3300	700	2200	950	1700	78	93	78						
		PAT	2.4×10^{6}	790	1700	490	130000	45	20	78						
1- 4 Dec 1975	172	PAT	700	170	3300	110	170	45	ID	ID	11.5	17.8	8.3	(5.2-11.6)	8.5	2.0
		PAT	2300	490	1700	130	230	110	45	ID						
		PAT		1400	3500	330		130	ID	20						
1- 4 Dec 1975	272	PAT	700	1300	330	40	170	40	20	ID						
		PAT	2300	1300	110	7900	230	45	ID	20	11.5	17.8	8.1	(5.2-11.6)	8.5	2.1
8-11 Dec 1975	173	PAT	1300	2200	1100	24000	110	490	45	68	10.1	17.8	8.7	(3.2- 9.2)	5.8	2.0
		PAT	3300	1300	3300	790	220	45	230	20						
		PAT	4900	490	1700	260	230	68	170	18						
8-11 Dec 1975	273	PAT	1300	2300	1300	790	110	170	68	ID	10.1	17.8	8.6	(3.2- 9.2)	5.8	2.1
		PAT	3300	2200	2200	490	220	490	40	ID						
			4900				230									

Table 30, Contd.

Date	Expt. No.	Tray No.	Total Coliforms MPN/100 g at Given Depuration Times in Hrs. 0	24	48	72	Fecal Coliforms MPN/100 g at Given Depuration Times in Hrs. 0	24	48	72	Mean Temp. (C)	Mean Sal. (°/oo)	Mean D.O. (mg/l)	Turbidity (mg/l) Range	Mean	Mean Flow (GPM/Bu)
10-13 Nov 75	170	1	3300	340	460	1700	2300	110	20	45	18.0	16.6	6.7	(4.4-12.4)	8.2	2.0
		3	7900	68	78	130	1300	ID	40	ID						
			49000				11000									
26-29 Apr 75	182	1	3300	61	ID	45	490	40	ID	20	15.6	18.0	4.9	(20.0-33.5)	25.1	1.0
		2	460	--	--	110	210	--	--	20						
		3	1100	--	--	≥24000	330	--	--	≥24000						
3- 6 May 75	183	1	11000	490	1300	790	1700	ID	130	20	17.4	18.3	4.8	(13.0-30.5)	20.3	1.0
		2	14000	--	--	140	700	--	--	18						
		3	13000	--	--	78	13000	--	--	45						
10-13 May 75	184	1	4900	110	20	78	3300	110	ID	ID	18.4	18.4	4.5	(8.5-70.0)	24.1	1.0
		2	4900	--	--	45	4900	--	--	20						
		3	4900	--	--	20	4900	--	--	20						

[1] When a group of trays had oysters from same batch, 0-hr values given apply to all trays enclosed within brackets. Values were used only once in computation of mean for combinations of more than one experiment.

[2] PAT = Sample pooled from all trays.

Table 31. Most probable number of total and fecal coliform bacteria in samples of oysters depurated in the flume. Mean values for environmental factors given for each experiment.

Date	Expt. No.	Tray No.	Total Coliforms MPN/100 g at Given Depuration Times in Hrs. 0	24	48	72	Fecal Coliforms MPN/100 g at Given Depuration Times in Hrs. 0	24	48	72	Mean Temp. (C)	Mean Sal. (°/oo)	Mean D.O. (mg/l)	Turbidity (mg/l) Range	Turbidity (mg/l) Mean	Mean Flow (GPM/Bu)
2- 5 Jun 1975	49	H[2]	3300[1]	790	1300	1100	330	20	ID	ID	24.4	16.4	6.0	(11.5-23.0)	17.7	1.5
		M[3]	4900	1100	2200	4900	460	40	ID	ID						
		E[4]	17000	490	54000	3300	11000	ID	110	ID			4.2			
2- 5 Jun 1975	149	H	3300	490	310	330	330	40	20	ID	24.4	16.4	5.7	(11.5-24.0)	18.8	1.5
		M	4900	790	490	2300	460	20	20	110						
		E	17000	4900	220	7900	11000	20	45	2800			4.5			
28-31 Jul 1975	55	H	13000	240000	230	7900	490	490	ID	1400	27.2	15.9	5.7	(3.5-19.2)	10.6	2.1
		M	22000	3300	22000	1400	1700	130	ID	1100			4.8			
		E	49000	490	790	1100	4900	68	ID	700			4.0			
			49000				13000									
28-31 Jul 1975	155	H	13000	230	54000	20	490	ID	330	ID	27.2	15.9	6.0	(3.5-19.2)	10.6	2.0
		M	22000	490	330	78	1700	20	78	ID			4.7			
		E	49000	24000	1700	170	4900	ID	210	20			3.8			
			49000				13000									
4- 7 Aug 1975	56	H	7000	15000	2200	45	220	260	45	ID	27.4	17.0	3.8	(2.0-27.2)	12.6	1.1
		M	7900	1300	1300	2300	1300	40	20	1300			3.0			
		E	7900	490	230	18	1300	140	ID	ID			1.5			
4- 7 Aug 1975	156	H	7000	2200	45	78	220	ID	ID	ID	27.4	17.0	6.2	(3.5-27.2)	13.8	2.3
		M	7900	700	40	78	1300	490	ID	ID			4.7			
		E	7900	220	61	130	1300	ID	ID	ID			3.8			
25-28 Aug 1975	59	H	4900	20	ID	ID	790	ID	ID	ID	29.0	19.1	5.1	(9.6-22.4)	15.8	1.6
		E	7000	3300	ID	ID	790	ID	ID	ID			3.3			
			33000				1300									
25-28 Aug 1975	159	H	4900	ID	--	--	790	ID	--	--	29.0	18.8	6.0	(10.4-22.4)	15.8	1.7
		E	7000	ID	--	--	790	ID	--	--			2.9			
			33000				1300									
25-28 Aug 1975	259	H	4900	130	--	--	790	20	--	--	28.9	18.8	5.3	(10.4-21.6)	14.8	1.7
		E	7000	78	--	--	790	45	--	--			2.2			
			33000				1300									

[1] When a group of trays had oysters from same batch, 0-hr values given apply to all trays enclosed within bracket. Values were used only once in computation of mean for combinations of more than one experiment.

[2] H = Head of flume.

[3] M = Middle of flume.

[4] E = Drain end of flume.

Table 32. Percent and mean total coliform (TC/100g) levels remaining after 24, 48, and 72 hours depuration in commercial-size tanks with respect to initial levels and depuration temperature.

Tank	Initial Level	Range Mean Temp. (C)	Mean 0 Hr. TC/100g	Percent TC/100g Remaining At			Mean TC/100g Remaining At			Experiment Number
				24 Hrs.	48 Hrs.	72 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.	
2 x 4	1,001-10,000/100g	10.1 - 11.5	2,000 (5)*	50	65	28	1,000 (10)	1,300 (10)	560 (10)	172, 173, 272, 273
		15.6 - 18.5	2,400 (6)	3.4	7.5	4.6	81 (8)	180 (8)	≥110 (12)	82, 84, 182, 184
	10,001-25,000/100g	17.4 - 18.0	12,000 (6)	2.0	1.5	1.9	240 (10)	<180 (10)	225 (12)	70, 83, 170, 183
	>25,000/100g	14.6	>30,000	5.0	5.0	4.7	1,500 (7)	1,500 (7)	1,400 (7)	171, 271,
2 x 8	1,001-10,000/100g	10.1	2,800 (3)	53.6	57.1	8.2	1,500 (3)	1,600 (3)	230 (3)	73
		17.6 - 26.1	4,000 (11)	10.5	6.0	4.5	420 (23)	<240 (23)	<180 (23)	60, 63, 65, 69
	10,001-25,000/100g	19.9 - 26.5	19,000 (6)	4.2	2.6	1.8	800 (10)	490 (10)	350 (10)	53 (trays 2, 4, 5, 7), 67
	>25,000/100g	11.5	49,000 (1)	1.6	1.4	0.88	800 (3)	690 (3)	430 (3)	72
		14.6 - 26.5	≥75,000 (15)	3.6	1.1	0.33	2,700 (22)	790 (22)	250 (22)	53 (trays 1, 8), 54, 61, 68, 71
4 x 8	0-1,000/100g	20.2	310 (1)	174	30.3	31.9	540 (4)	94 (4)	99 (4)	48 (trays 1,4, 5, 8)
	1,001-10,000/100g	20.2 - 27.5	4,300 (10)	14	6.5	2.8	600 (20)	<280 (20)	120 (20)	48 (trays 3, 7), 50, 51, 57
	10,001-25,000/100g	23.1 - 28.8	15,000 (6)	1.7	0.39	0.67	260 (12)	58 (12)	100 (12)	58, 62

*Number of samples constituting mean value

Table 33. Percent and mean fecal coliforms (FC/100g) levels remaining after 24, 48, and 72 hours depuration in commercial-size tanks with respect to initial levels and depuration temperatures.

Tank	Initial Level	Range Mean Temp. (C)	Mean 0 Hr. FC/100g	Percent FC/100g Remaining At			Mean FC/100g Remaining At			Experiment Number
				24 Hrs.	48 Hrs.	72 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.	
2 x 4	0-500/100g	10.1 - 11.5	190 (5)*	52.6	22.6	11.1	100 (10)	<43 (10)	<21 (10)	172, 173, 272, 273
		15.6	320 (3)	18.1	7.8	20.3	58 (4)	<25 (4)	65 (6)	82, 182
	1,001-5,000/100g	14.6 - 18.5	3,300 (12)	2.1	1.0	0.94	<68 (21)	<34 (21)	<31 (25)	70, 83, 84, 170, 171, 183, 184, 271
2 x 8	0-500/100g	10.1 - 11.5	180 (4)	26.1	17.2	13.9	<47 (6)	<31 (6)	<25 (6)	72, 73
		17.6 - 23.7	210 (8)	11.0	10.0	9.5	<23 (18)	<21 (18)	<20 (18)	63, 65, 69
	1,001-5,000/100g	14.6 - 26.6	3,000 (16)	1.5	0.83	0.90	<44 (23)	<25 (23)	<27 (23)	53 (trays 2, 4, 5, 7), 60, 67, 68, 71
	5,001-10,000/100g	25.9	7,000 (3)	1.2	0.59	0.26	<85 (6)	<41 (6)	<18 (6)	61
	>10,001/100g	26.2 - 26.5	39,000 (5)	0.41	0.09	0.06	<160 (8)	<36 (8)	<24 (8)	53 (trays 1, 8), 54
4 x 8	0-500/100g	20.2 - 23.3	380 (2)	6.6	4.7	4.7	<25 (5)	<18 (5)	<18 (5)	48 (trays 1, 4, 5, 8), 50 (tray 1)
	501-1,000/100g	23.3 - 28.8	700 (7)	3.6	2.6	2.6	<25 (17)	<18 (17)	<18 (17)	50 (trays 2, 4, 5, 7, 8), 57, 58
	1,001-5,000/100g	20.2 - 25.9	2,200 (6)	1.0	0.91	0.82	<23 (8)	<20 (8)	<18 (8)	48 (trays 7, 3), 51

*Number of samples constituting mean values

Table 34. Summary of reduction of total coliforms in commercial-size tanks with respect to initial levels, depuration temperature and water quality of harvest area.

No. Expts.	Range Mean Temp. (C)	Range Mean 0 hr TC/100 g	Calculated* Range TC/100 ml Water	Actual Mean TC/100 ml Water	Average Percent TC/100 g Remaining At			Range Mean TC/100 g Remaining At		
					24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
A. Initial TC level = 1,001-10,000/100 g										
5	10.1-11.5	2,000-2,800	1,500 1,700	280	51.8	61.1	18.1	1,000-1,500	1,300-1,600	230-560
12	15.6-27.5	2,400-4,300	6,000-6,300	200	9.3	6.7	4.0	81-600	<180-<280	≥110-<180
B. Initial TC level = 10,001-25,000/100 g										
8	17.4-28.8	12,000-19,000	7,400-8,500	700	2.6	1.5	1.5	240-800	58-490	100-350
C. Initial TC level = >25,000/100 g										
1	11.5	49,000	13,000	23	1.6	1.4	0.88	800	690	430
7	14.6-26.5	>30,000-75,000	10,000-17,000	5,000	4.3	2.0	2.5	1,500-2,700	790-1,500	250-1,400

* Calculated range TC/100 ml water - values derived from data compiled by Hope and Wiley (1961) and presented for comparison with actual mean coliform levels in harvest water; coefficient of regression at temperatures ≤10 C = 0.18, slope = 0.248 and y intercept (TC/100 ml water) = 992, number paired samples = 24 and coefficient of regression for temperatures ≥13 C = 0.23, slope = 0.153 and y intercept (MPN/100 ml water) = 5,608, number paired samples = 95.

Table 35. Summary of reduction of fecal coliforms in commercial-size tanks with respect to initial levels, depuration temperature and water quality of harvest area.

No. Expts.	Range Mean Temp. (C)	Range Mean 0 hr FC/100 g	Calculated* Range FC/100 ml Water	Actual Mean FC/100 ml Water	Average Percent FC/100 g Remaining At			Range Mean FC/100 g Remaining At		
					24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
A. Initial FC level = 0-500/100 g										
6	10.1-11.5	180-190	240-250	170	39.4	19.9	12.5	<47-100	<31-<43	<21-<25
7	15.6-23.7	210-380	2,000-2,100	57	11.9	7.5	11.5	<23-58	<18-<25	<18-65
B. Initial FC level = 501-1,000/100 g										
3	23.3-28.8	700	2,200	120	3.6	2.6	2.6	<25	<18	<18
C. Initial level = 1,001-5,000/100 g										
15	14.6-26.6	2,200-3,300	2,800-3,200	350	1.5	0.91	0.89	<23-<68	<20-<31	<18-<31
D. Initial level = 5,001-10,000/100 g										
1	25.9	7,000	4,500	700	1.2	0.59	0.26	<85	<41	<18
E. Initial level = >10,000/100 g										
2	26.2-26.5	39,000	16,000	1,300	0.41	0.09	0.06	<160	<36	<24

* Calculated range FC/100 ml water - values derived from data compiled by Hope and Wiley (1961) and presented for comparison with actual mean coliform levels in harvest water; coefficient of regression at temperatures ≤10 C = 0.37, slope = 0.969 and y intercept (FC/100 ml water) = 69, number paired samples = 24 and coefficient of regression for temperatures ≥13 C = 0.23, slope = 0.365 and y intercept (FC/100 ml water) = 1,973, number of paired samples = 95.

Table 36. Correlation of total coliform concentration in oysters and shellfish growing waters.

Temperature (C)	N[1]	R^2	b[3]	m[4]	UCL[5]	LCL[6]	Mean Oyster TC/100 g	Mean Water TC/100 ml	Oyster Water Index
A. 1.5-15.0	13	0.34	3,337	1.07	3.01	-0.87	940	360	2.61
B. 15.1-20.0	19	-0.06	2,364	-0.00	0.00	-0.01	5,200	320	16.25
C. 20.1-25.0	17	-0.08	1,443	-0.01	0.04	-0.06	5,800	790	7.34
D. >25.1	18	-0.11	664	-0.00	0.01	-0.02	7,900	560	14.11

[1] N = number samples
[2] R = coefficient of linear regression
[3] b = y (water total or fecal coliform level) intercept
[4] m = slope
[5] UCL = upper limit of slope] at 95% probability
[6] LCL = lower limit of slope]

Table 37. Correlation of fecal coliform concentrations in oysters and shellfish growing waters.

Temperature (C)	N[1]	R[2]	b[3]	m[4]	UCL[5]	LCL[6]	Mean Oyster FC/100 g	Mean Water FC/100 ml	Oyster Water Index
A. 1.5-15.0	13	0.76	-3,371	46.13	71.62	20.65	77	100	0.77
B. 15.1-20.0	19	0.66	37	0.25	0.40	0.11	580	97	5.98
C. 20.1-25.0	17	0.12	321	0.04	0.22	-0.14	630	190	3.32
D. >25.1	18	0.13	265	0.07	0.36	-0.22	420	140	3.00

[1] N = number samples
[2] R = coefficient of linear regression
[3] b = y (water total or fecal coliform level) intercept
[4] m = slope
[5] UCL = upper limit of slope ⎤ at 95% probability
[6] LCL = lower limit of slope ⎦

Table 38. Mean MPN level, 90% confidence interval and cumulative percentages for total coliforms of oysters depurated in the 2 x 8 tank. Grouped according to initial oyster MPN level and frequency of tank flushing. Oysters contaminated in nature.

Initial level in oysters (MPN/100g)	Flushing Interval	Depuration Time (Hrs.)	No. Samples	MPN/100 g Mean	MPN/100 g 90% Confidence Interval[1]		Percent of samples with MPN levels ≤ stated values 500	230	100	Percent of samples with MPN levels >1000
1,001-10,000	6 hr	0	8	4,300	2,400	7,800	---	---	---	100
		24	17	320	50	1,900	65	47	29	24
		48	17	<190	34	1,100	71	71	47	18
		72	17	<91	26	330	88	76	53	6
10,001-25,000	6 hr	0	3	17,000	---	---	---	---	---	100
		24	6	610	---	---	33	---	---	---
		48	6	350	---	---	100	17	---	---
		72	6	540	160	1,700	50	17	---	33
1,001-10,000	24 hr	0	3	3,300	---	---	---	---	---	100
		24	6	950	370	2,300	33	17	---	67
		48	6	460	130	1,600	67	33	---	17
		72	6	1,200	460	3,000	33	---	---	50
10,001-25,000	24 hr	0	3	21,000	---	---	---	---	---	100
		24	4	1,200	210	6,400	50	25	---	50
		48	4	810	350	1,900	50	---	---	25
		72	4	180	95	310	100	75	25	---
>25,000	6/12 hr	0	4	81,000	23,000	280,000	---	---	---	100
		24	6	9,600	4,300	20,000	---	---	---	100
		48	6	3,600	520	23,000	---	---	---	67
		72	6	2,200	950	5,000	---	---	---	83
>25,000	24 hr	0	3	75,000	---	---	---	---	---	100
		24	6	1,100	63	17,000	50	33	17	33
		48	6	240	40	1,500	67	67	33	33
		72	6	47	20	110	100	100	83	---

Footnotes:

[1]Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 39. Mean MPN level, 90% confidence interval and cumulative percentages for fecal coliforms of oysters depurated in the 2 x 8 tank. Grouped according to initial oyster MPN level and frequency of tank flushing. Oysters contaminated in nature.

Initial level in oysters (MPN/100g)	Flushing Interval	Depuration Time (Hrs.)	No. Samples	MPN / 100 g Mean	90% Confidence Interval[1]		Percent of samples with MPN levels ≤stated values			Percent of samples with MPN levels >200
							100	50	20	
0-500	6 hr	0	5	140	---	---	20	---	---	20
		24	12	<23	---	---	100	92	75	---
		48	12	<18	---	---	100	100	100	---
		72	12	<18	---	---	100	100	100	---
1,001-5,000	6 hr	0	6	2,600	1,000	6,700	---	---	---	100
		24	11	<39	20	73	91	91	45	9
		48	11	<18	---	---	100	100	100	---
		72	11	<36	---	---	82	82	64	9
0-500	24 hr	0	3	410	---	---	---	---	---	100
		24	6	<22	---	---	100	100	83	---
		48	5	<29	---	---	100	60	60	---
		72	6	<18	---	---	100	100	100	---
1,001-5,000	24 hr	0	3	4,400	---	---	---	---	---	100
		24	4	59	24	140	100	100	---	
		48	4	<49	---	---	50	50	50	---
		72	4	<23	---	---	100	100	75	---
1,001-5,000	6/12 hr	0	4	2,500	1,100	5,500	---	---	---	100
		24	6	<85	16	470	67	50	50	17
		48	6	<19	---	---	100	100	100	---
		72	6	<18	---	---	100	100	100	---

Footnotes:

[1]Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 40. Mean MPN level, 90% confidence interval and cumulative percentages for total coliforms in oysters and biodeposits depurated in the 2 x 4 tank. Tank A flushed at 24 hr intervals. Tank B not flushed. Oysters contaminated in nature.

Type Sample	Depuration Time (Hrs.)	No. Samples	MPN / 100 g Mean	MPN / 100 g 90% Confidence Interval		Percent of samples with MPN levels ≤stated values 500	230	100	Percent of samples with MPN levels >1000
Oysters Tank A	0	9	4,200	1,100	15,000	11	---	---	89
	24	9	91	38	200	89	89	56	---
	48	9	<290	51	1,500	56	33	22	22
	72	9	100	41	250	100	89	56	---
Oysters Tank B	0	9	4,200	1,100	15,000	11	---	---	89
	24	3	150	---	---	100	67	33	---
	48	3	<76	---	---	67	67	67	33
	72	9	160	40	620	78	78	56	11
Biodeposits Tank A	0	---	---	---	---	---	---	---	---
	4	6	780,000	44,000	12,000,000	---	---	---	100
	24	6	140,000	45,000	440,000	---	---	---	100
	48	6	64,000	11,000	360,000	---	---	---	100
	72	4	25,000	6,300	97,000	---	---	---	100

Footnotes:

[1] Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 41. Mean MPN level, 90% confidence interval and cumulative percentages for fecal coliforms in oysters and biodeposits depurated in the 2 x 4 tank. Tank A flushed at 24 hr intervals. Tank B not flushed. Oysters contaminated in nature.

Type Sample	Depuration Time (Hrs.)	No. Samples	MPN / 100 g Mean	MPN / 100 g 90% Confidence Interval		Percent of samples with MPN levels ≤stated values 100	50	20	Percent of samples with MPN levels >200
Oysters Tank A	0	9	1,500	440	5,100	---	---	---	89
	24	9	<40	---	---	89	78	44	11
	48	9	<34	---	---	89	67	67	11
	72	9	<22	---	---	100	100	78	---
Oysters Tank B	0	9	1,500	440	5,100	---	---	---	89
	24	3	<42	---	---	67	67	33	---
	48	3	<33	---	---	67	67	67	---
	72	9	<47	20	110	89	89	78	11
Biodeposits Tank A	0	---	---	---	---	---	---	---	---
	4	6	110,000	29,000	450,000	---	---	---	100
	24	6	29,000	7,600	110,000	---	---	---	100
	48	6	1,000	320	3,300	---	---	---	100
	72	4	820	---	---	---	---	---	100

Footnotes:

[1]Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 42 . Mean MPN level, 90% confidence interval and cumulative percentages for total coliforms in feces, pseudofeces and oysters depurated in shallow (plexiglass) trays. Oysters contaminated in nature.

Type Sample	Depuration Time (Hrs.)	No. Samples	MPN / 100 g Mean	MPN / 100 g 90% Confidence Interval[1]		Percent of samples with MPN levels <stated values 500	230	100	Percent of samples with MPN levels >1000
Oysters	0	6	27,000	5,000	140,000	---	---	---	100
	4	4	1,200	330	4,500	25	---	---	50
	24	4	370	82	1,600	75	50	---	25
	48	4	460	180	1,100	50	25	---	---
	72	4	130	25	690	100	75	25	---
Feces	0	---	---	---	---	---	---	---	---
	4	4	2,500,000	48,000	13,000,000	---	---	---	100
	24	4	35,000	8,200	150,000	---	---	---	100
	48	4	2,100	300	13,000	---	---	---	50
	72	4	2,000	820	5,100	---	---	---	75
Pseudofeces	0	---	---	---	---	---	---	---	---
	4	4	67,000	10,000	440,000	---	---	---	100
	24	4	4,300	1,400	12,000	50	50	---	50
	48	4	<820	130	4,800	50	50	---	50
	72	4	1,000	210	4,700	50	---	---	50

Footnotes:

[1]Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 43. Mean MPN level, 90% confidence interval and cumulative percentages for fecal coliforms in feces, pseudofeces and oysters depurated in shallow (plexiglass) trays. Oysters contaminated in nature.

Type Sample	Depuration Time (Hrs.)	No. Samples	MPN/100 g Mean	MPN/100 g 90% Confidence Interval[1]		Percent of samples with MPN levels <stated values 100	50	20	Percent of samples with MPN levels >200
Oysters	0	6	750	180	3,100	---	---	---	50
	4	4	<49	---	---	75	50	50	25
	24	4	<22	---	---	100	100	75	---
	48	4	<18	---	---	100	100	100	---
	72	4	<22	---	---	100	100	75	---
Feces	0	---	---	---	---	---	---	---	---
	4	4	150,000	18,000	1,300,000	---	---	---	100
	24	4	2,100	430	11,000	---	---	---	100
	48	4	<230	---	---	---	---	---	25
	72	4	<180	---	---	---	---	---	---
Pseudofeces	0	---	---	---	---	---	---	---	---
	4	4	5,600	760	41,000	---	---	---	100
	24	4	480	110	2,000	---	---	---	75
	48	4	<180	---	---	---	---	---	---
	72	4	<180	---	---	---	---	---	---

Footnotes:

[1]Absence of confidence intervals throughout table due to either a lack of variation other than that related to the MPN technique or to skewness of the data as a result of depuration.

Table 44 . Survival of coliform organisms in depuration tank drain water with and without biodeposits. Flask A = tank drain water. Flask B = tank drain water plus feces. Flask C = tank drain water plus pseudofeces

	No. Hours	Flask A		Flask B		Flask C		
	Incubation	TC/100ml	FC/100ml	TC/100ml	FC/100ml	TC/100ml	FC/100ml	Temperature
Trial 1*	0	1.8	ID	23	4.5	11	4.0	20 ± 1 C
	3	4.5	ID	7.8	7.8	4.5	2.0	"
	6	4.5	ID	13	4.5	2.0	2.0	"
	24	2.0	ID	7.8	4.5	6.8	6.8	"
	48	ID	ID	4.5	2.0	4.5	2.0	"
	72	7.8	ID	11	4.5	ID	ID	"
Trial 2**	0	ID	ID	3,300	170	790	46	20 C
	3	4.5	2.0	12,000	140	110	49	19.7 C
	6	1.8	ID	1,300	230	310	49	23 C
	24	4.5	ID	3,100	170	220	23	28 C
	48	4.5	ID	430	17	49	4.5	24 C
	72	ID	ID	110	49	13	2.0	19 C

*Trial 1 - Ratio of volume feces or pseudofeces to volume tank drain water = 1:1000

**Trial 2 - Ratio of volume feces or pseudofeces to volume tank drain water = 1:100

Table 45 . Coliform types isolated from positive brilliant green (BG) tubes representing oyster samples prior to and after 72 hrs depuration in shallow plexiglass trays.*

Sample Type	Coliform Type Isolated**							Number Positive BG Tubes	Number Samples
	Escherichia coli	Klebsiella pneumoniae	Enterobacter cloacae	Enterobacter aerogenes	Enterobacter agglomerans	Citrobacter freundii	Citrobacter diversus		
Oysters									
0 Hr. (TC/100g = 11,000)	58.3 (14)	91.7 (22)			8.3 (2)		8.3 (2)	24	2
72 Hrs. (TC/100g = 2,100)	2.4 (1)	14.3 (6)	11.9 (5)	2.4 (1)		81 (34)		42	4

*Experiment numbers - 169 and 270

**Percent occurrence based on number brilliant green tubes from which organism was isolated ( ) and number brilliant green tubes examined.

Table 46 . Coliform types isolated from positive brilliant green (BG) tubes representing oyster and biodeposit samples prior to and after 72 hrs depuration in the 2 x 4 tank.*

Sample Type	Coliform Type Isolated**									
	Escherichia coli	Klebsiella pneumoniae	Enterobacter cloacae	Enterobacter aerogenes	Enterobacter hafniae	Enterobacter agglomerans	Citrobacter freundii	Citrobacter diversus	Number Positive BG Tubes	Number Samples
Oysters										
0 Hr. (TC/100g = 3,100)	79.5 (35)	59.1 (26)	45.5 (20)	9.1 (4)	4.5 (2)	2.3 (1)	9.1 (4)	2.3 (1)	44	4
72 Hrs. (TC/100g = 130)	23.5 (4)	70.6 (12)	11.8 (2)				23.5 (4)		17	4
Biodeposits										
4 Hr. (TC/100g = 500,000)	68.6 (48)	61.4 (43)	44.3 (31)	8.6 (6)		2.9 (2)	11.4 (8)		70	4
72 Hrs. (TC/100g = 25,000)	22.7 (10)	68.2 (30)	31.8 (14)	2.3 (1)	2.3 (1)		36.4 (16)		44	4

*Experiment numbers - 82 and 84

**Percent occurrence based on number brilliant green tubes from which organism was isolated ( ) and number brilliant green tubes examined.

Table 47. Variability of total and fecal coliform (TC and FC/100g) levels from samples of pooled oysters within specific lots.

Oyster Lot	Collection Date	Number Pooled Oysters	TC/100g	FC/100g
A	5-12-75	6	310	310
		6	1,300	1,300
		6	4,600	1,300
B	6-09-75	5	2,300	330
		5	3,300	790
C	6-16-75	3	4,900	2,300
		7	4,900	3,300
		3	7,900	7,900
D	7-07-75	7	4,900	2,300
		7	22,000	4,600
		7	92,000	7,900
E	7-14-75	7	130,000	79,000
		5	170,000	33,000
		7	240,000	79,000
F	8-11-75	5	7,000	700
		5	7,000	1,100
G	8-18-75	5	7,900	700
		5	13,000	2,200
		5	17,000	260
H	9-02-75	6	3,300	790
		6	4,900	2,200
		6	14,000	4,600
I	9-08-75	6	33,000	7,900
		6	79,000	13,000
		6	160,000	3,300
J	9-22-75	6	13,000	2,300
		6	17,000	3,300
		6	33,000	33,000
K	9-29-75	8	1,100	78
		8	3,300	170
		8	4,900	230

Table 47 cont.

Oyster Lot	Collection Date	Number Pooled Oysters	TC/100g	FC/100g
L	10-14-75	9	4,900	130
		9	6,400	140
M	10-20-75	8	13,000	1,100
		8	17,000	7,900
		8	22,000	4,900
N	10-27-75	6	33,000	1,700
		7	70,000	2,200
		6	240,000	4,900
O	11-03-75	8	2,200	790
		8	2,300	110
		8	7,000	790
P	11-17-75	8	3,300	170
		8	3,300	1,700
		8	2,400,000	130,000
Q	12-08-75	8	1,300	230
		8	3,300	220
		8	4,900	110

Table 48. Variability in total and fecal coliform levels of individual oysters as determined using the graphical method of Velz (1951).

Lot Number	Number Individual Oysters	Total Coliforms/100g Mean	Total Coliforms/100g Range 90% Confidence Limits	Total Coliforms/100g Upper Limit at 95 percentile Actual*	Total Coliforms/100g Upper Limit at 95 percentile Theoretical**	Fecal Coliforms/100g Mean	Fecal Coliforms/100g Range 90% Confidence Limits	Fecal Coliforms/100g Upper Limit at 95 percentile Actual*	Fecal Coliforms/100g Upper Limit at 95 percentile Theoretical**
A	12	<100	36-280	690	260	<41	20-81	200	100
B	12	690	170-2,700	6,700	1,700	<40	12-130	320	100
C	20	19,000	6,000-54,000	110,000	48,000	1,700	420-6,400	16,000	4,300
D	23	1,700	720-4,200	11,000	4,400	<200	62-600	1,500	490
E	24	1,700	600-4,400	11,000	4,200	<470	120-1,800	4,500	1,200

*Actual upper limit: The value obtained where the graphical line passing through the data and the calculated mean value intersected the 95 percentile line.

**Theoretical upper limit: The value obtained where the theoretical line for a 5 portion MPN test and passing through the calculated mean value intersected the 95 percentile line.

Table 49. Comparison of the standard fecal coliform test and the ETCP method in the examination of oyster samples prior to depuration with respect to the concentration of oyster brei analyzed and time of incubation.*

Trial No.	Standard Method Fecal Coliforms /100g	ETCP Method Elevated Temperature Coliforms/100g			No. Hrs. Incubation
		1g oyster/plate	0.5g oyster/plate	0.1g oyster/plate	
1	4,900	<17		1,170	22
		384		1,340	29
		568		1,340	48
		568		1,340	72
2	390	50	752	1,840	22
		117	935	1,840	27
		317	1,020	1,840	48
		701	1,070	1,840	72
3	340	200	685	1,340	24
		802	885	1,500	48
		952	919	1,500	72

* Data concerning coliform levels in depurated oysters is not presented since fecal coliforms as determined by both procedures were reduced to low or indeterminate levels.

Table 50. Enumeration of fecal and elevated temperature coliforms in clean and polluted shellfish.

Trial No.	Standard Method Fecal Coliforms /100g	ETCP Method Elevated Temperature Coliforms/100g			No. Hrs. Incubation
		1g oyster/plate	0.5g oyster/plate	0.1g oyster/plate	
A. Relatively clean oysters					
1	20	<17	100	<167	18
		33	100	<167	24
		67	100	<167	48
		84	100	<167	72
2	20	67	84	<167	18
		100	117	<167	24
		100	117	167	48
		100	117	167	72
3	<18	<17	17	<167	18
		17	17	<167	24
		17	17	<167	48
		17	17	<167	72
4	<18	17	17	<167	18
		17	33	167	24
		83	33	167	48
		83	33	167	72

Table 50 con't.

Trial No.	Standard Method Fecal Coliforms /100g	ETCP Method Elevated Temperature Coliforms/100g			No. Hrs. Incubation
		1g oyster/plate	0.5g oyster/plate	0.1g oyster/plate	
5	<18	<17	33	167	18
		33	50	334	24
		33	67	334	48
		50	67	334	72
6	<18	17	17	<167	18
		50	50	<167	24
		50	50	<167	48
		50	50	<167	72
7	20	17	33	<167	18
		17	84	<167	24
		33	84	167	48
		50	117	167	72
8	78	84	84	167	18
		167	117	167	24
		167	117	167	48
		167	117	334	72
9	18	17	67	167	18
		33	100	167	24
		134	134	334	48
		184	150	334	72
10	18	<17	67	<167	18
		17	100	<167	24
		65	184	<167	48
		65	184	<167	72

Table 50 con't.

Trial No.	Standard Method Fecal Coliforms /100g	ETCP Method Elevated Temperature Coliforms/100g			No. Hrs. Incubation
		1g oyster/plate	0.5g oyster/plate	0.1g oyster/plate	
B. Moderately polluted oysters					
1	110	17	184	501	18
		117	334	501	24
		200	401	501	48
		217	418	501	72
2	1,100	17	134	167	18
		251	317	334	24
		501	434	501	48
		501	484	668	72
3	170	33	167	668	18
		100	334	668	24
		334	401	668	48
		384	418	668	72
4	330	50	301	334	18
		184	601	668	24
		701	752	835	48
		752	752	835	72
5	140	84	1,000	2,000	18
		184	1,170	2,170	24
		1,020	1,250	2,170	48
		1,100	1,250	2,170	72
6	170	284	551	1,000	18
		1,200	919	1,170	24
		1,290	1,000	1,170	48
		1,290	1,000	1,170	72

Table 51. ETCP plate counts/ml *E. coli* suspension inoculated into different concentrations oyster brei and counted after various intervals of incubation.

Trial No.	Concentration oyster/plate	Plate count/ml inoculum			
		19 hr.	24 hr.	48 hr.	72 hr.
1	1.0 g		94	135	142
	0.5 g		143	156	157
	0.1 g		168	169	169
	0.01 g		161	162	162
	0.0 g		155	155	155
2	1.0 g	31	94	124	124
	0.5 g	130	134	134	134
	0.1 g	135	141	141	141
	0.01 g	117	117	118	118
	0.0 g	138	139	139	139
3	1.0 g	47	87	162	167
	0.5 g	157	183	192	192
	0.1 g	210	210	210	210
	0.01 g	174	175	175	175
	0.0 g	201	202	202	203

Table 52 . Isolation of *E. coli* and coliforms from positive EC and Medium A-1 tubes representing naturally-polluted oysters.

Test Medium	No. Positive Tubes	*E. coli*$^+$ only		*E. coli*$^+$ + FP* Group		FP Group only	
		No. Tubes	Percent	No. Tubes	Percent	No. Tubes	Percent
E.C.	259	244	94.2	13	5.0	2	0.8
Medium A-1	287	284	99.0	3	1.0	0	0.0

* FP = False Positive

FIGURES

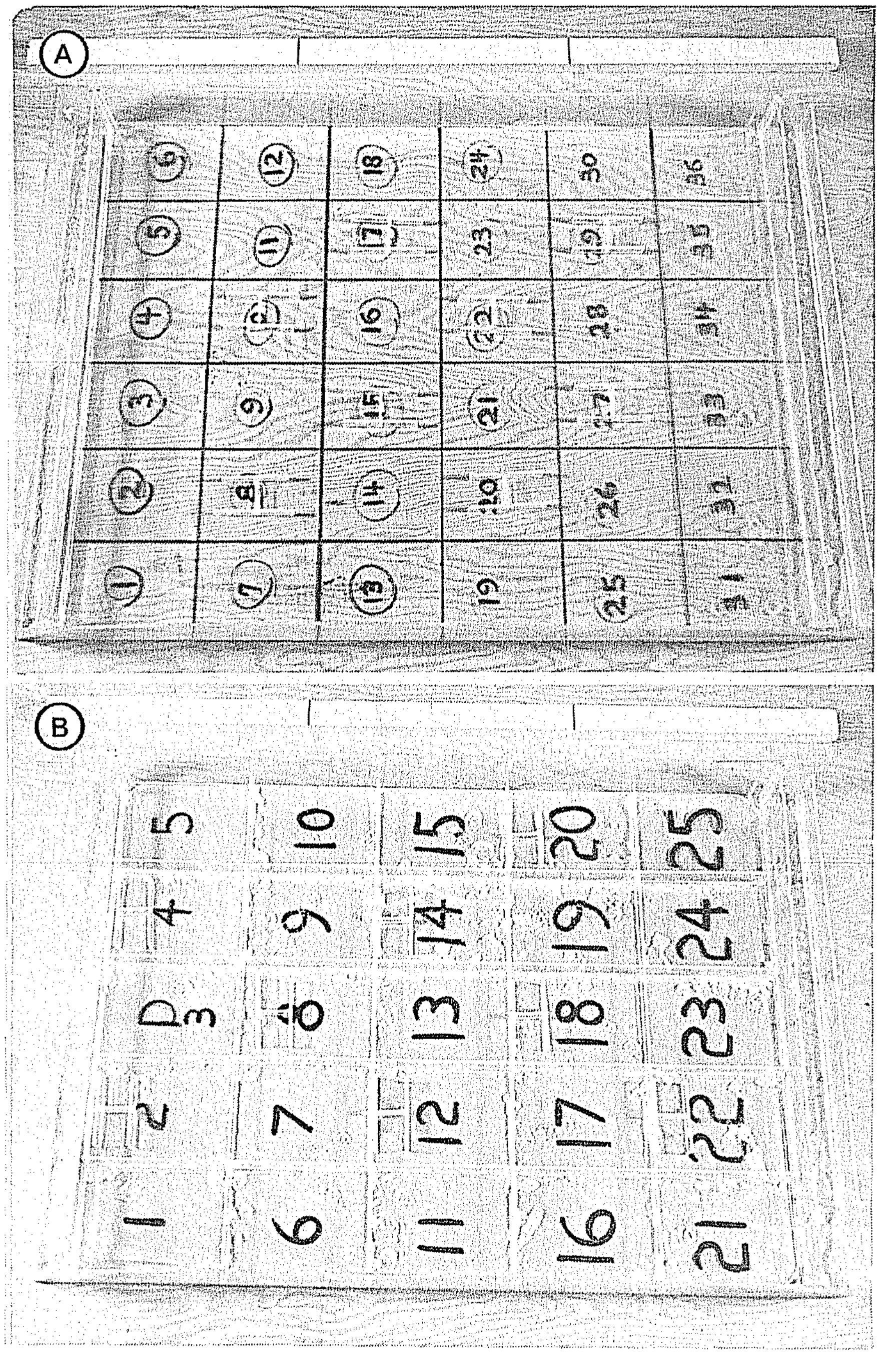

Figure 1. Shallow trays made of acrylic plastic. H-shape supports were used to raise oysters off the bottom. (A) Used with 36 oysters without partitions. (B) Used with 25 oysters separated by partitions.

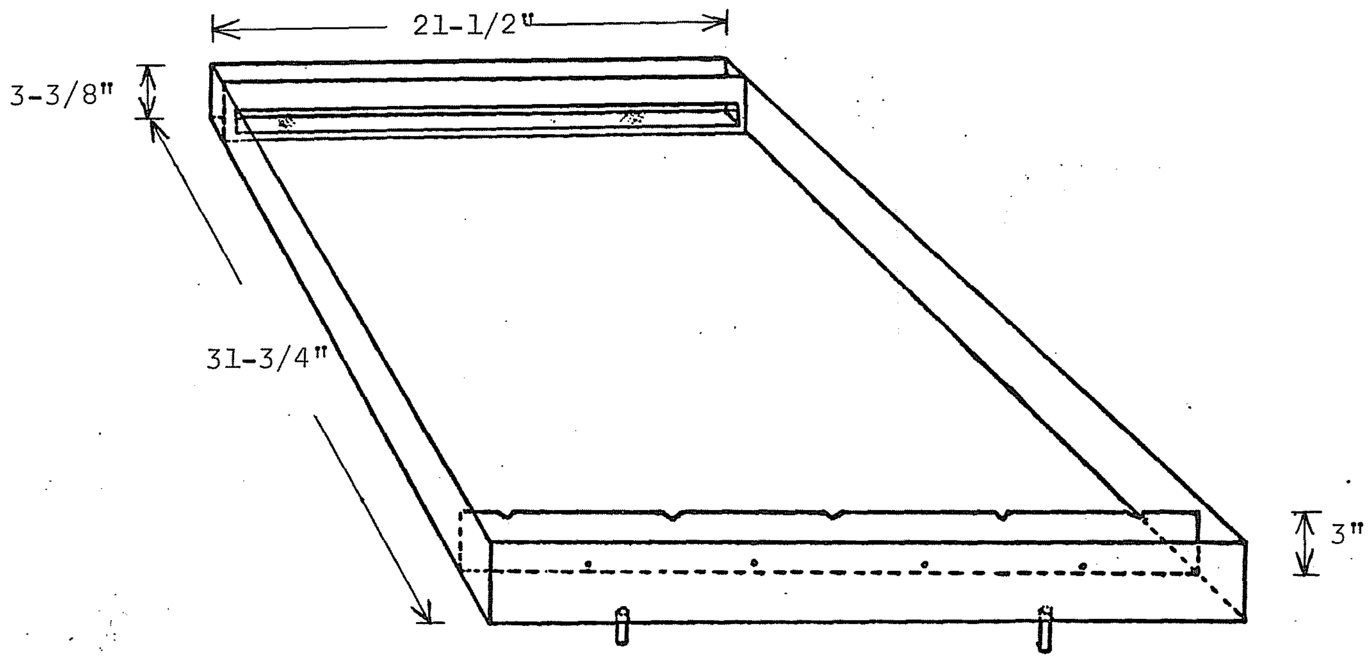

Figure 2. Diagram of shallow tray made of acrylic plastic showing construction details.

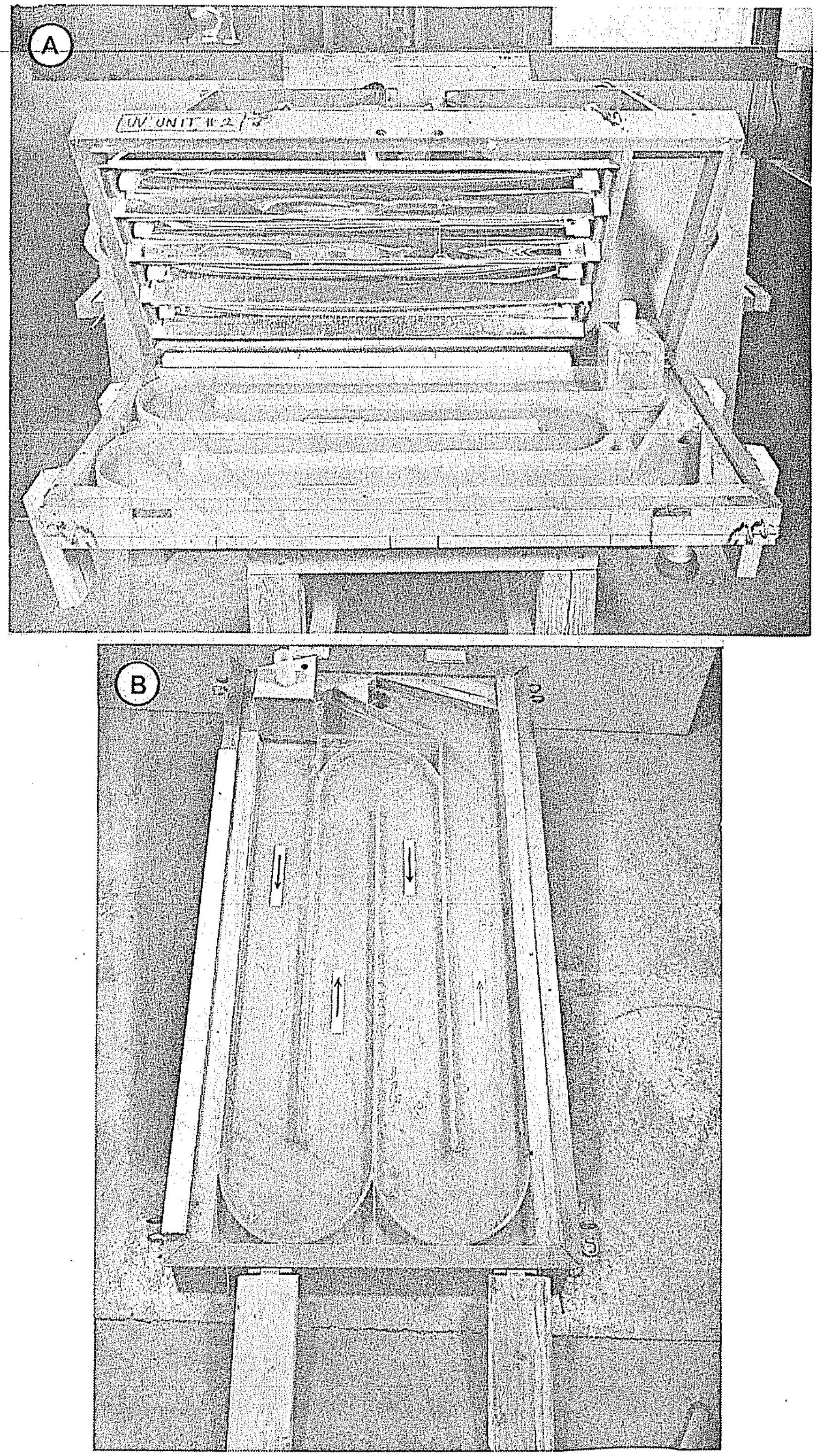

Figure 3. Four-lamp ultraviolet treatment unit used in shallow tray experiments.

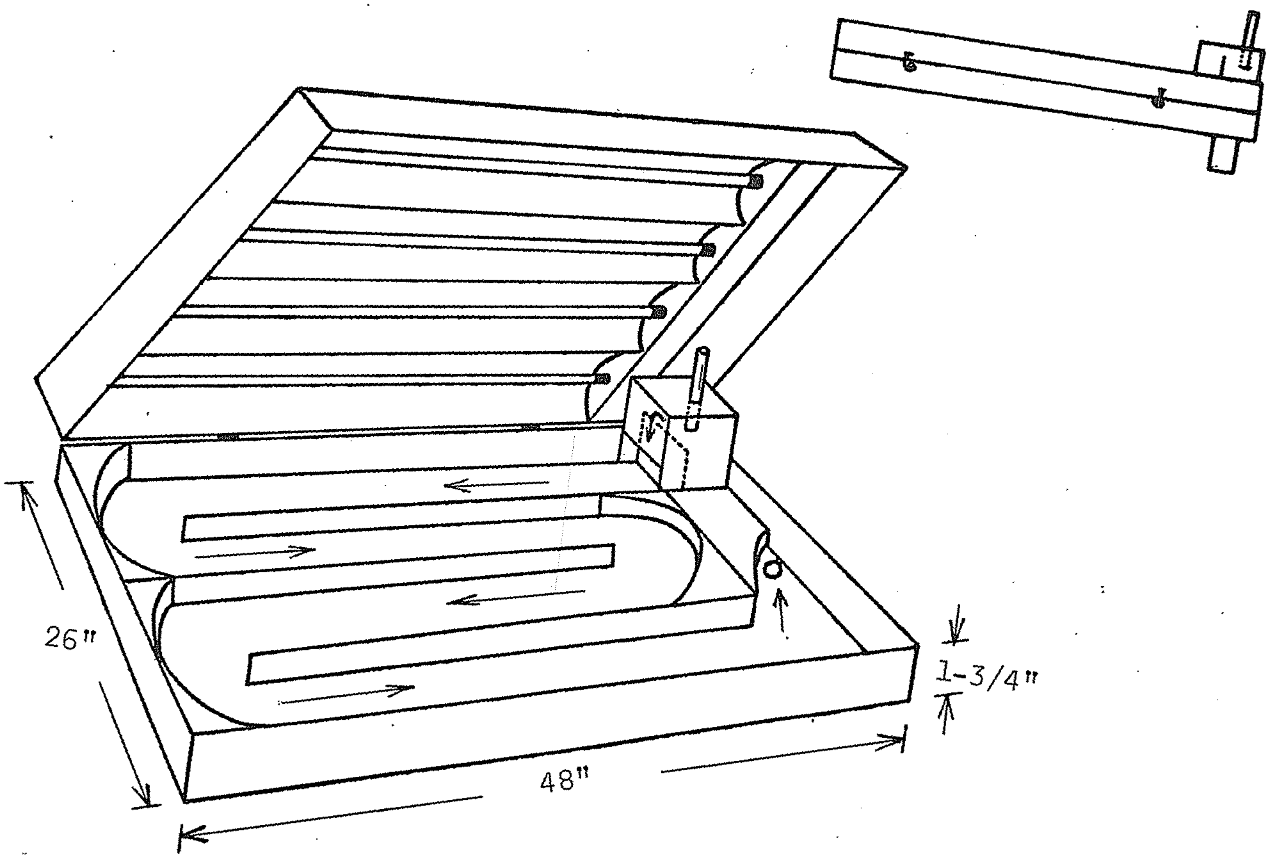

Figure 4. Diagram of four-lamp ultraviolet unit showing construction details.

Experiments included in construction of Figure 5

Legend	Experiment Numbers
○	35, 36
●	13-1, 13-2, 13-3, 46-2
▲	46-1
□	27, 31

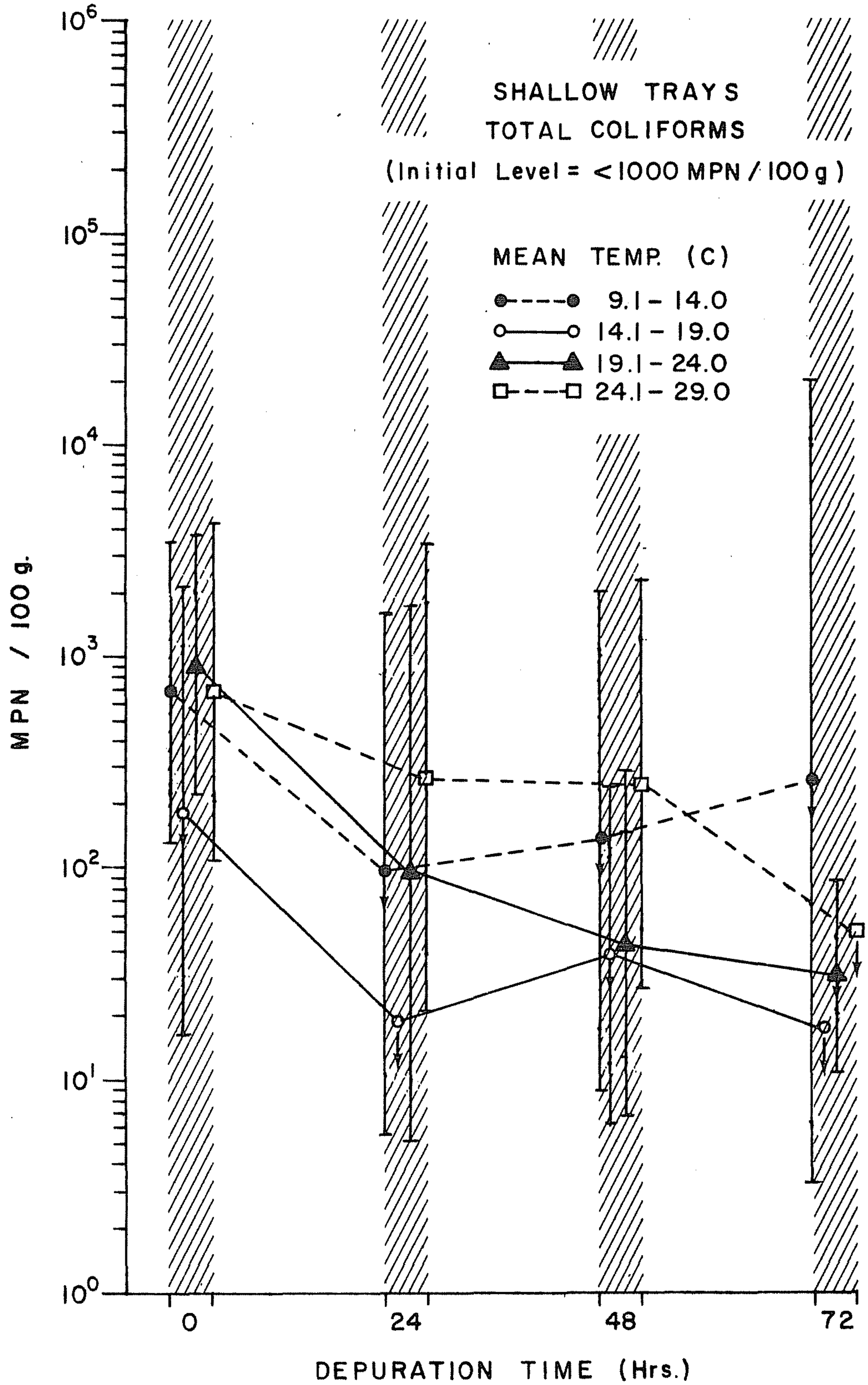

Figure 5. Mean total coliform levels in oysters depurated in shallow trays with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 6

Legend	Experiment Numbers
○	12, 16, 17-1, 17-2, 18-1, 18-2, 19-1, 19-2
●	11
▲	20-1, 20-2, 20-3
□	21 (tray 3), 21 (tray 1), 22, 23, 26, 30-1, 30-2

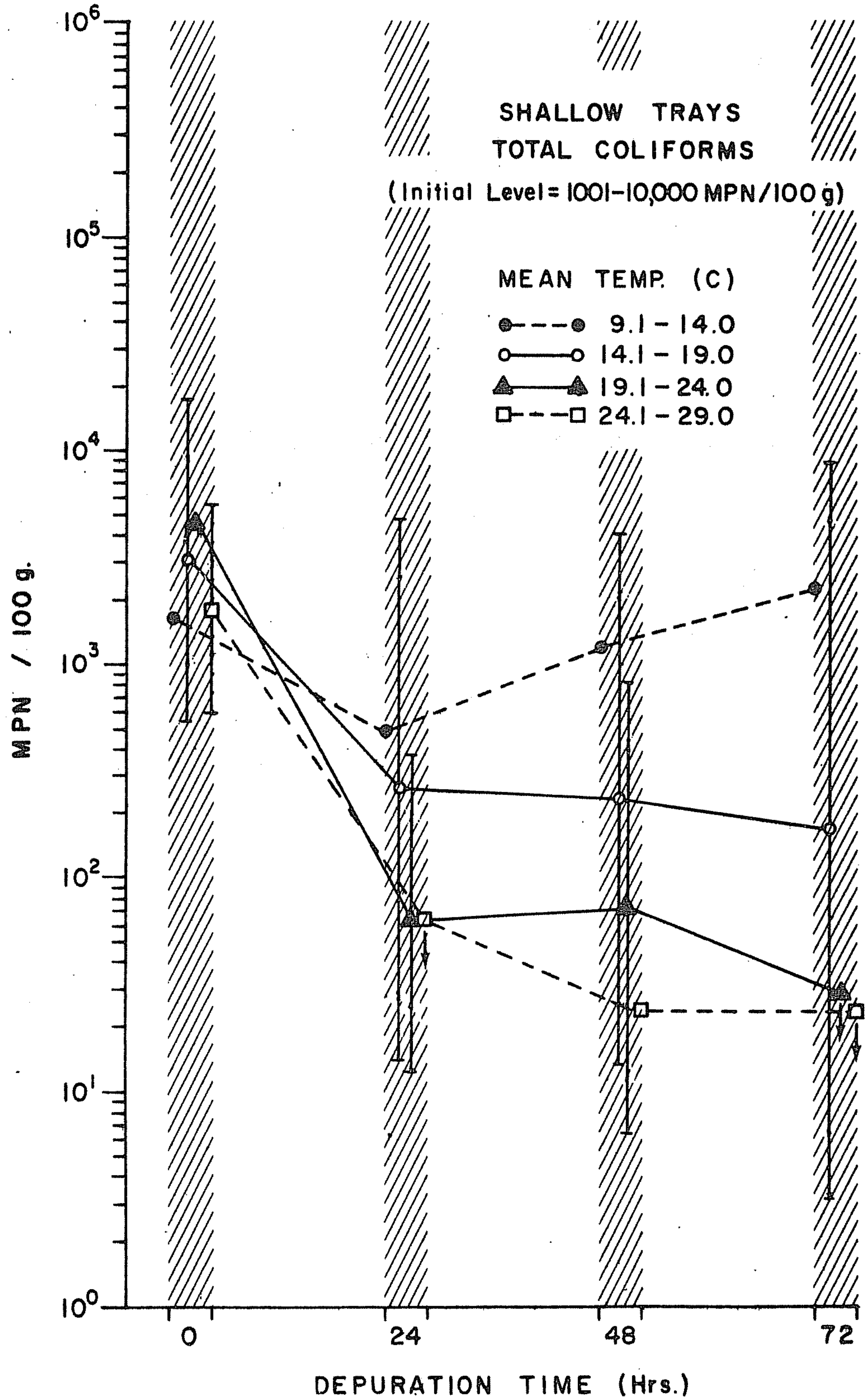

Figure 6. Mean total coliform levels in oysters depurated in shallow trays with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 8

Legend	Experiment Numbers
○	9, 12, 18-1, 18-2, 35, 36
●	11, 13-1, 13-2, 13-3, 46-2
▲	20-3, 46-1
□	21 (tray 3), 22, 23, 30-1, 30-2, 31

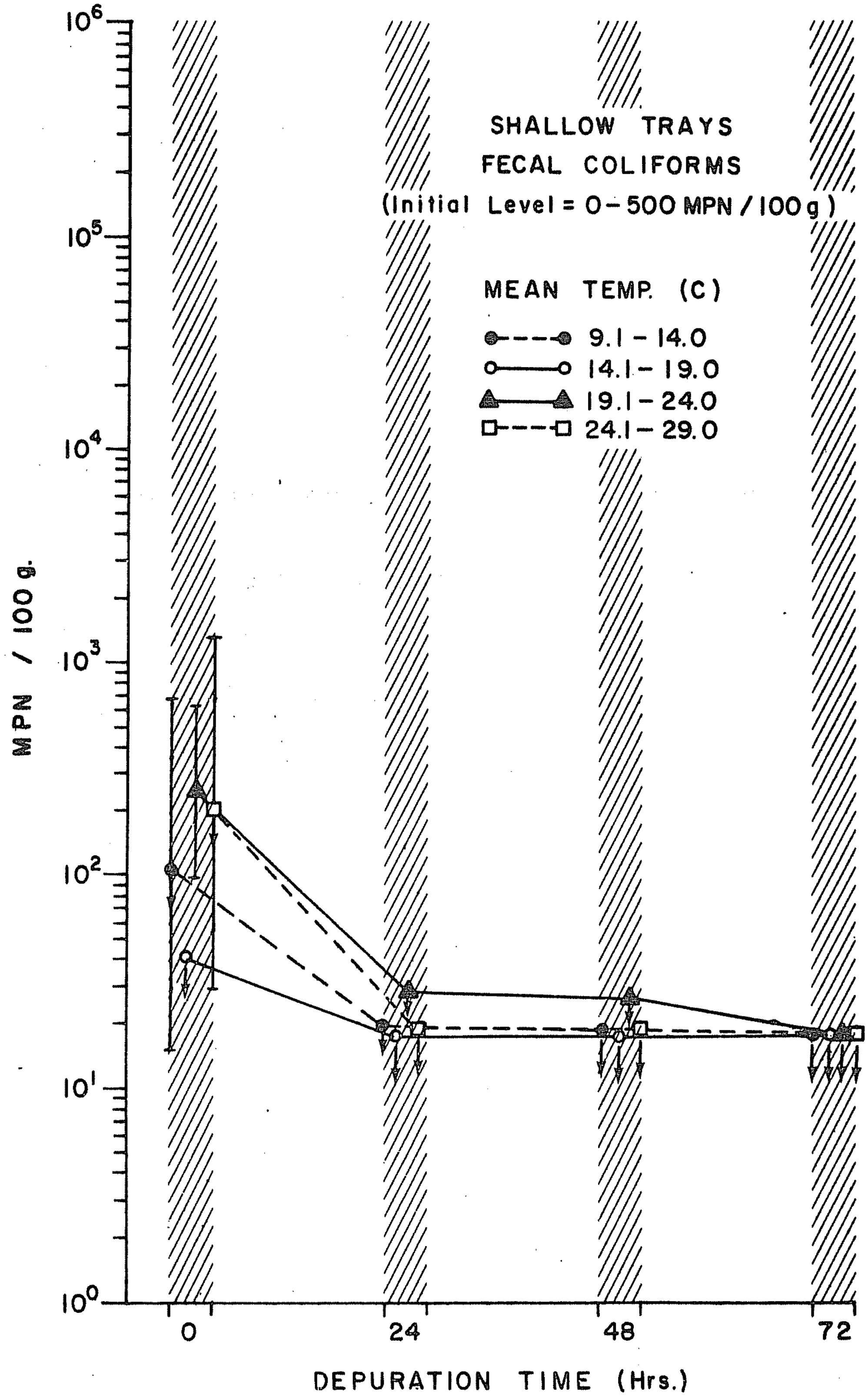

Figure 8. Mean fecal coliform levels in oysters depurated in shallow trays with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 9

Legend	Experiment Numbers
o	17-1, 17-2, 17-3, 19-1, 19-2
□	21 (tray 2), 27

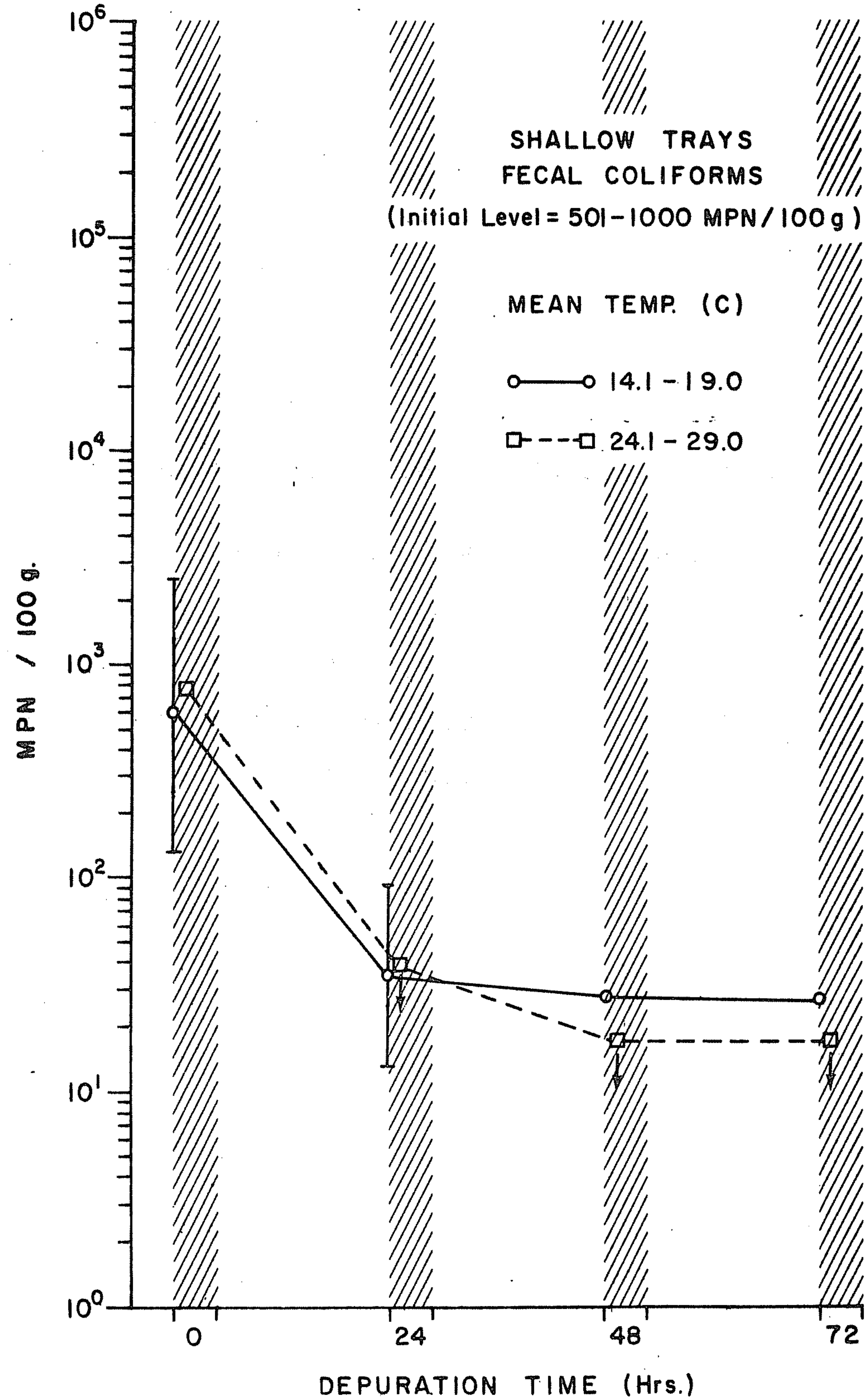

Figure 9. Mean fecal coliform levels in oysters depurated in shallow trays with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 10

Legend	Experiment Numbers
o	16, 270
▲	20-1, 20-2
□	21 (tray 1), 26, 28-1, 28-2, 28-4, 32
×	15-2, 15-3

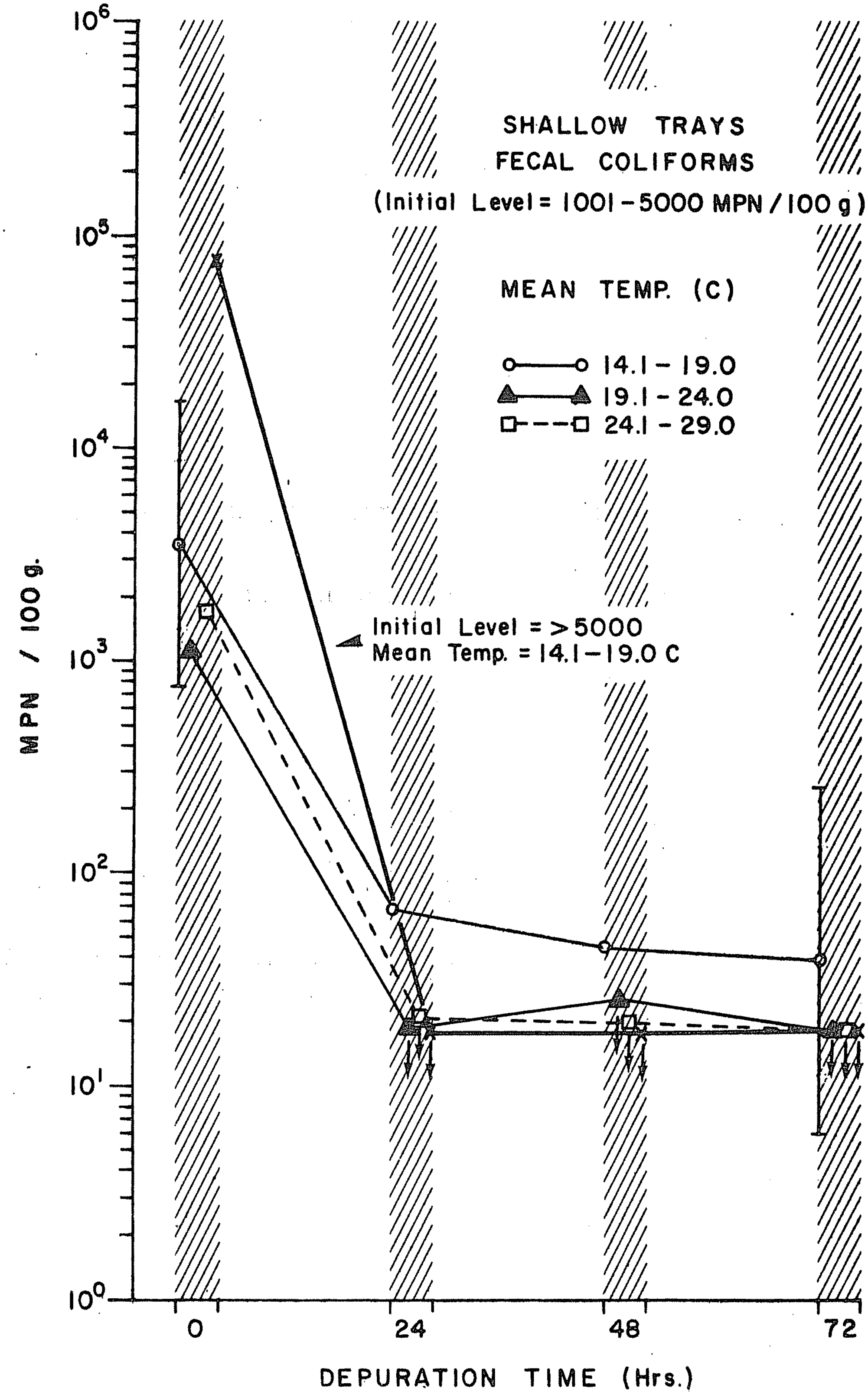

Figure 10. Mean fecal coliform levels in oysters depurated in shallow trays with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 11

Legend	Experiment Numbers
●	28-3
○	30-3
▲	28-4
△	30-4
■	23, 26, 27, 28-1, 28-2, 30-1, 30-2, 31, 32
□	20-1, 20-2, 20-3, 21, 22, 46-1

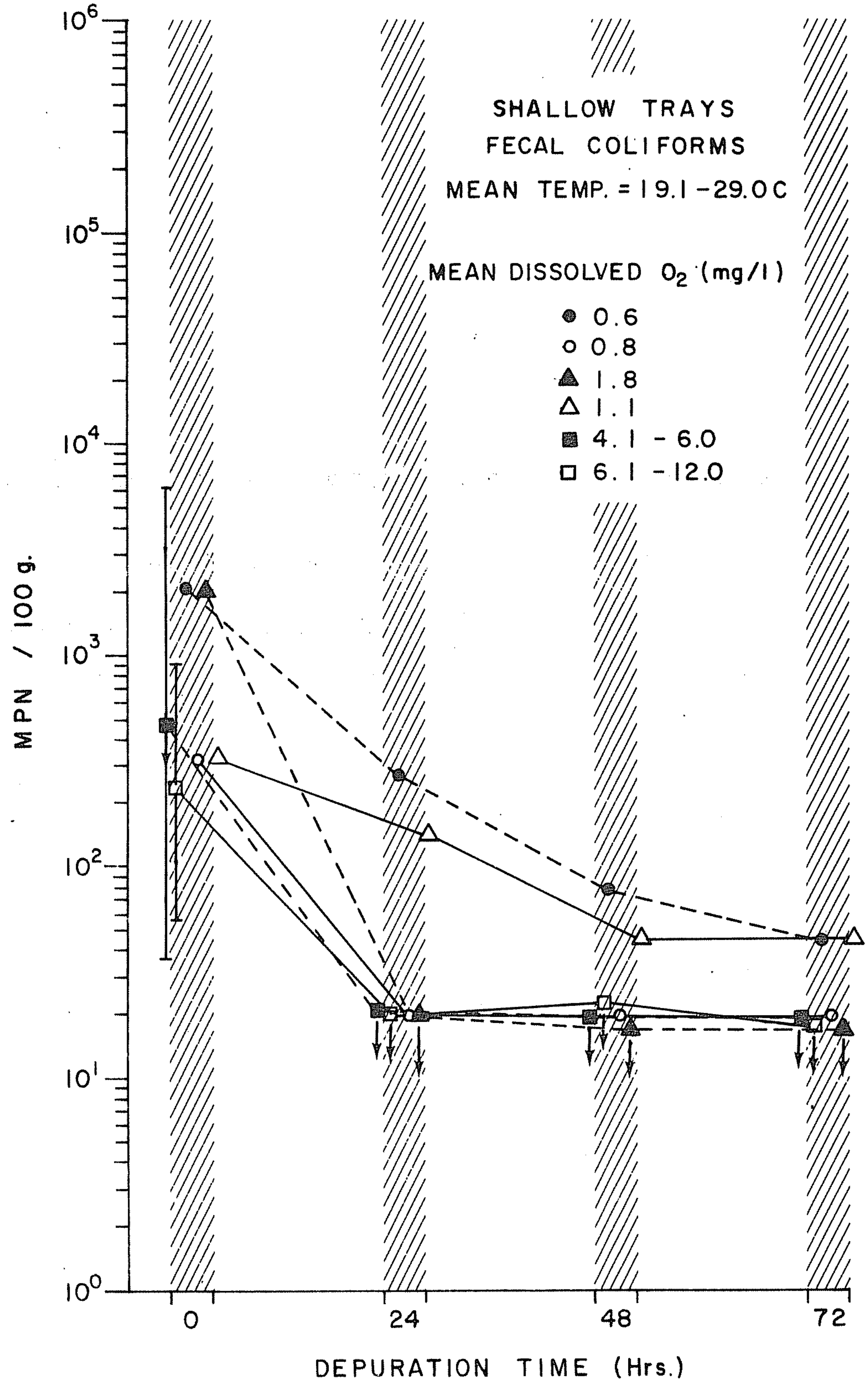

Figure 11. Mean total and fecal coliform levels in oysters depurated in shallow trays with respect to mean dissolved oxygen concentration. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 12

Legend	Experiment Numbers
●	9, 10, 15-2, 15-3, 16, 17-1, 17-2, 17-3
○	18-1, 18-2, 19-1, 19-2

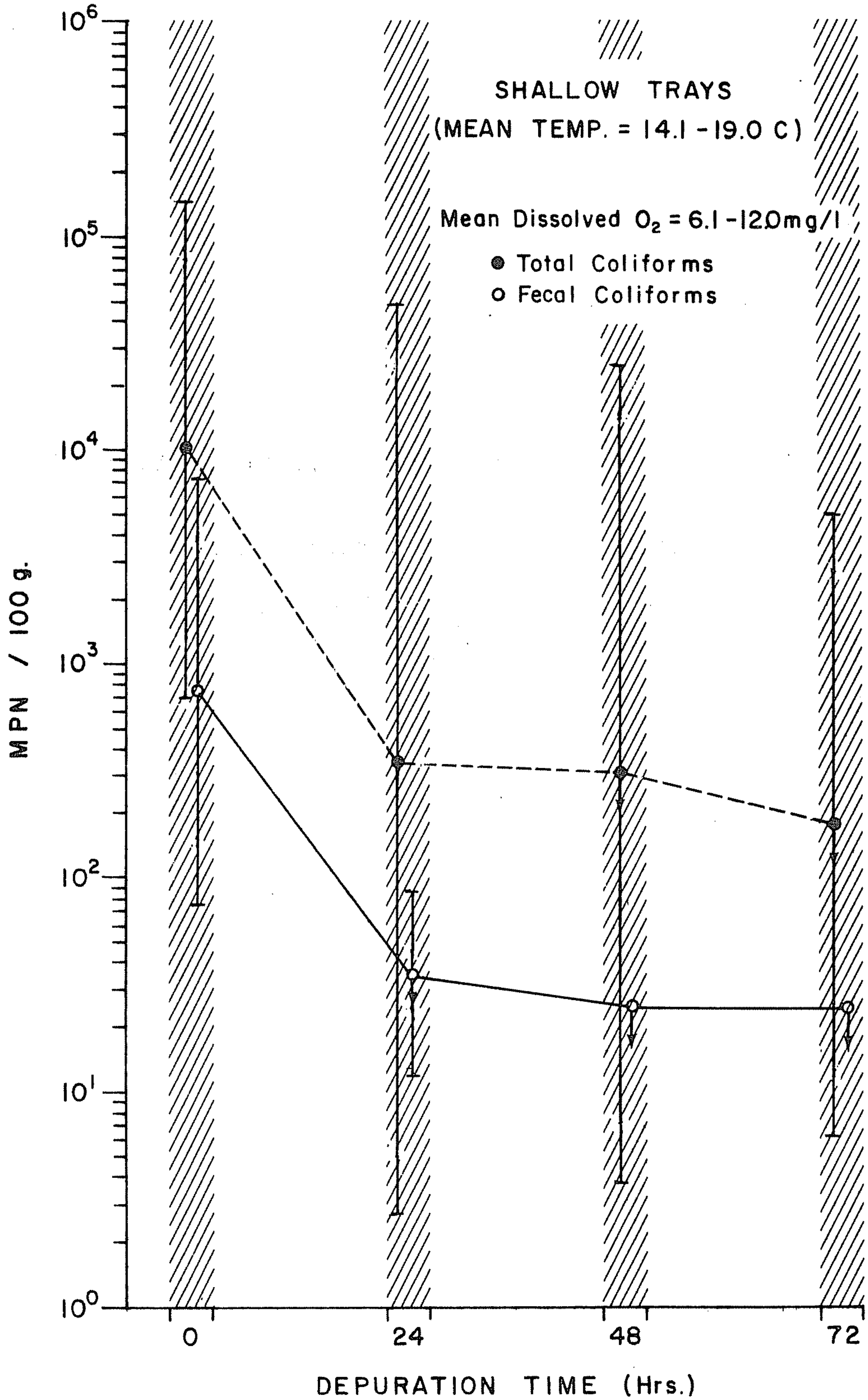

Figure 12. Mean total and fecal coliform levels in oysters depurated in shallow trays with respect to mean dissolved oxygen concentration and temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 13

Legend	Experiment Numbers
●	11, 13-1, 13-2, 13-3
○	

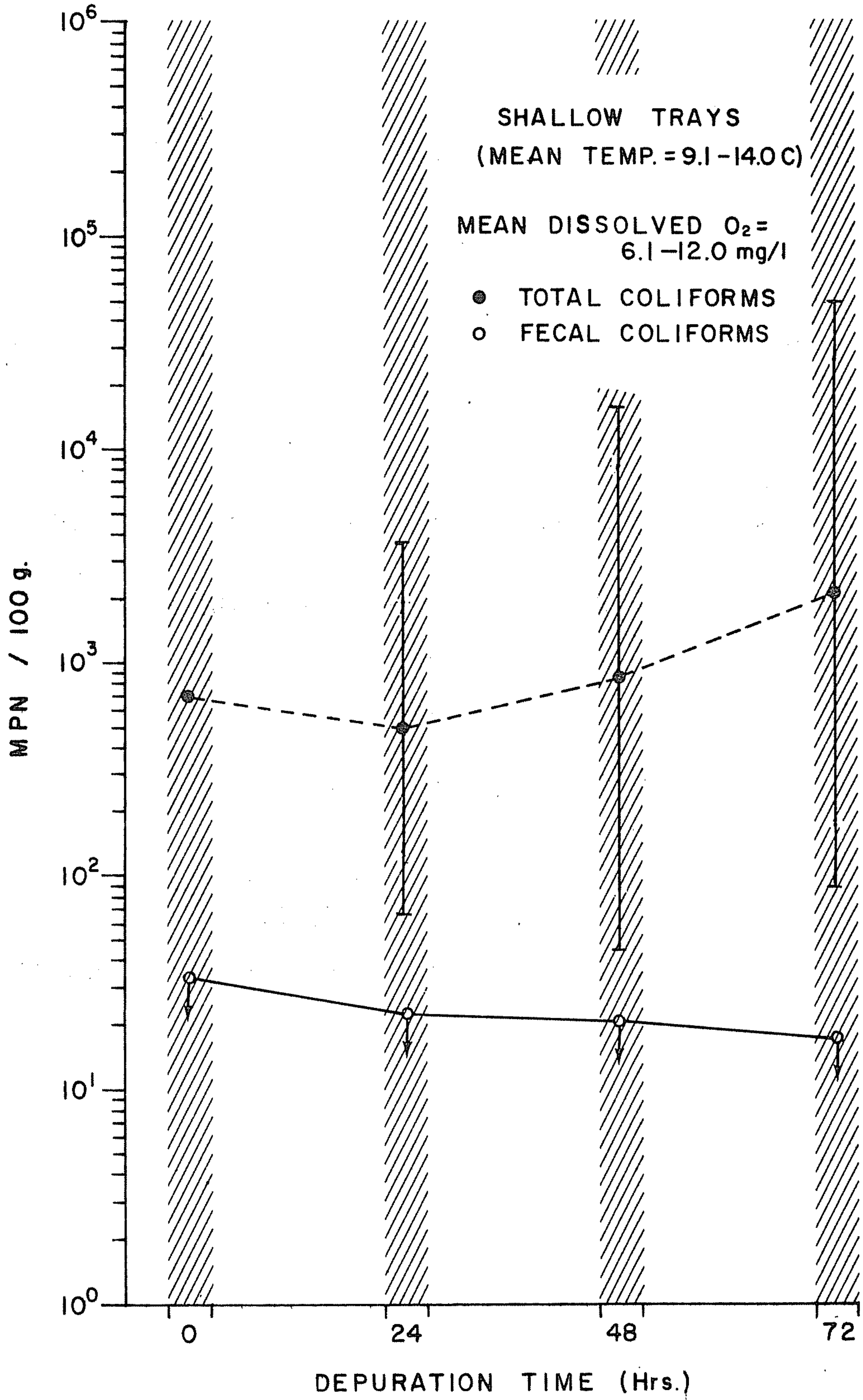

Figure 13. Mean total and fecal coliform levels in oysters depurated in shallow trays with respect to mean dissolved oxygen concentration and temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 15

Legend	Experiment Numbers
●	28-1, 28-2, 28-4, 30-1, 30-2, 30-4
○	19-2, 20-3
▲	11, 31, 32
△	18-1, 19-1, 46-1, 46-2
■	20-1, 20-2, 21, 22, 23, 26, 27
□	16, 18-2, 12, 35, 36

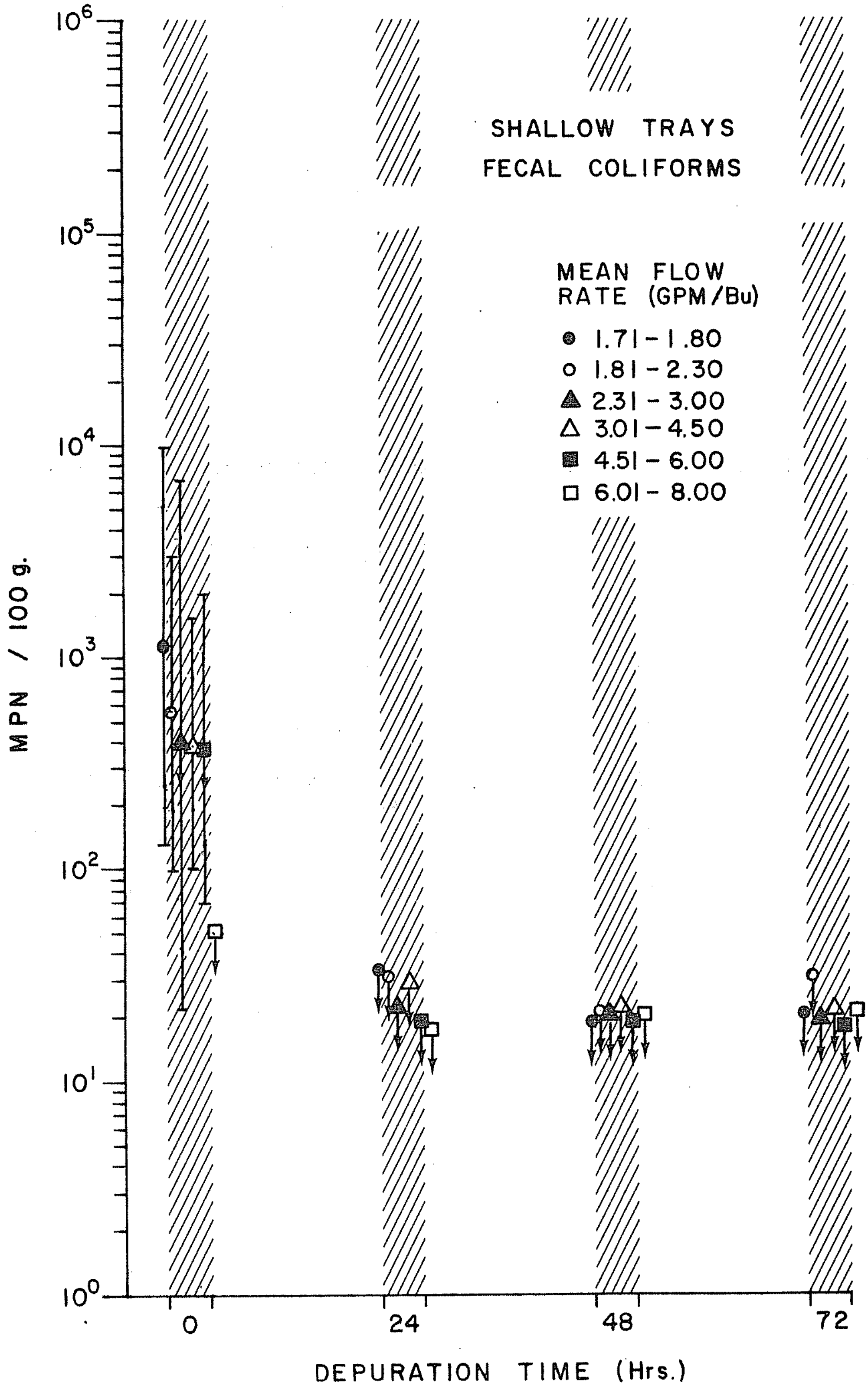

Figure 15. Mean fecal coliform levels in oysters depurated in shallow trays with respect to mean flow rates. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 16

Legend	Experiment Numbers
●	28-1, 28-2, 28-4, 30-1, 30-2, 30-4
○	19-2, 20-3
▲	11, 31, 32
△	18-1, 19-1, 46-1, 46-2
■	20-1, 20-2, 21, 22, 23, 26, 27
□	16, 18-2, 12, 35, 36

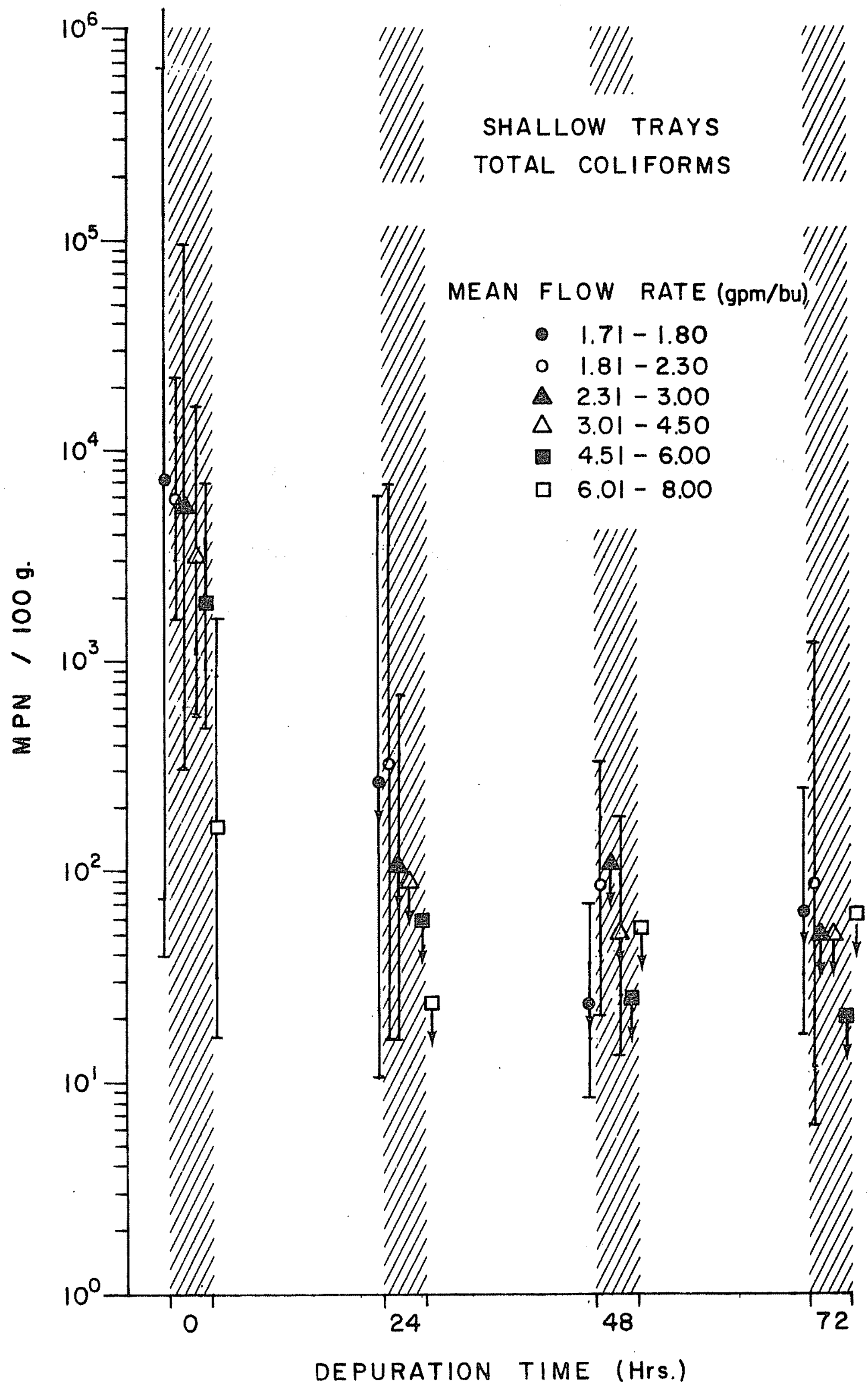

Figure 16. Mean total coliform levels in oysters depurated in shallow trays with respect to mean flow rates. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 17

Legend	Experiment Numbers
●	13-1, 13-3, 46-2

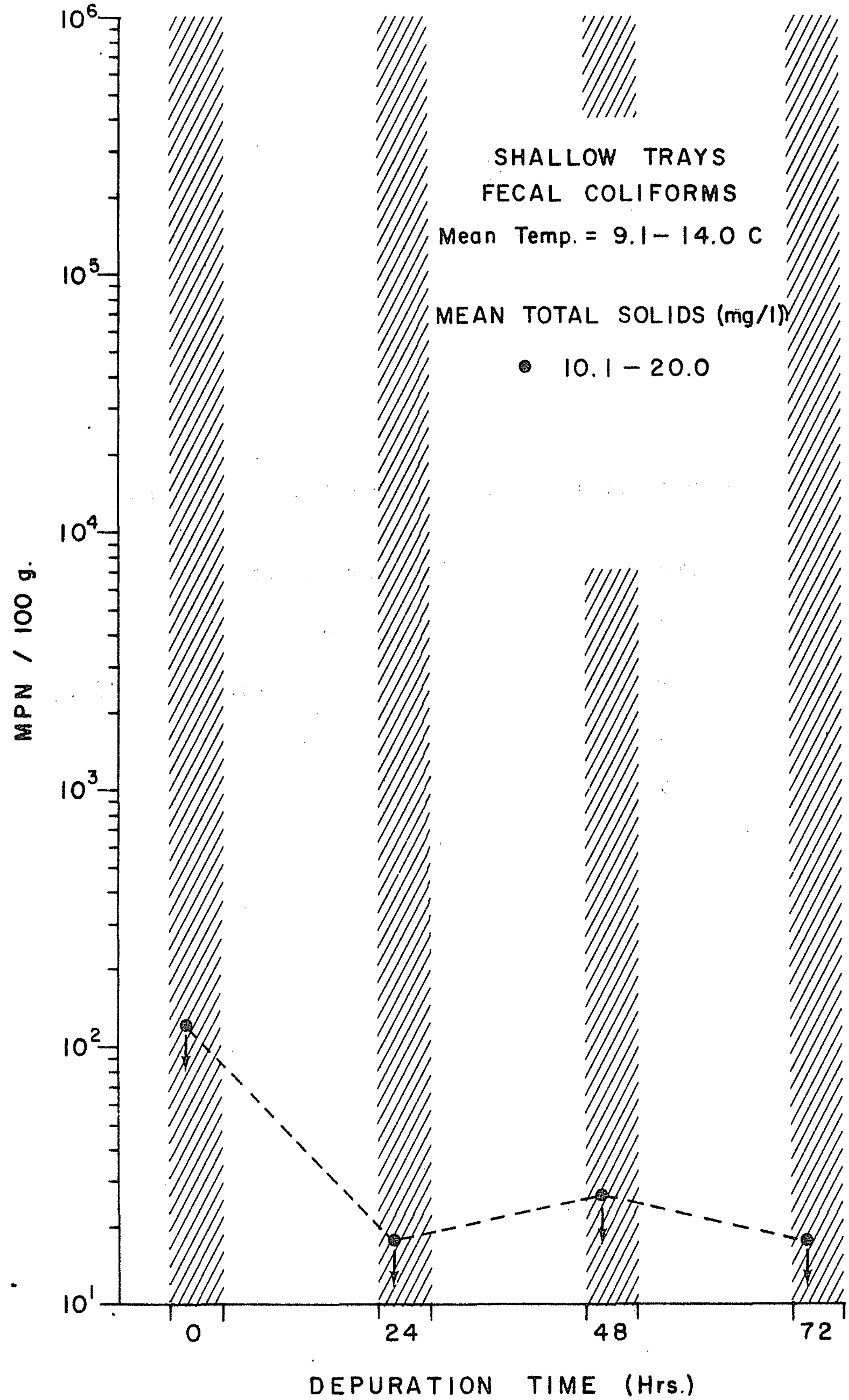

Figure 17. Mean fecal coliform levels in oysters depurated in shallow trays with respect to mean total suspended solids and temperature. Oysters contaminated in nature.

Experiments included in construction of Figure 18

Legend	Experiment Numbers
○	15-3, 270
●	15-2, 16, 17-3, 19-1, 19-2, 35, 36
△	18-1, 18-2
▲	17-1
□	17-2

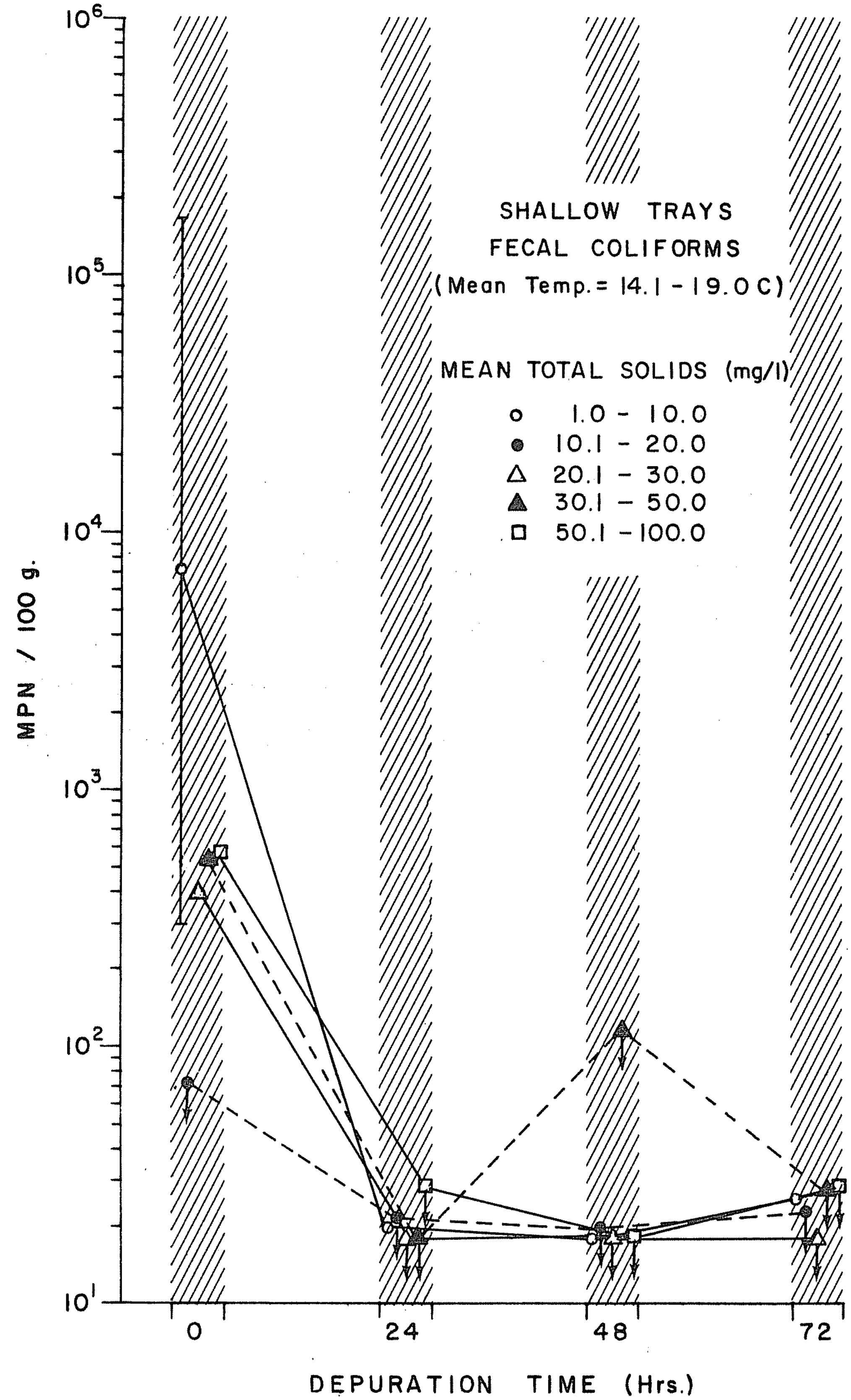

Figure 18. Mean fecal coliform levels in oysters depurated in shallow trays with respect to mean total suspended solids and temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 19

Legend	Experiment Numbers
○	20-1
●	21, 22, 23, 27, 28-1, 28-2, 28-4, 32, 46-1
△	20-2, 20-3, 26, 30-1, 30-2, 30-4, 31

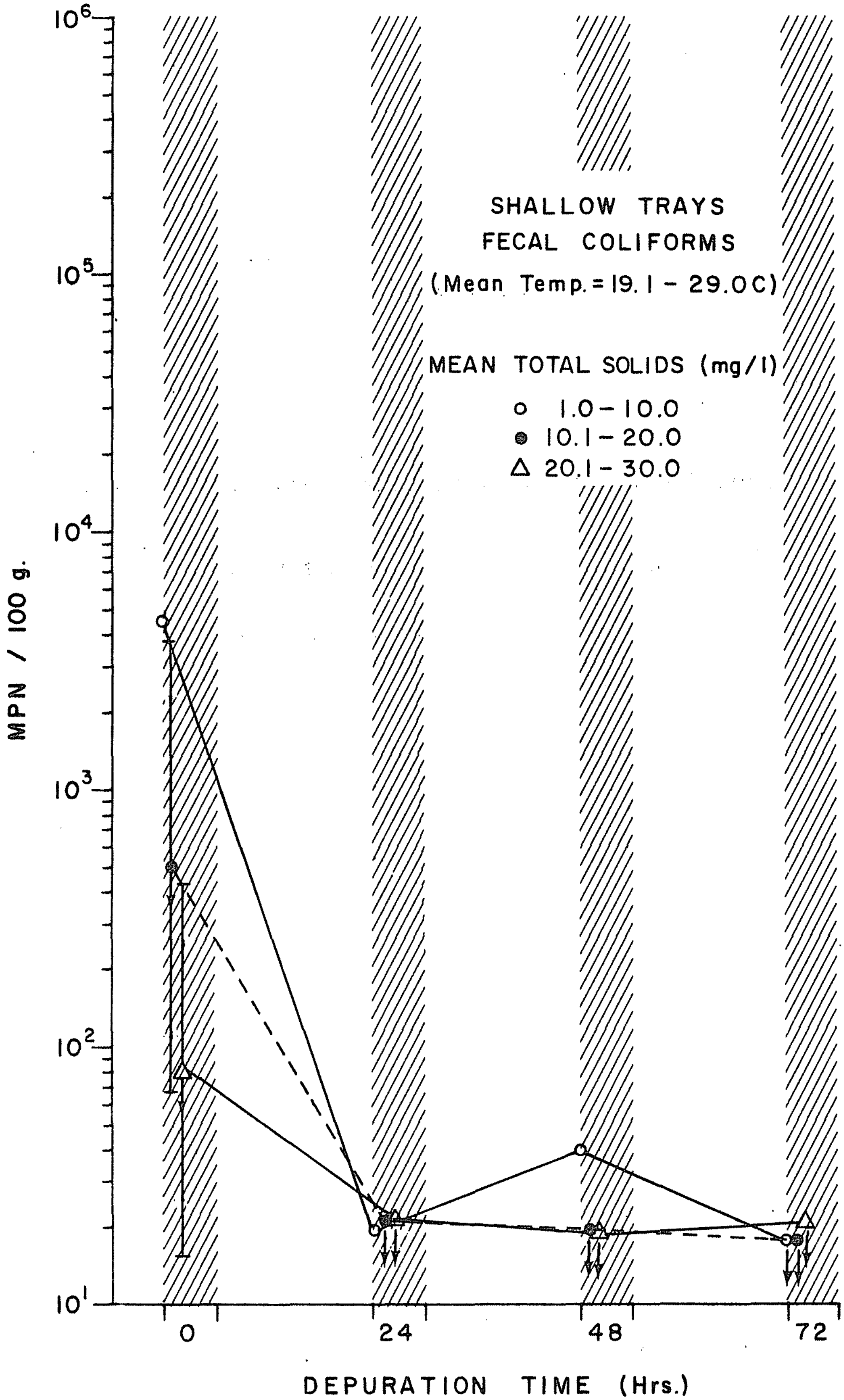

Figure 19. Mean fecal coliform levels in oysters depurated in shallow trays with respect to mean total suspended solids and temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 20

Legend	Experiment Numbers
●	13-1, 13-3, 46-2
□	13-2

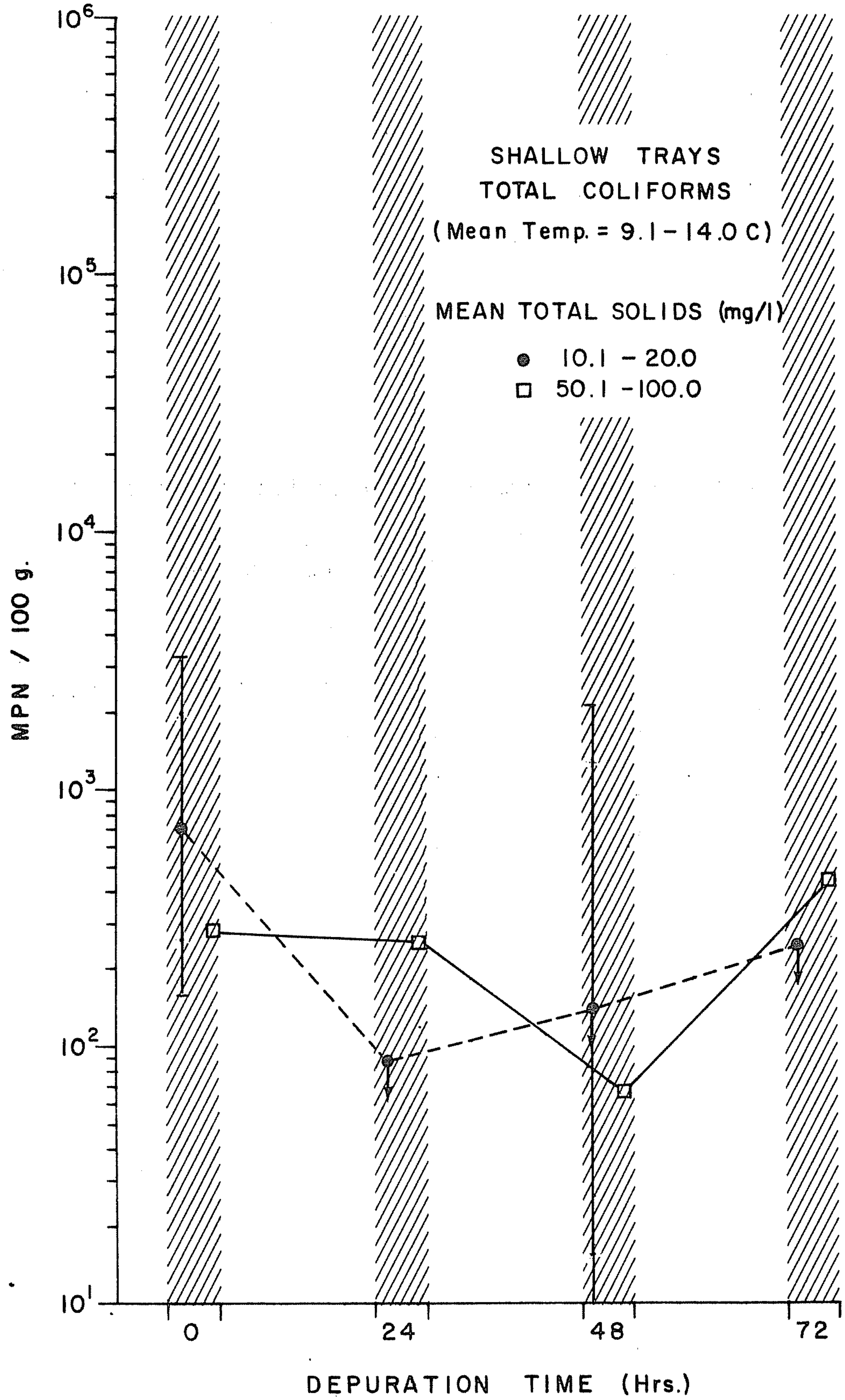

Figure 20. Mean total coliform levels in oysters depurated in shallow trays with respect to mean total suspended solids and temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 21

Legend	Experiment Numbers
○	15-3, 270
●	15-2, 16, 17-3, 19-1, 19-2, 25, 36
△	18-1, 18-2
▲	17-1
□	17-2

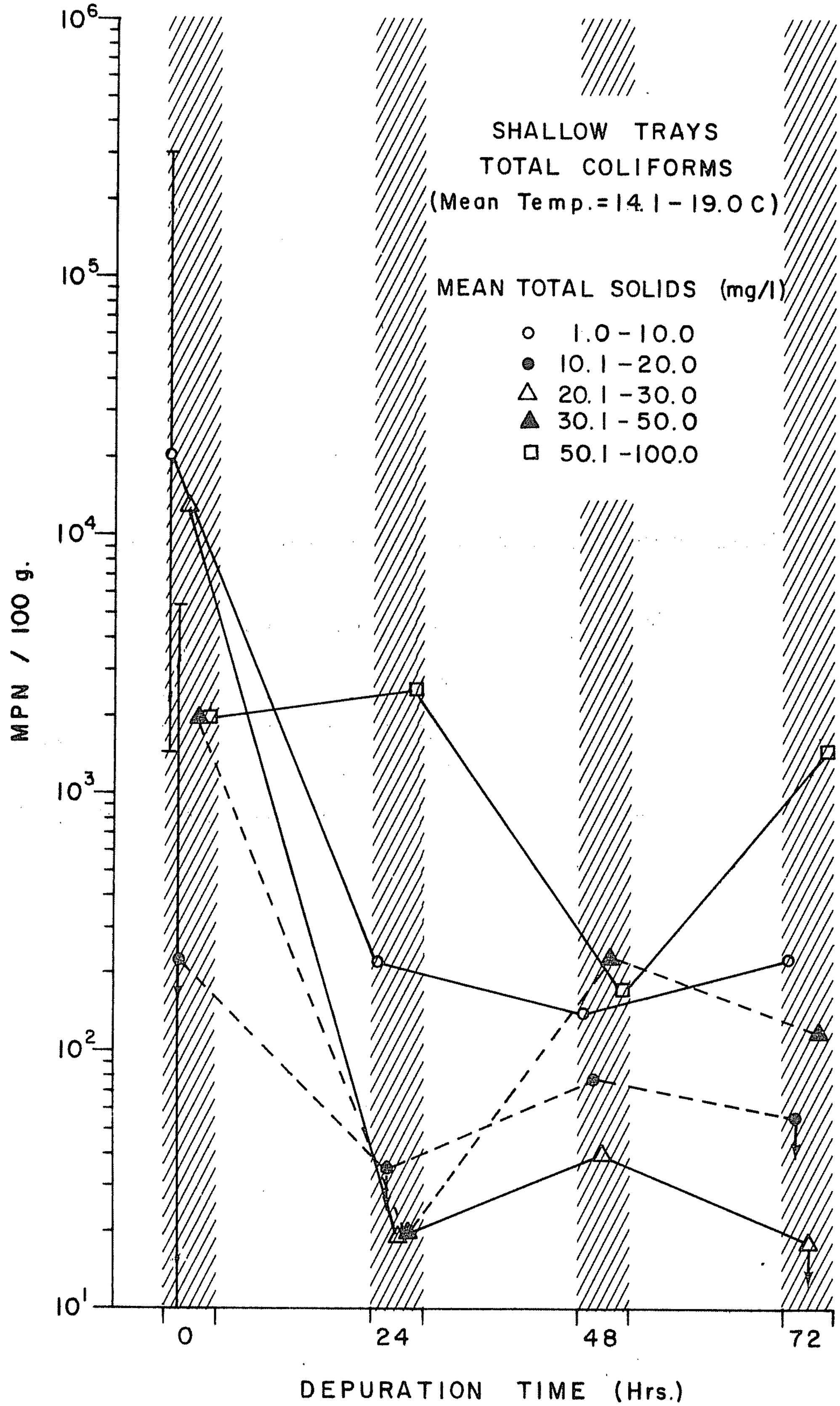

Figure 21. Mean total coliform levels in oysters depurated in shallow trays with respect to mean total suspended solids and temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 22

Legend	Experiment Numbers
○	20-1
●	21, 22, 23, 27, 28-1, 28-2, 28-4, 32, 46-1
△	20-2, 20-3, 26, 30-1, 30-2, 30-4, 31

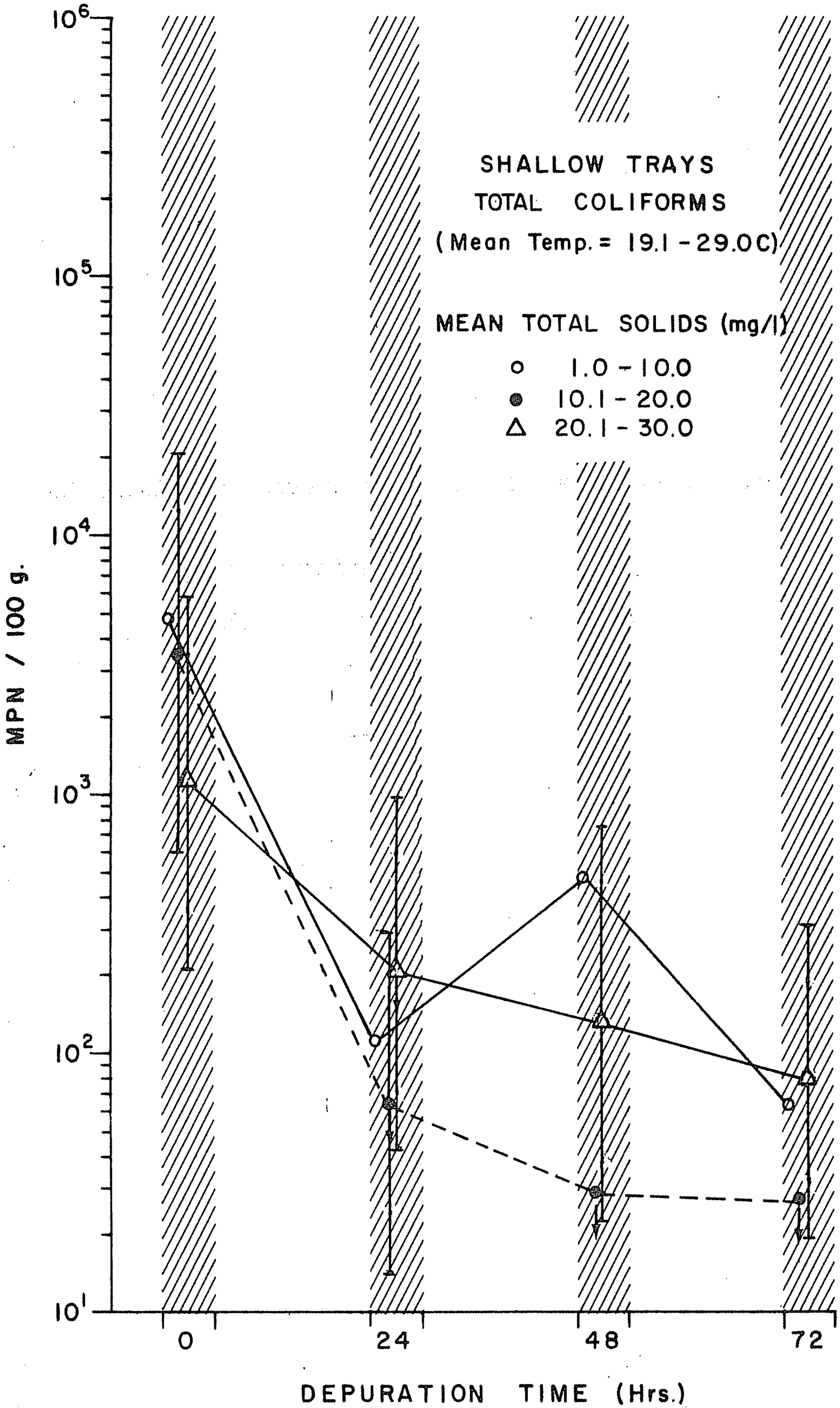

Figure 22. Mean total coliform levels in oysters depurated in shallow trays with respect to mean total suspended solids and temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 23

Legend	Experiment Numbers
■	2, 22, 23, 31, 32, 36
△	22, 32
●	2, 23
o	36

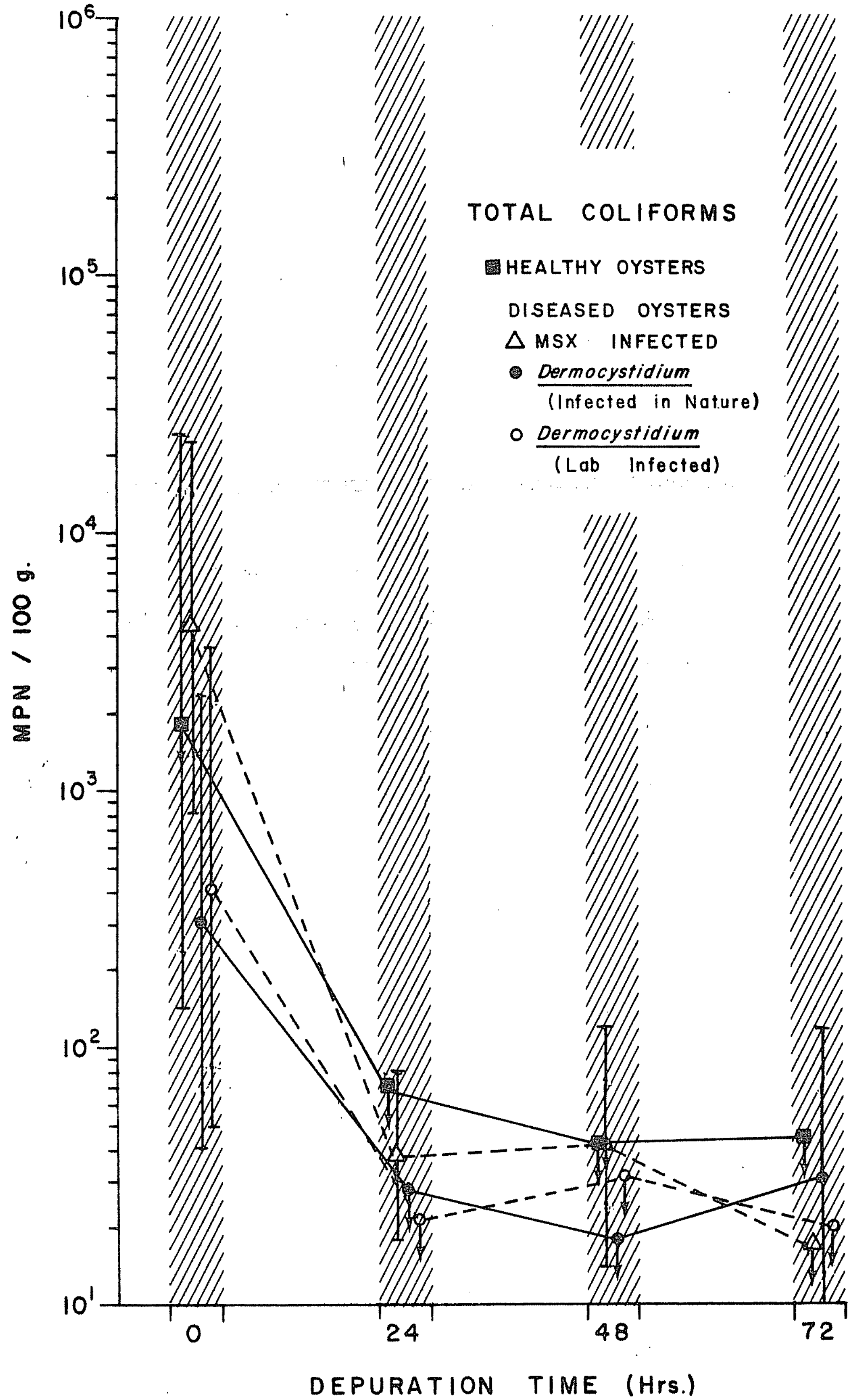

Figure 23. Mean total coliform levels in infected oysters depurated in shallow trays. Oysters were infected with Dermocystidium marinum and Minchinia nelsoni (MSX). Oysters contaminated in nature.

Experiments included in construction of Figure 24

Legend	Experiment Numbers
■	2, 22, 23, 31, 32, 26
△	22, 32
●	2, 23
○	36

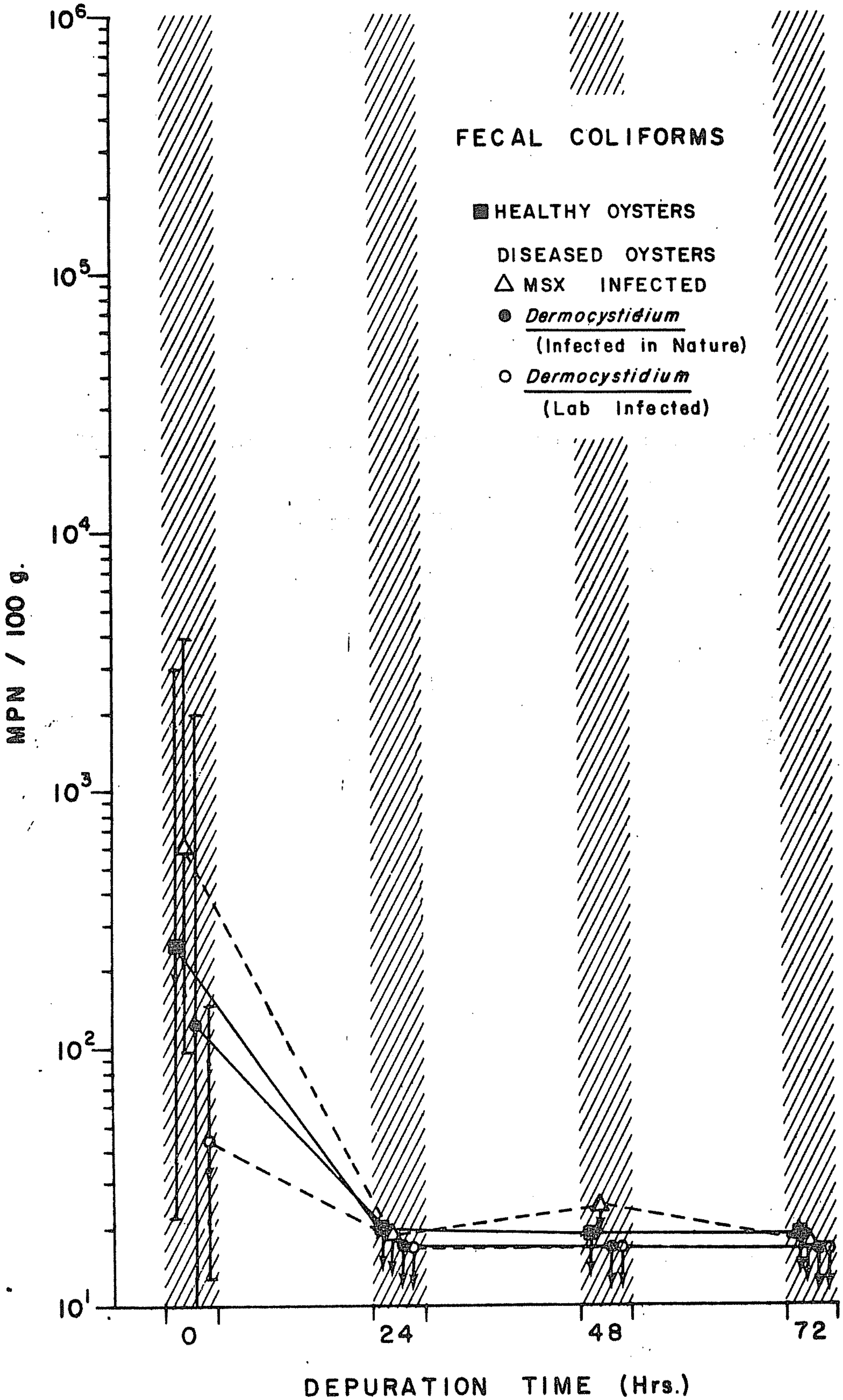

Figure 24. Mean fecal coliform levels in infected oysters depurated in shallow trays. Oysters were infected with Dermocystidium marinum and Minchinia nelsoni (MSX). Oysters contaminated in nature.

Experiments included in construction of Figure 25

Legend	Experiment Numbers
■	40
o	40
△	40

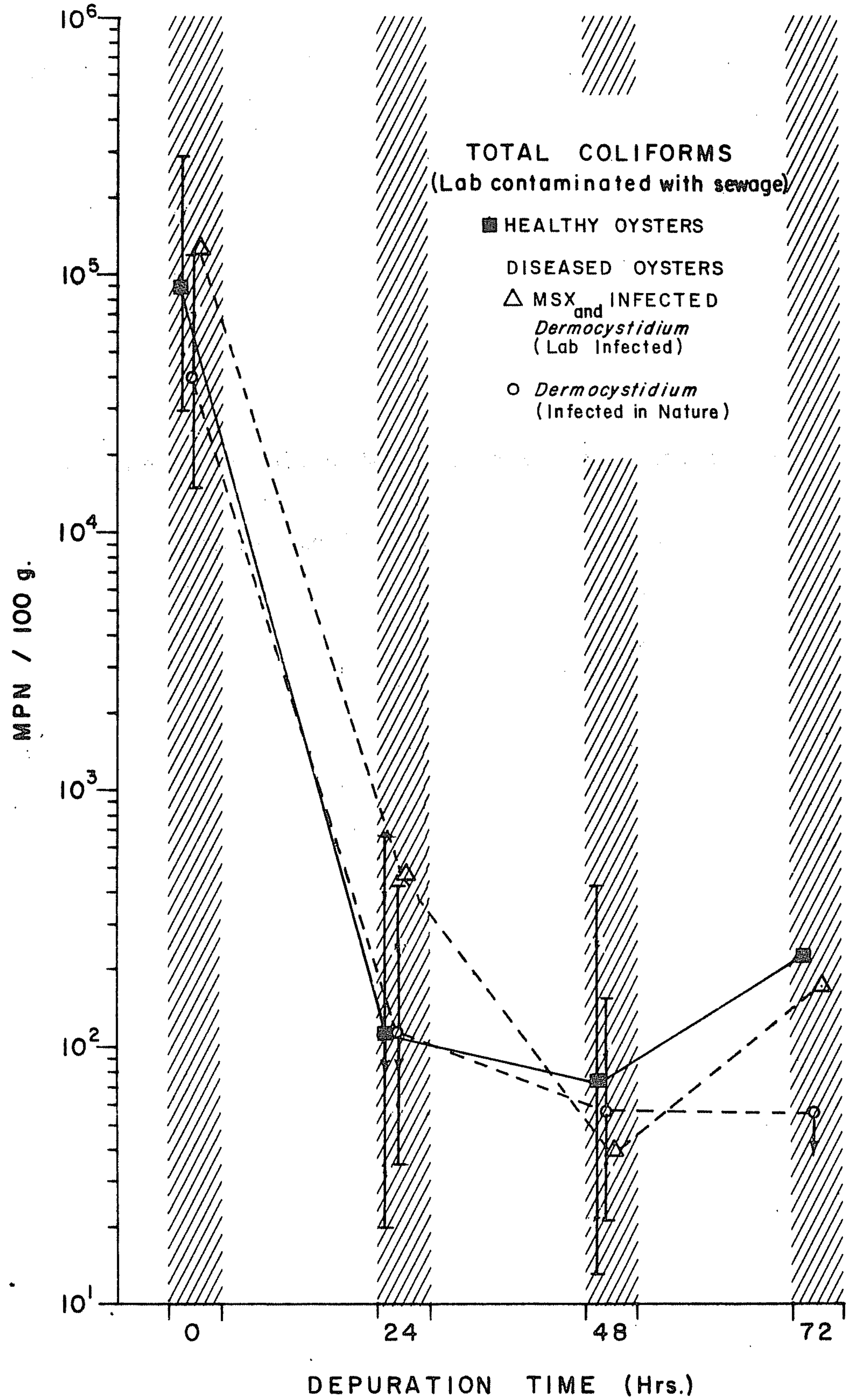

Figure 25. Mean total coliform levels in oysters depurated in shallow trays with respect to infection with Dermocystidium marinum and Minchinia nelsoni (MSX). Oysters contaminated with raw sewage.

Experiments included in construction of Figure 26

Legend	Experiment Numbers
■	40
o	40
△	40

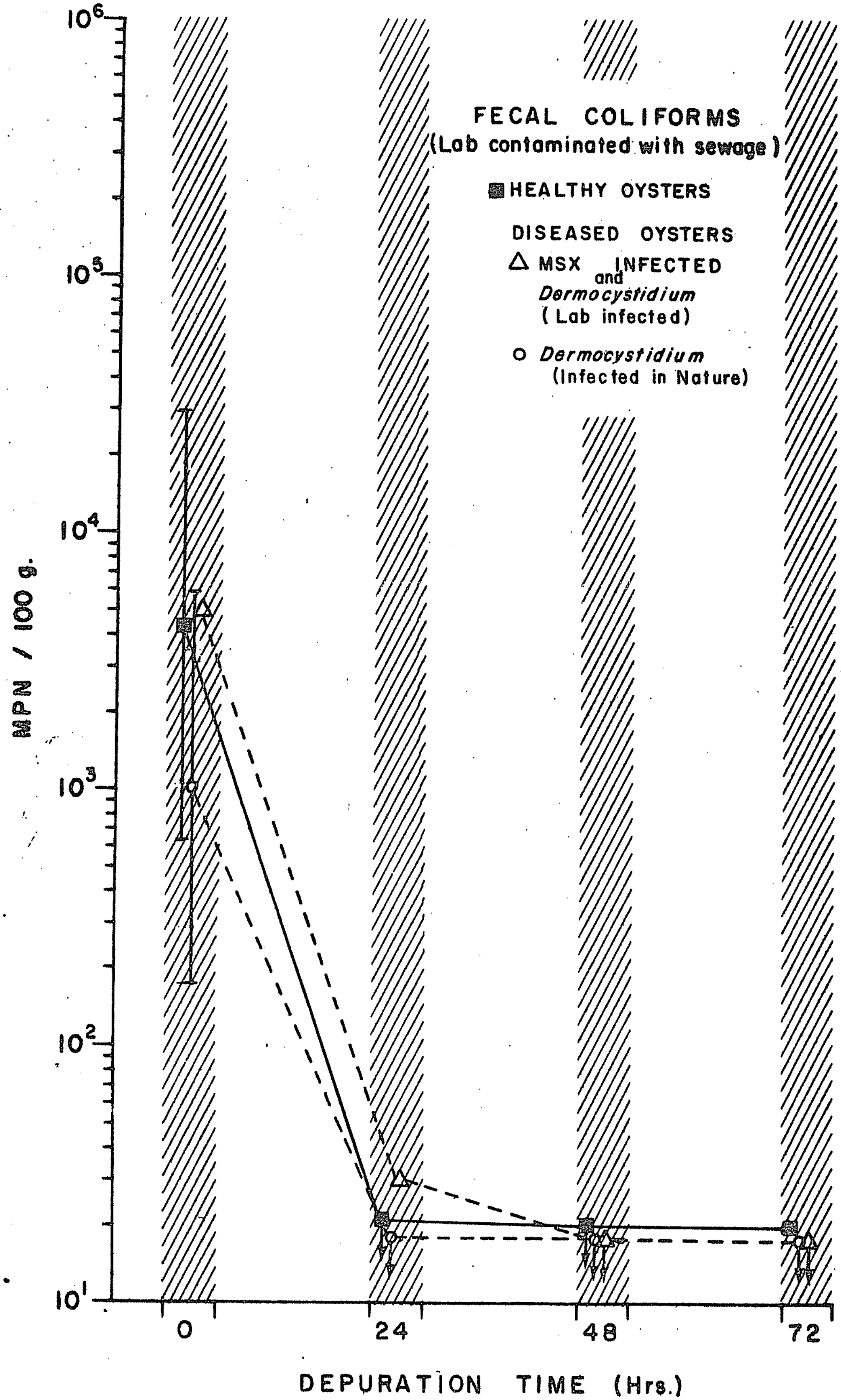

Figure 26. Mean fecal coliform levels in oysters depurated in shallow trays with respect to infection with Dermocystidium marinum and Minchinia nelsoni (MSX). Oysters contaminated with raw sewage.

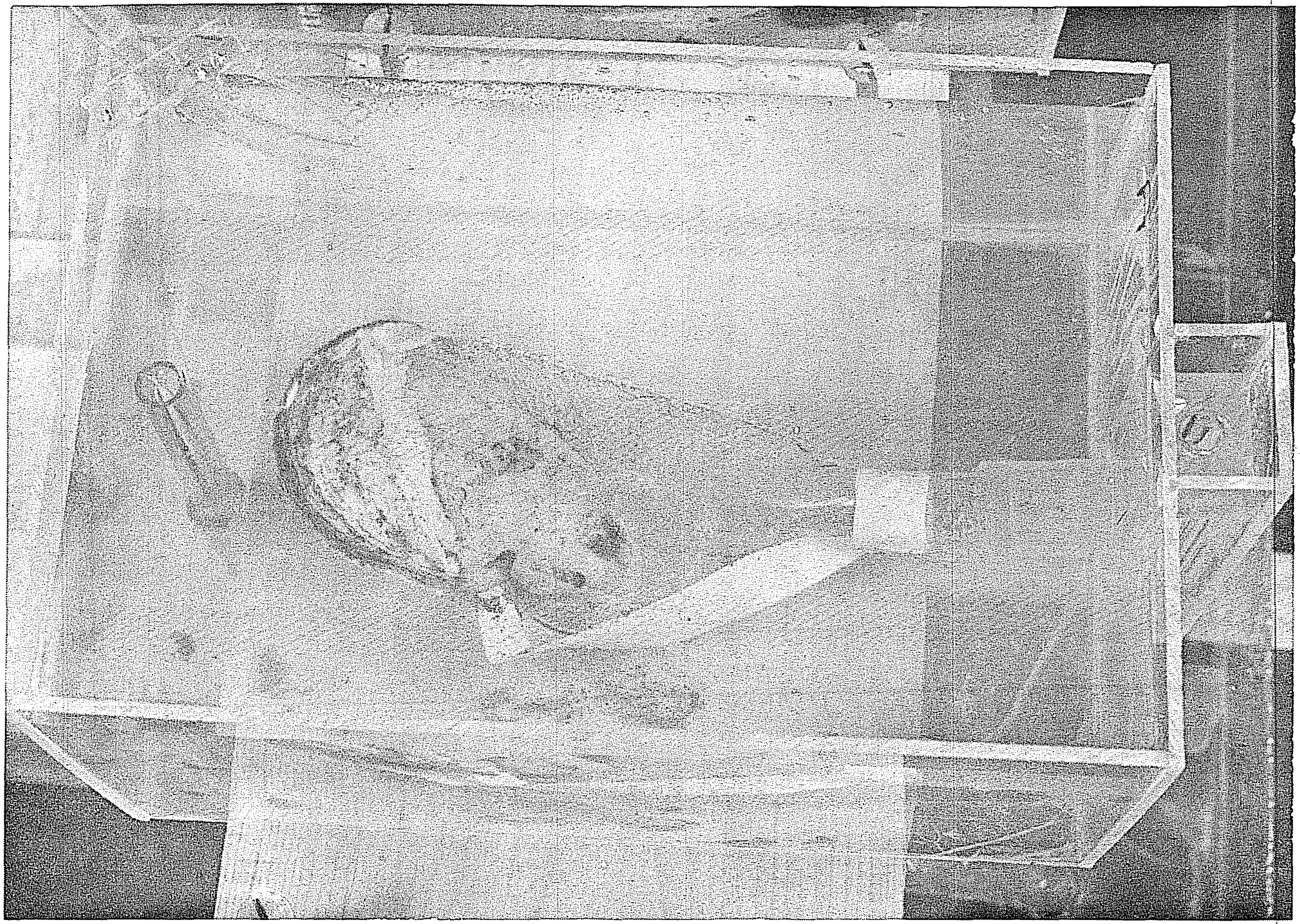

Figure 27. Oyster with plastic funnel attachment in aquarium used in pumping rate experiments.

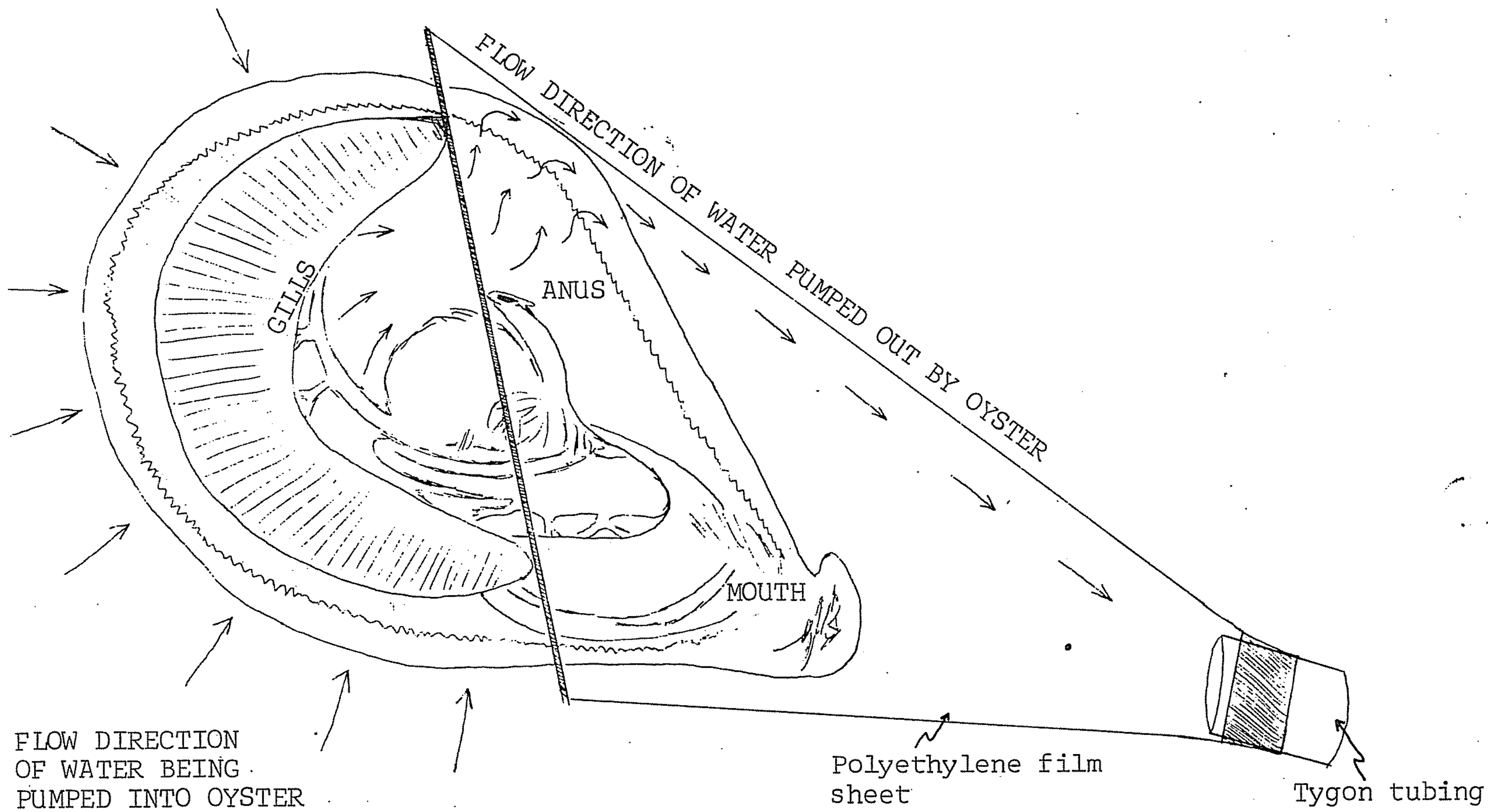

Figure 28. Diagram showing the areas of an oyster covered by the plastic bag used in measurement of pumping rates.

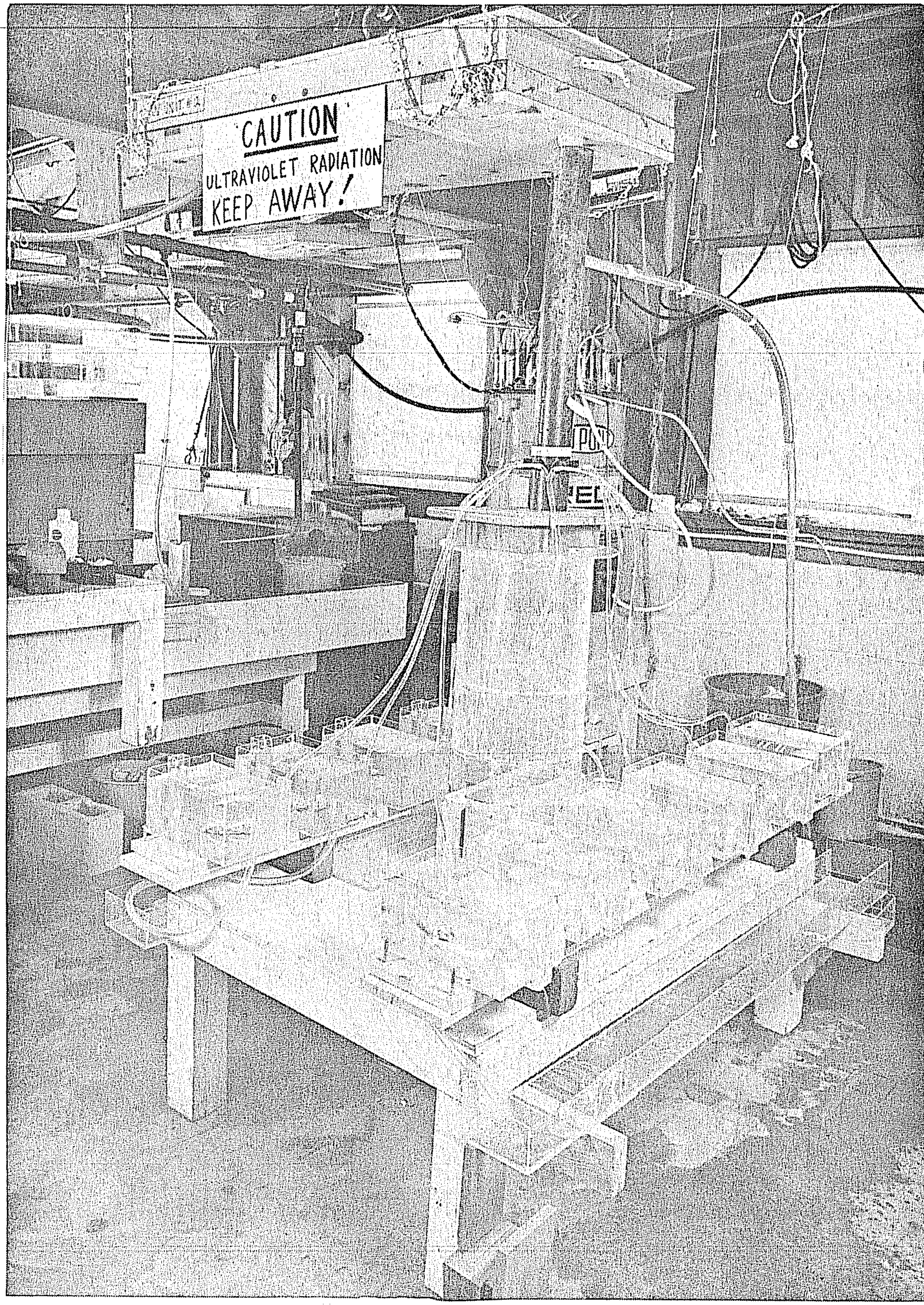

Figure 29. Arrangement of ultraviolet lamp unit and aquaria used in pumping rate experiments.

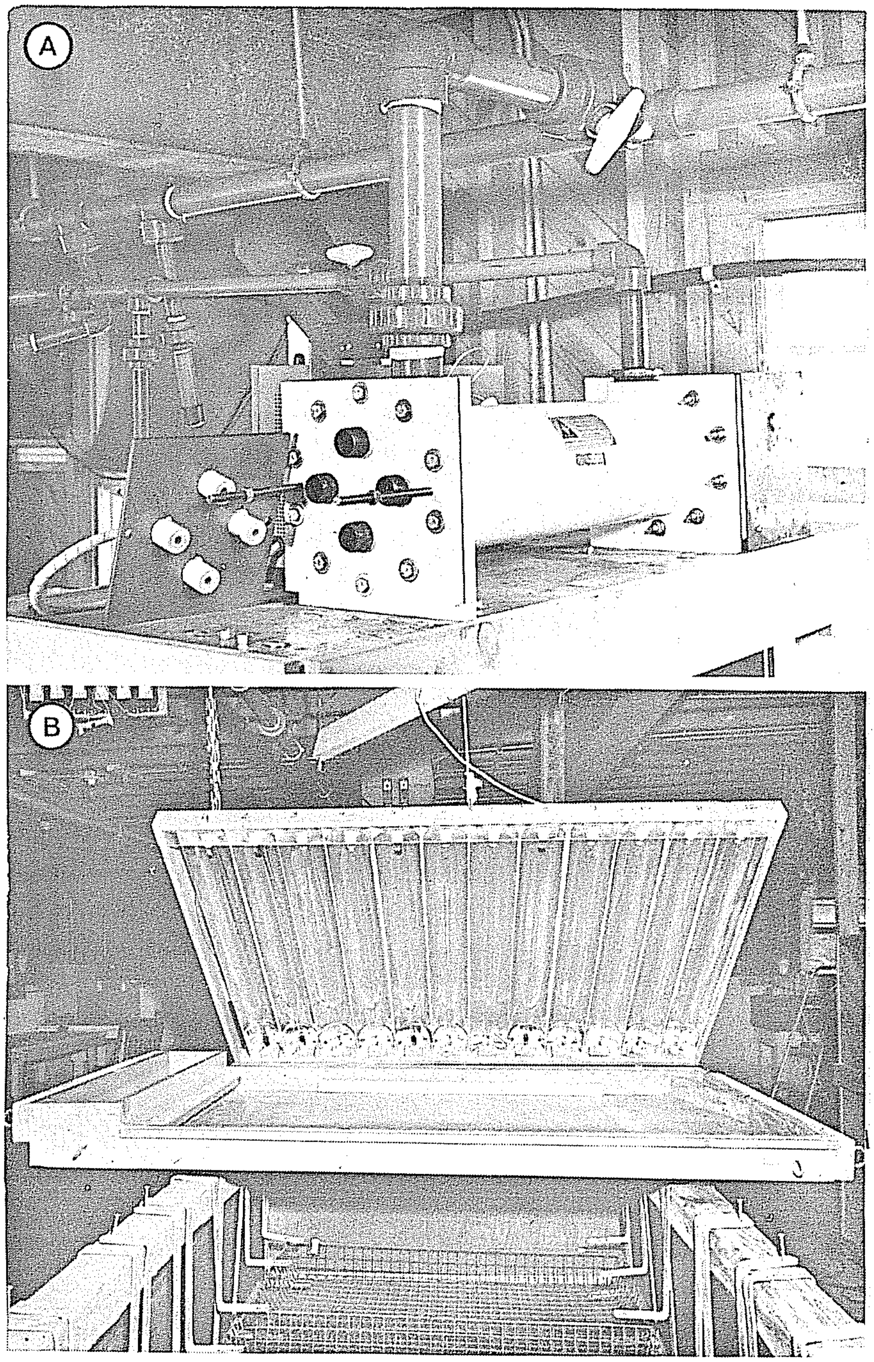

Figure 30. Ultraviolet lamp units used in experiments with commercial-size tanks. (A) 4-lamp commercial-type unit used with 2 x 8 and 2 x 4 tanks. (B) 12-lamp Kelly-Purdy type unit used with 4 x 8 tank and flumes.

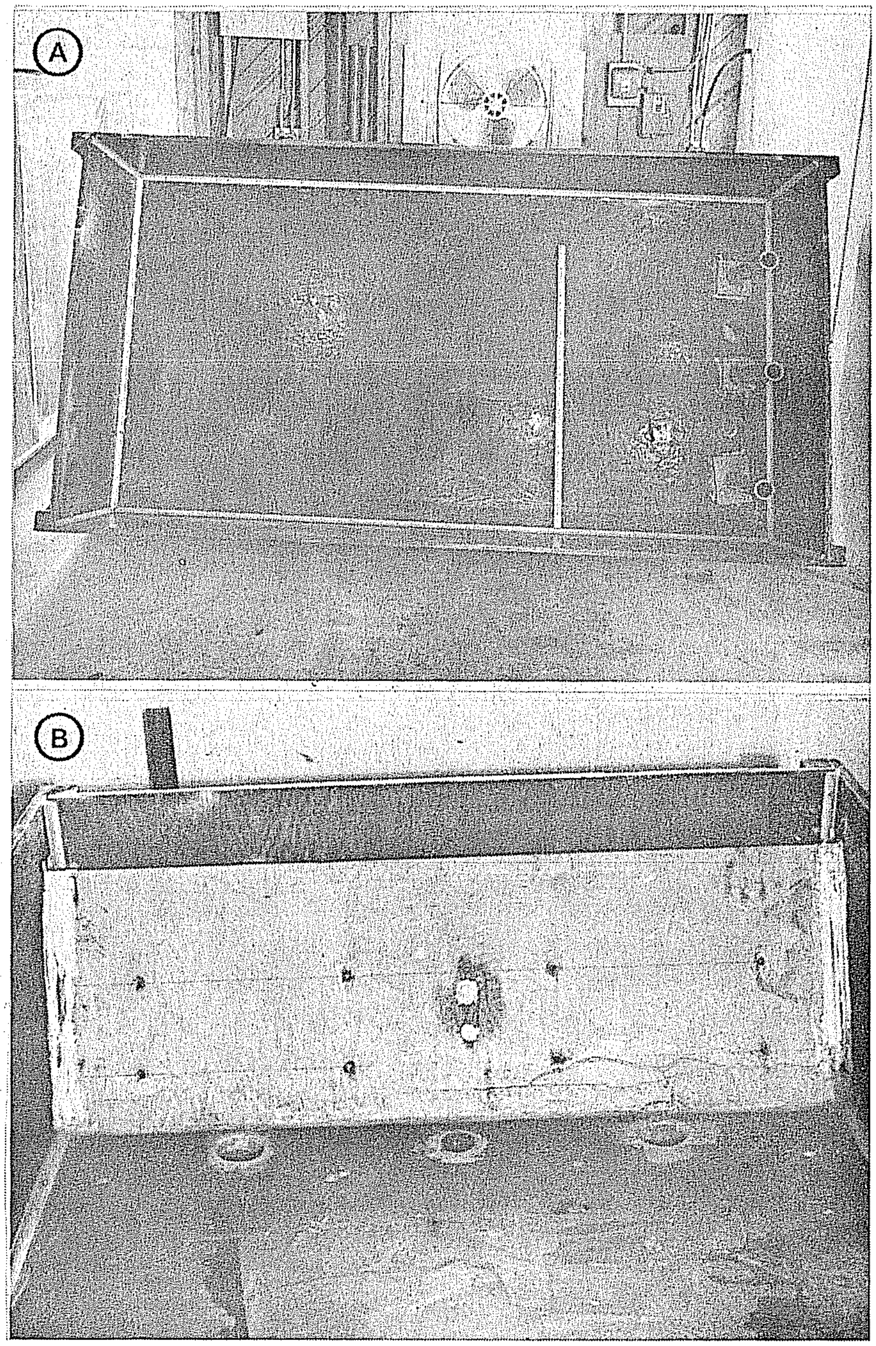

Figure 31. 4 x 8 tank. (A) With three standpipes for water overflow. (B) Drain end of tank showing slanted overflow baffle with eight holes.

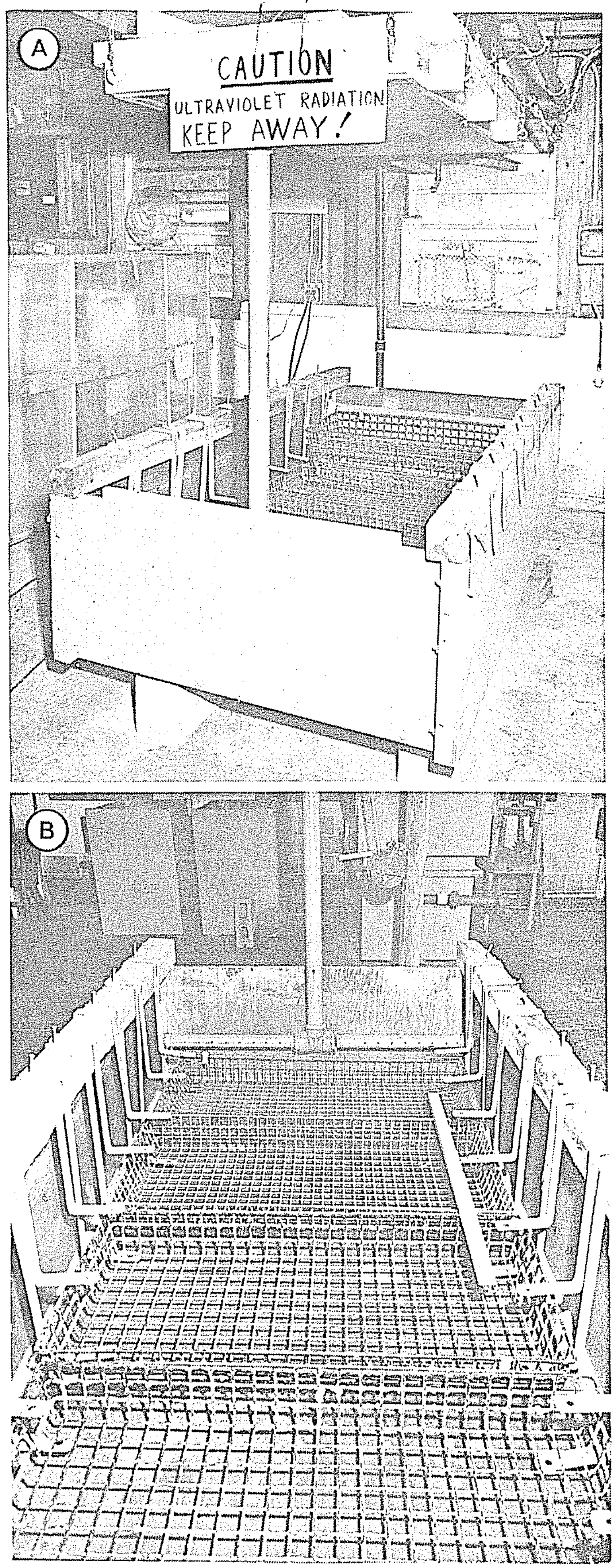

Figure 32. 4 x 8 tank. (A) Arrangement of ultraviolet lamp unit and tank. (B) Arrangement of trays, tray supports and water-inflow pipe.

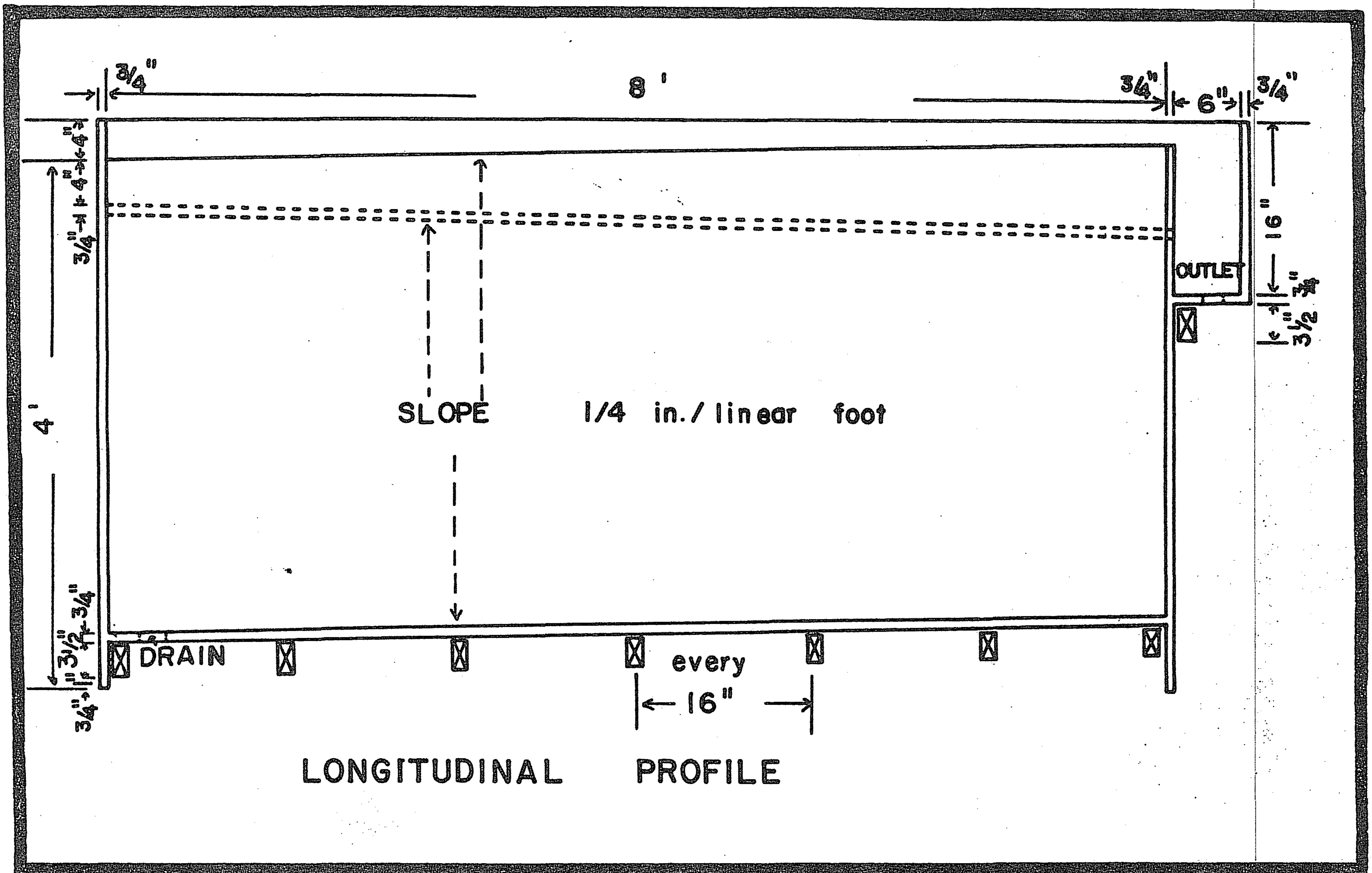

Figure 33D. Diagram of longitudal profile of 2 x 8 tank.

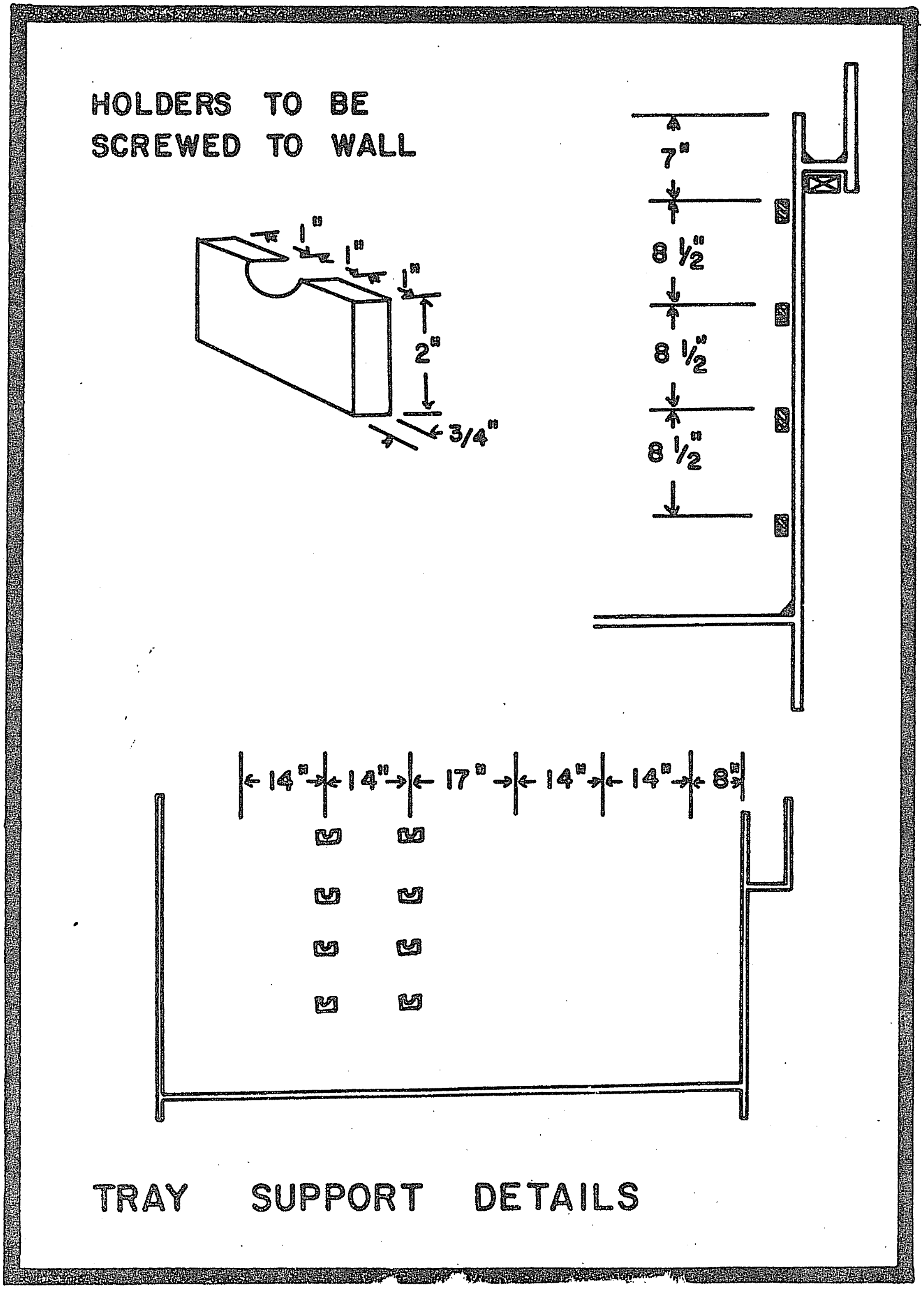

Figure 33E. Diagram of tray support details in 2 x 8 tank.

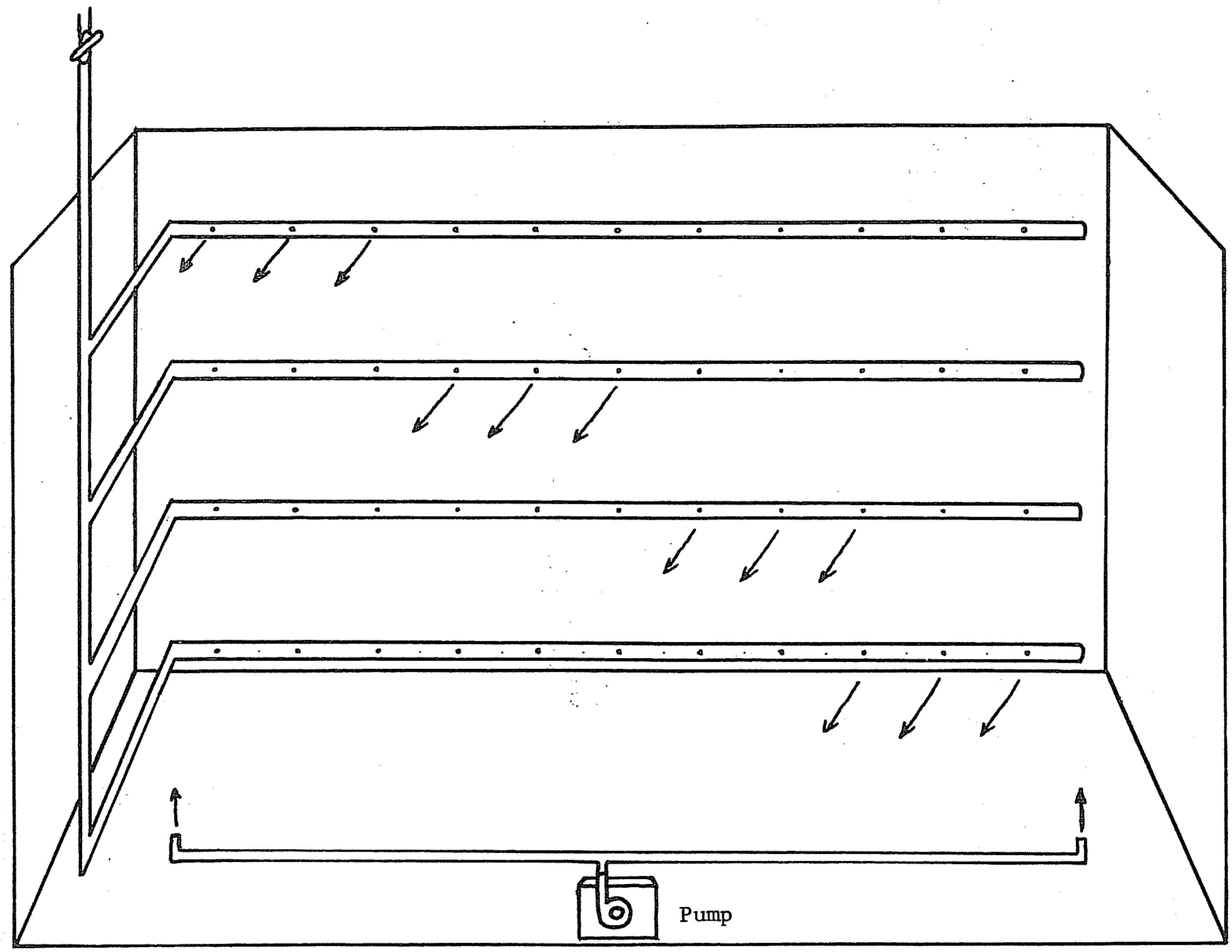

Figure 33F

Diagram of 2 X 8 tank with one side wall removed. Inflowing water pipes are arranged against one side wall and small pump with pipe arms is set against opposite wall. Flow from holes drilled in wall pipes is directed parallel to the bottom. Flow from ends of pump pipe arms is directed parallel to side wall.

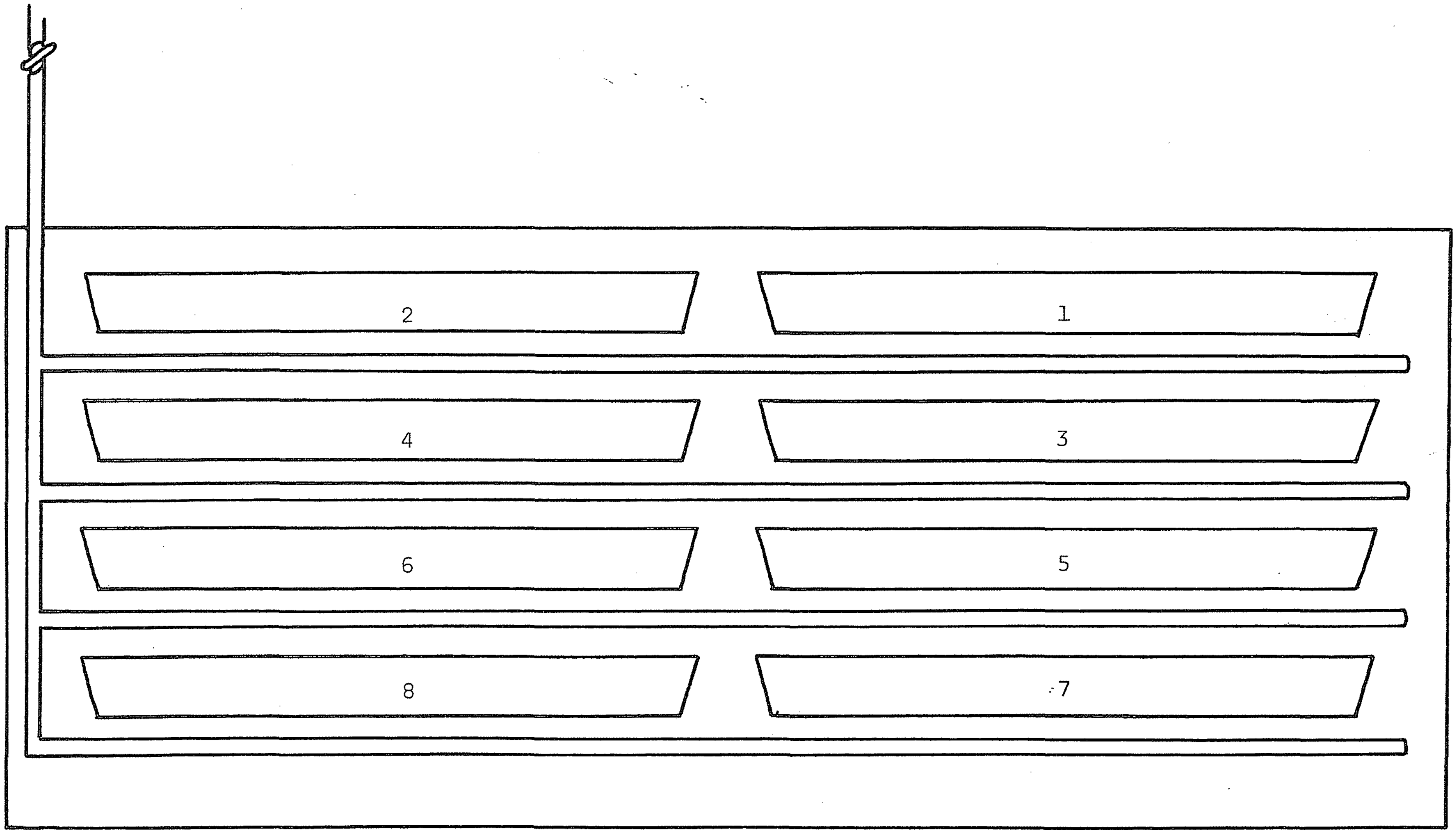

Figure 33G

Diagram of 2 X 8 tank showing arrangement of trays and water pipes.

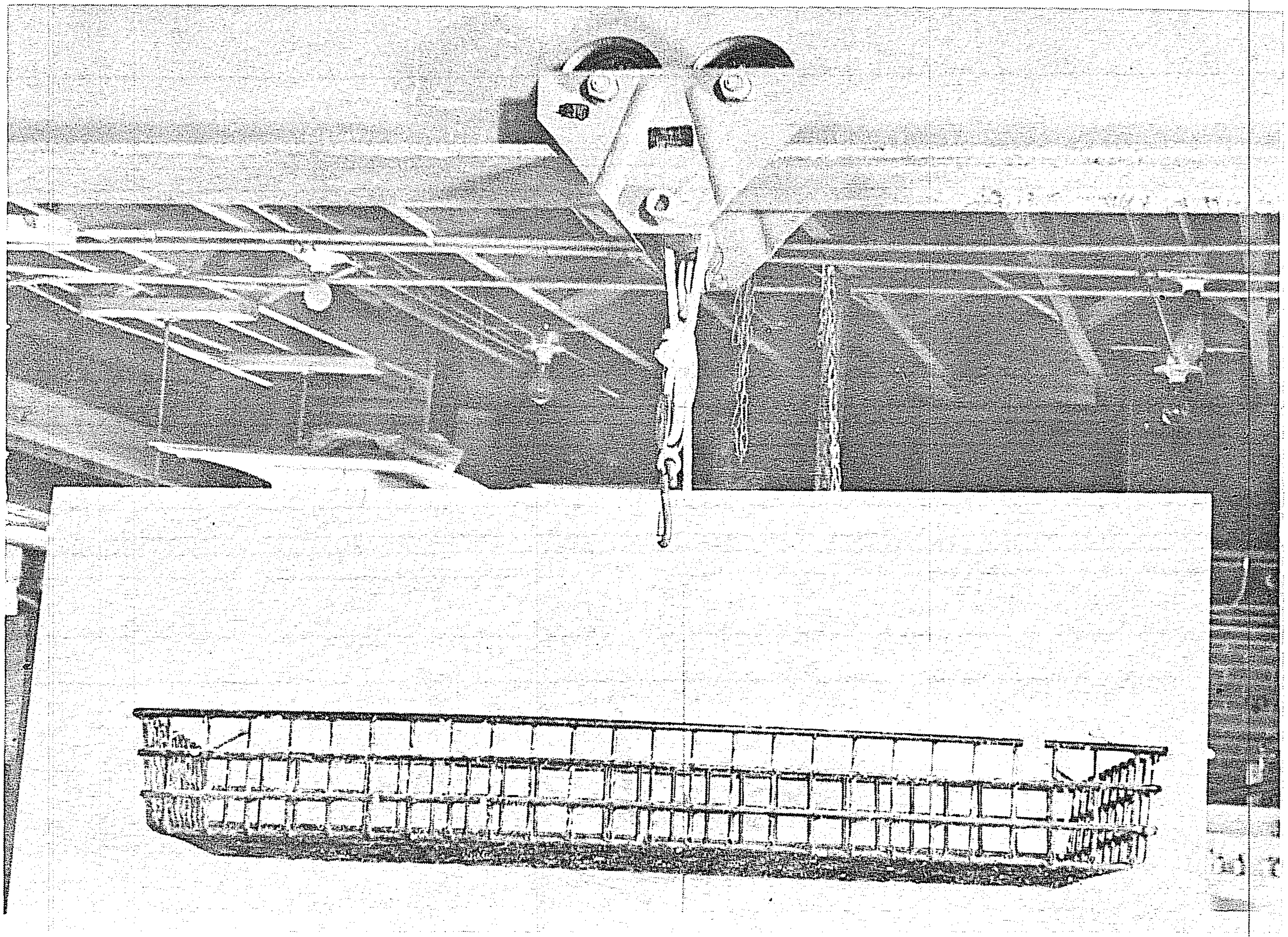

Figure 33H. Hoist used to lift trays in and out of 2 x 8 tank.

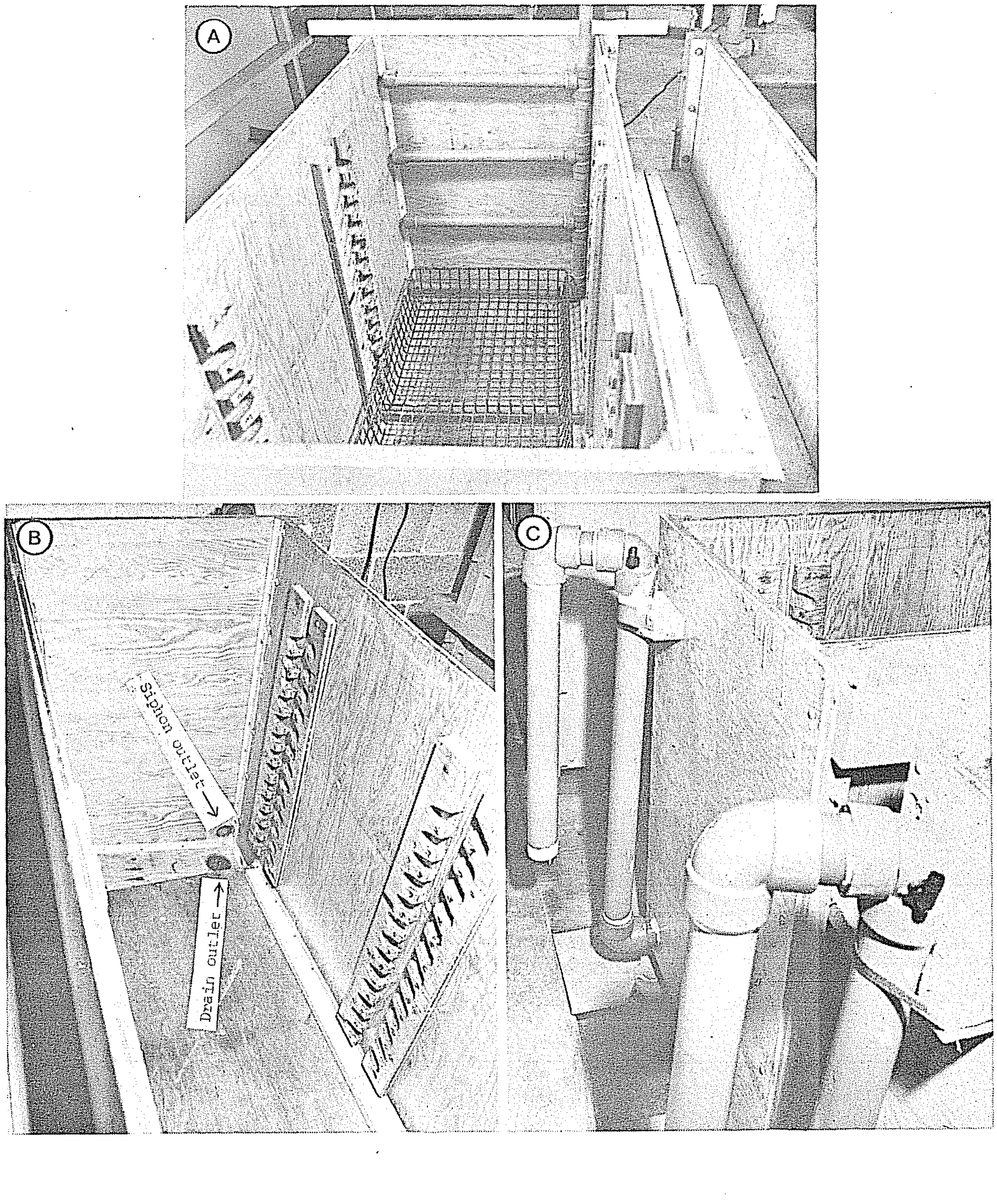

Figure 34. 2 x 4 tank. (A) Arrangement of tray and supports and inflowing water pipes. (B) Drain end showing water outlet holes. (C) Outside view of drain end showing siphon pipes.

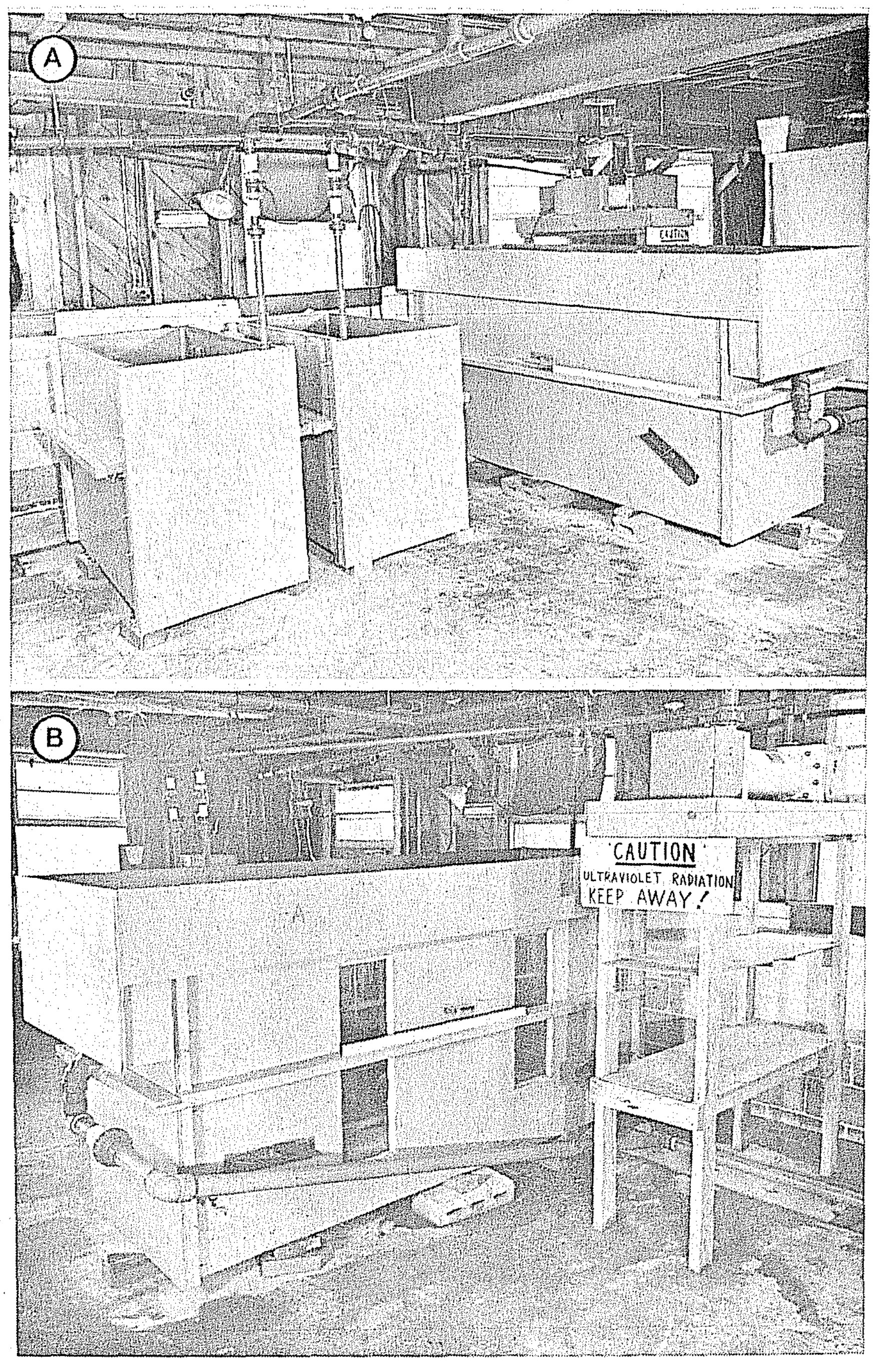

Figure 35. (A) Arrangement of 2 x 8 and 2 x 4 tanks hooked up to commercial-type UV unit. (B) Side view of 2 x 8 tank and UV unit on stand.

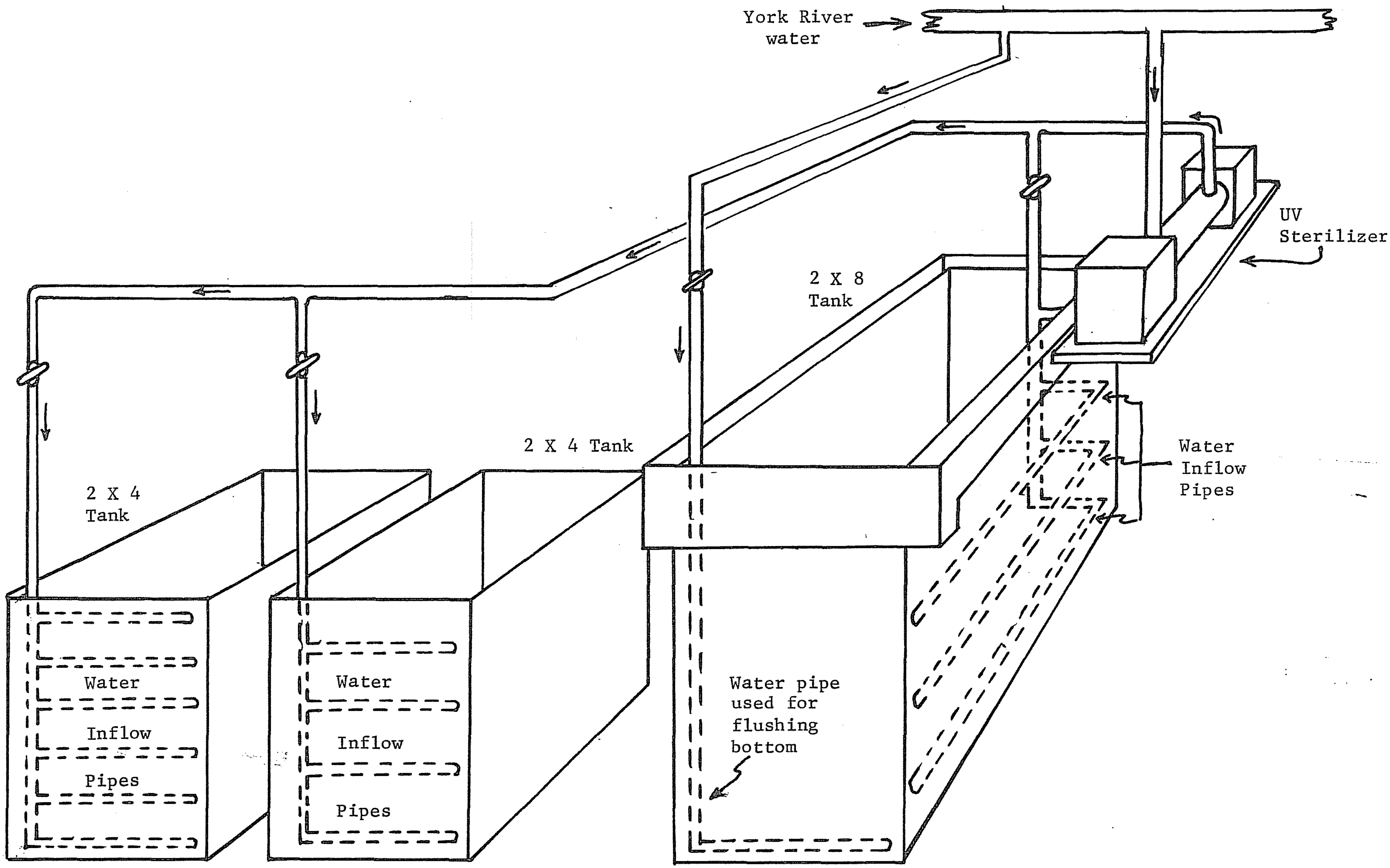

Figure 35C

Diagram showing arrangement of 2 X 8 and 2 X 4 tanks during simultaneous depuration runs in the three tanks.

Experiments included in construction of Figure 37

Legend	Experiment Numbers
△	50, 48 (3, 7)
▲	48 (1, 4, 5, 8)
□	57, 51

Figures in parentheses indicate tray numbers.

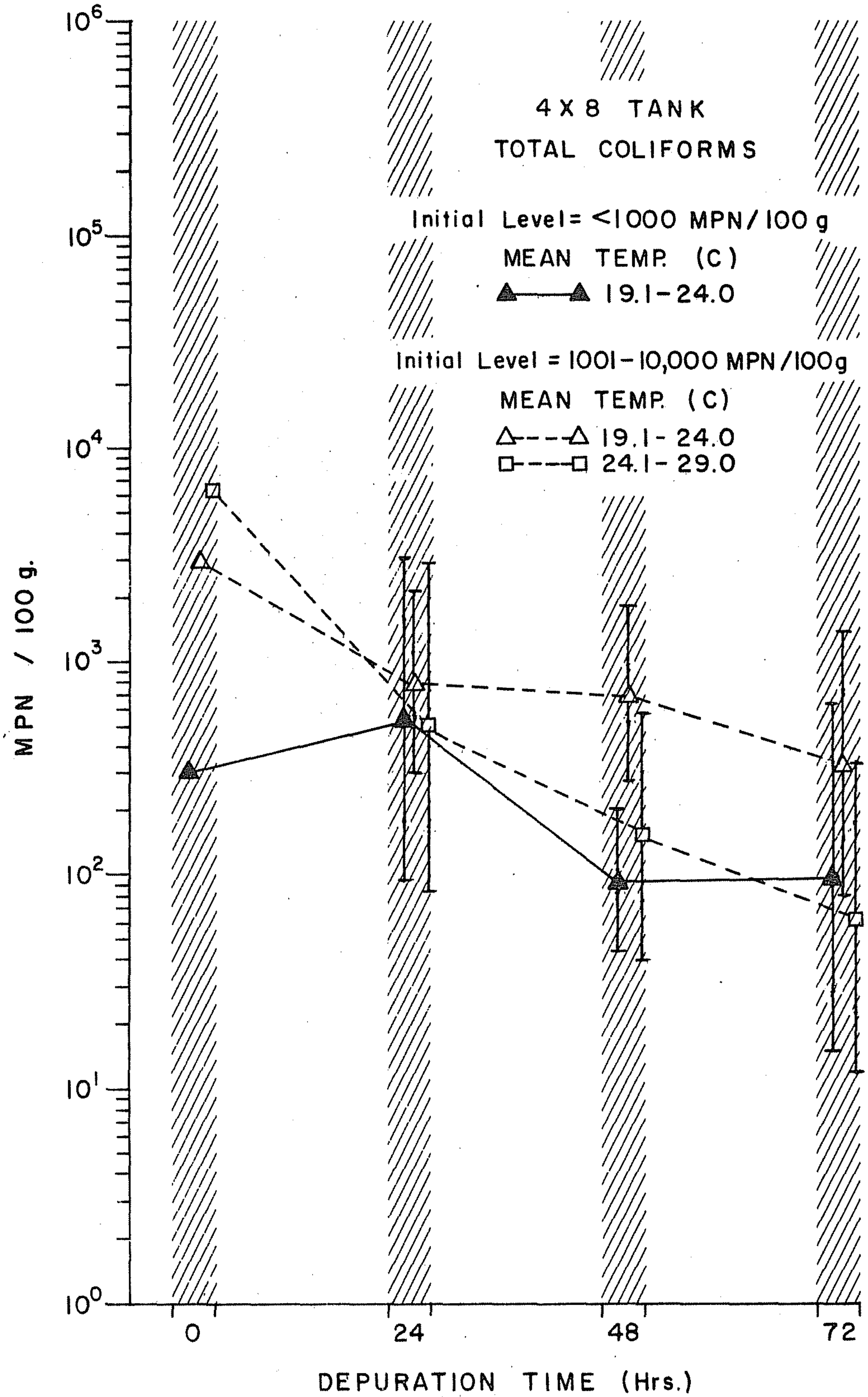

Figure 37. Mean total coliform levels in oysters depurated in the 4 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 38

Legend	Experiment Numbers
▲	62
□	58

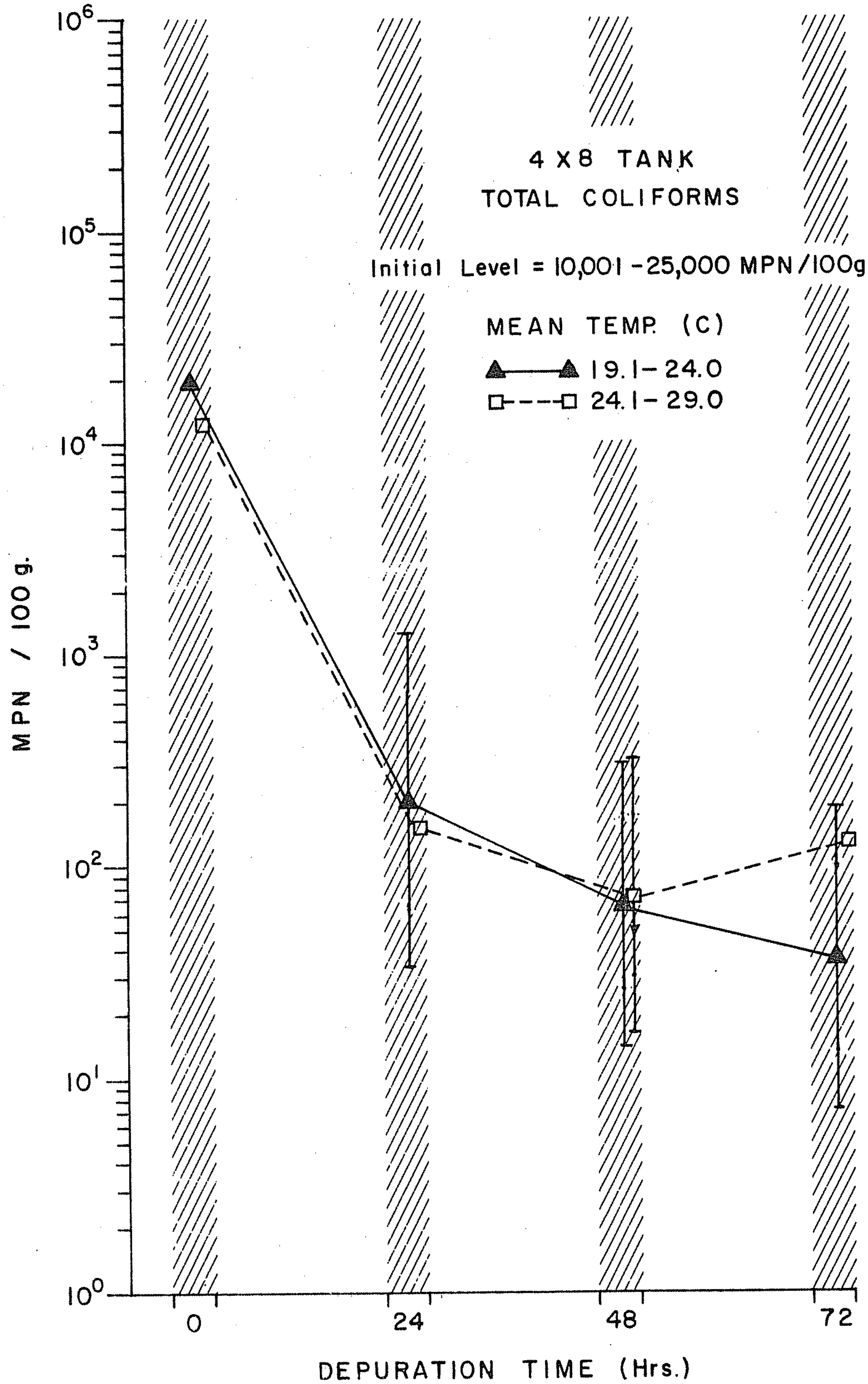

Figure 38. Mean total coliform levels in oysters depurated in the 4 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 39

Legend	Experiment Numbers
○	69
●	73
▲	63, 65
□	60

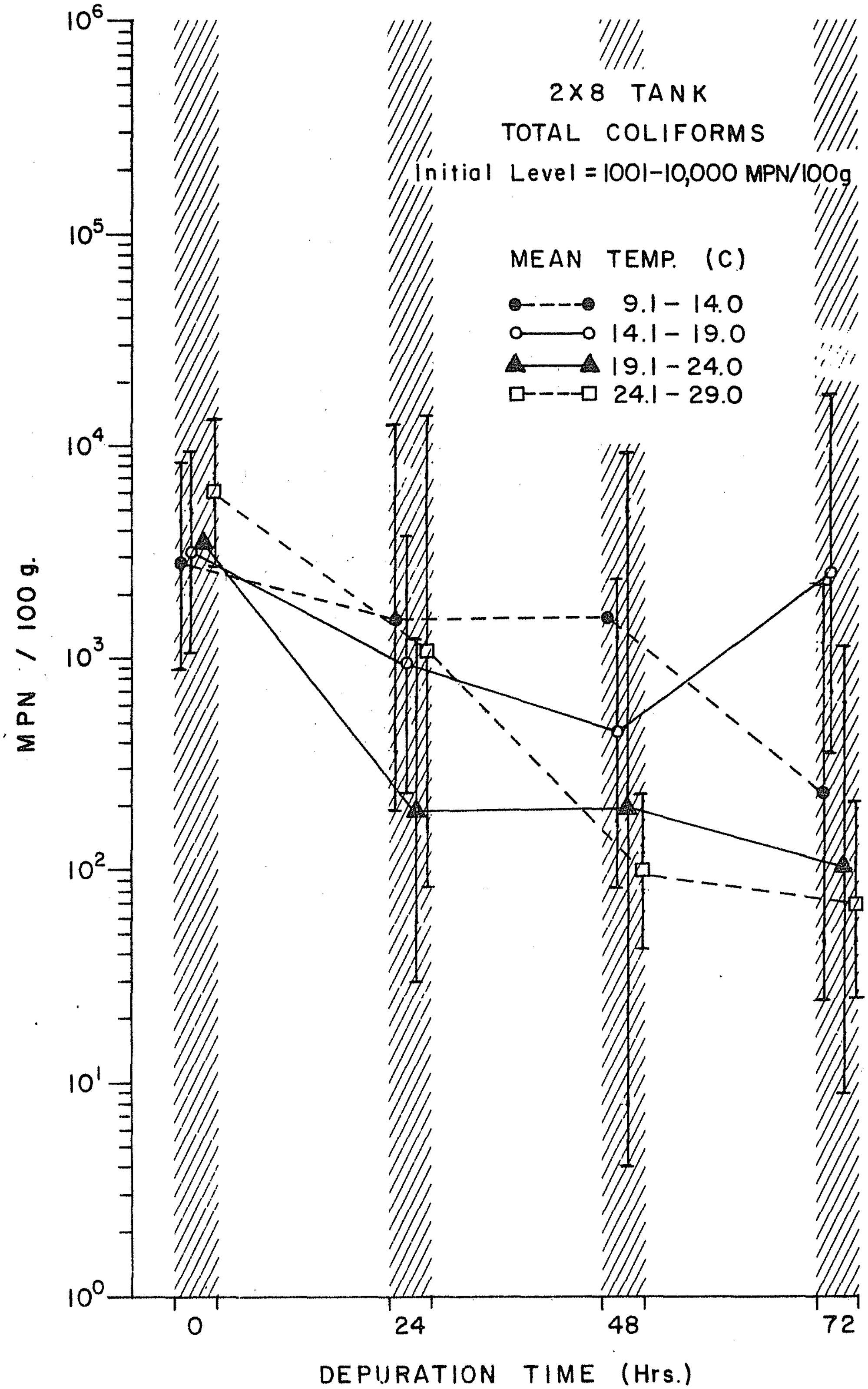

Figure 39. Mean total coliform levels in oysters depurated in the 2 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 40

Legend	Experiment Numbers
▲	67
■	53 (1, 8), 54 (3, 6, 7, 8)
□	53 (2, 4, 5, 7)

Figures in parentheses indicate tray numbers.

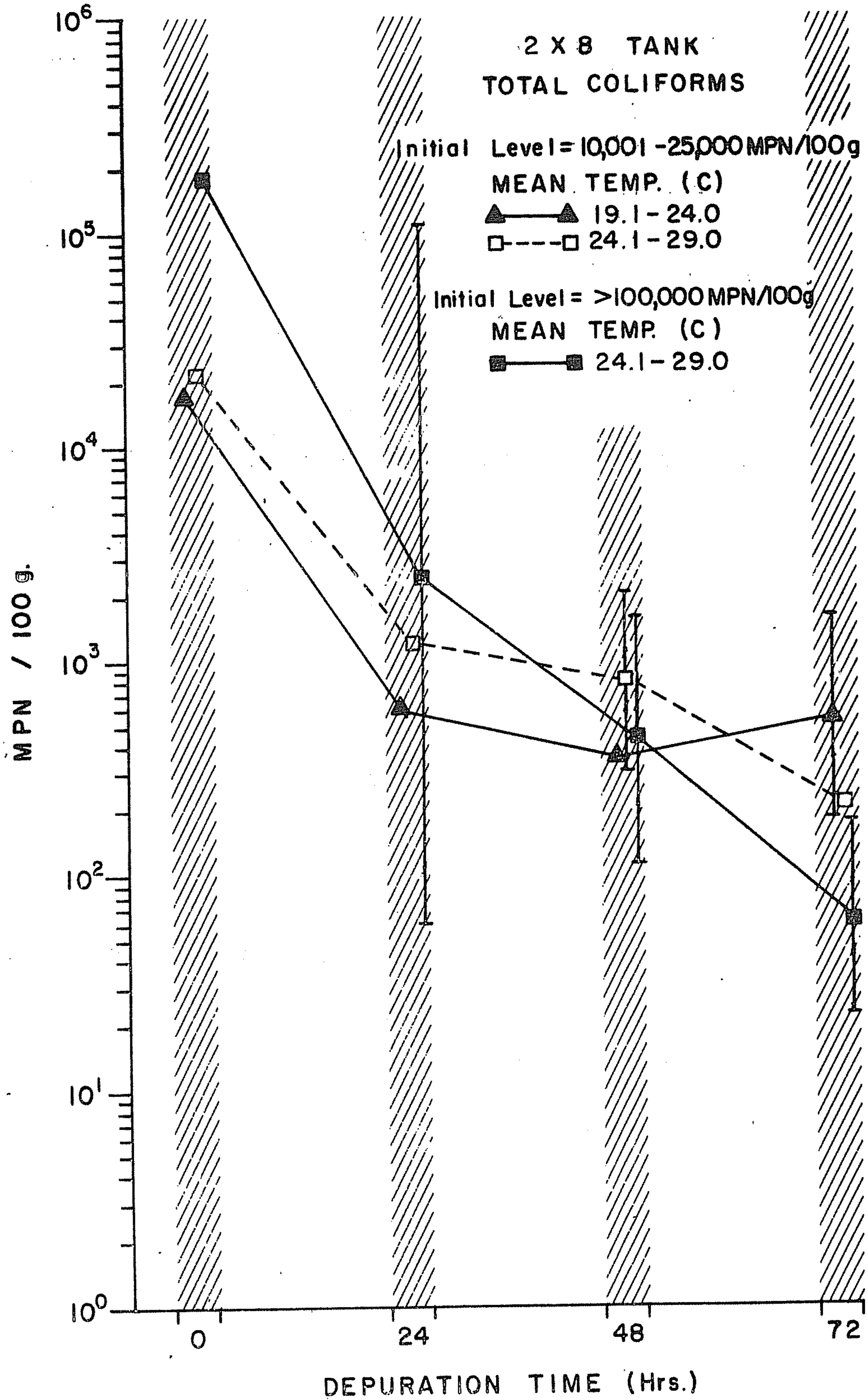

Figure 40. Mean total coliform levels in oysters depurated in the 2 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 41

Legend	Experiment Numbers
○	71
●	72
▲	68
□	54 (1, 2), 61

Figures in parentheses indicate tray numbers.

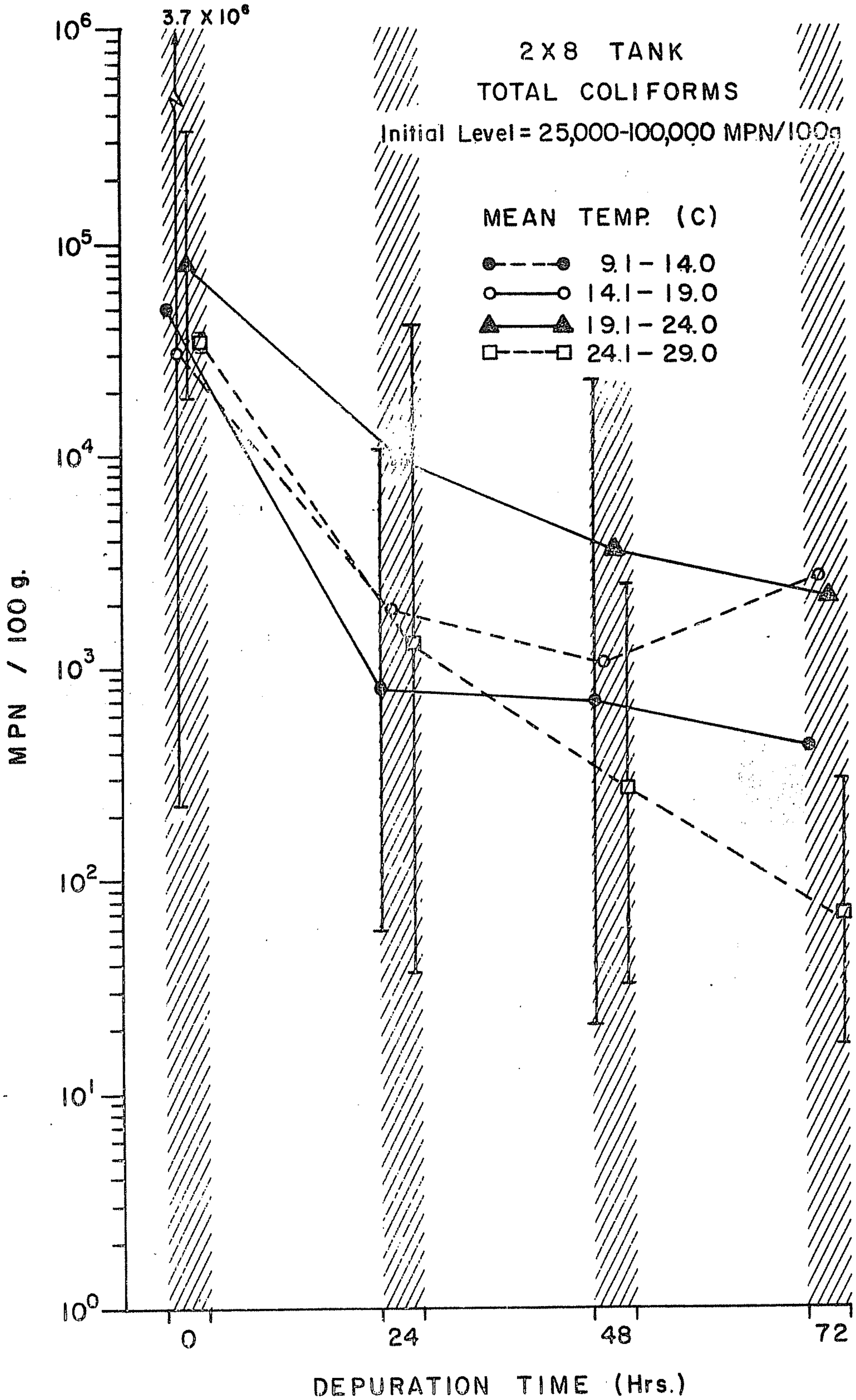

Figure 41. Mean total coliform levels in oysters depurated in the 2 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 42

Legend	Experiment Numbers
●	172, 272, 173, 273
○	82, 182, 84, 184
△	171, 271
■	70, 170, 83, 183

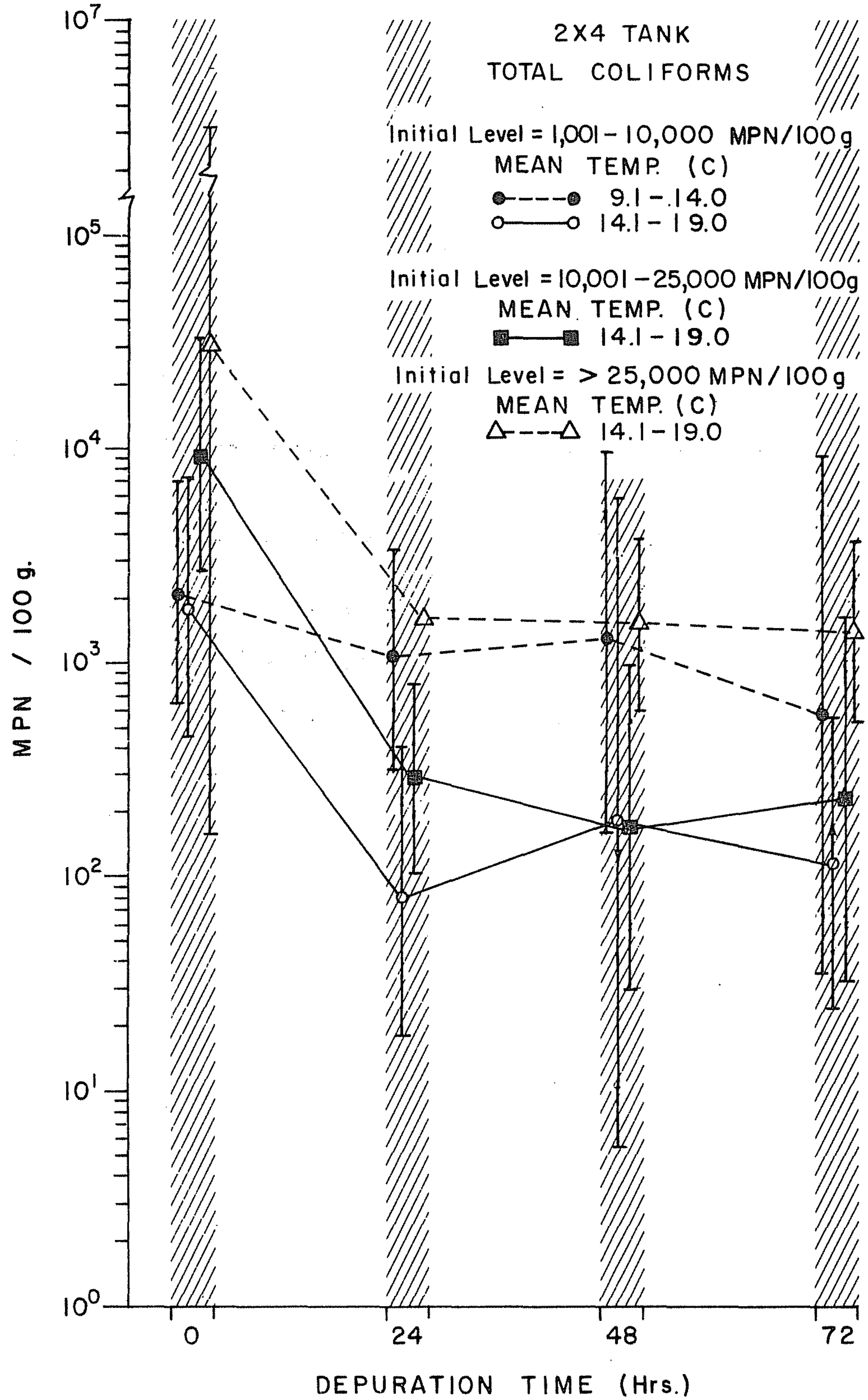

Figure 42. Mean total coliform levels in oysters depurated in the 2 x 4 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 43

Legend	Experiment Numbers
●	49, 149, 56, 156
○	59, 159, 259
▲	55, 155

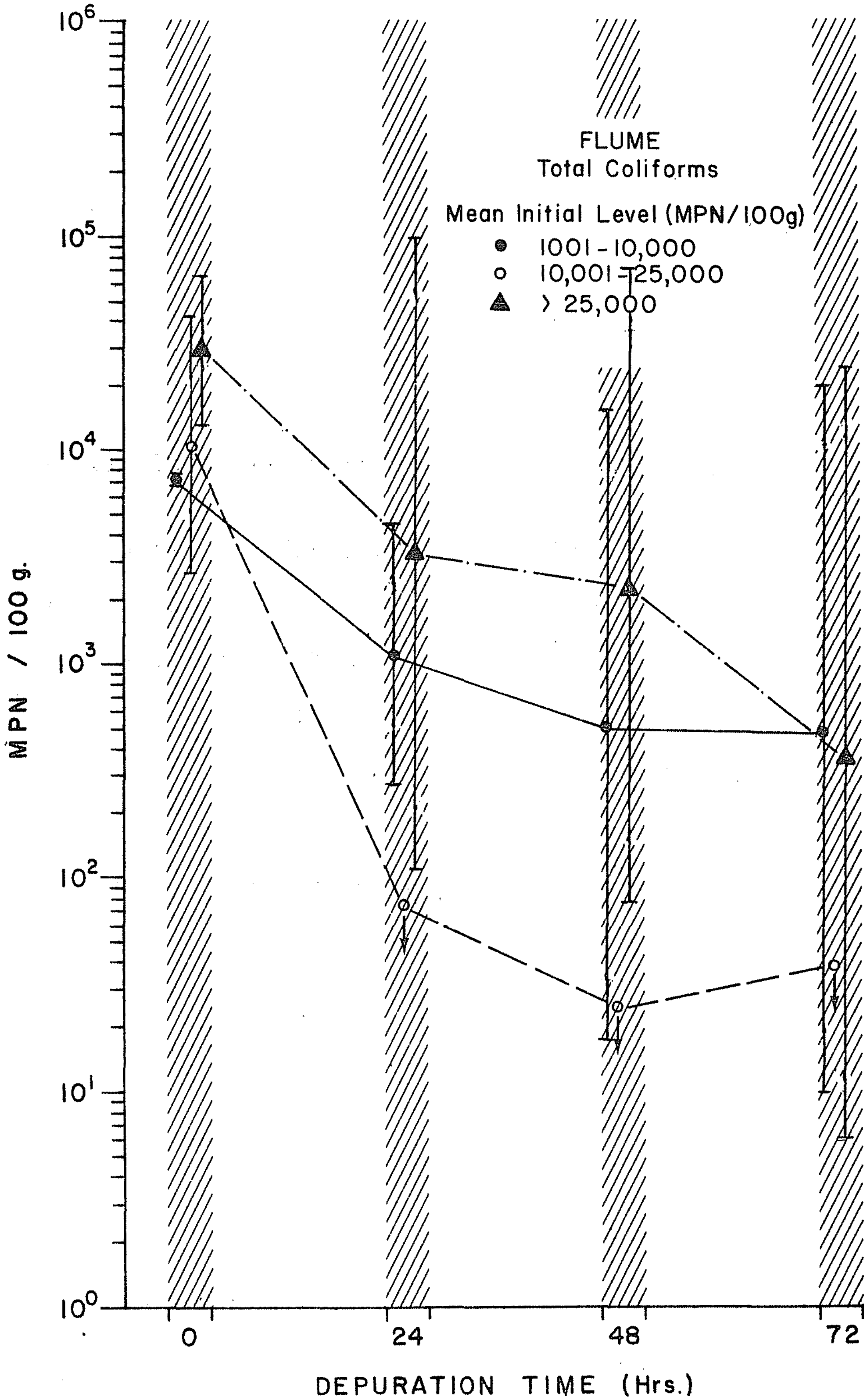

Figure 43. Mean total coliform levels in oysters depurated in the flume with respect to initial MPN level (mean temp. = 24.1-29.0 C). Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 44

Legend	Experiment Numbers
△	48 (1, 4, 5, 8), 50 (1)
▲	50 (2, 4, 5, 7, 8)
□	57, 58

Figures in parentheses indicate tray numbers

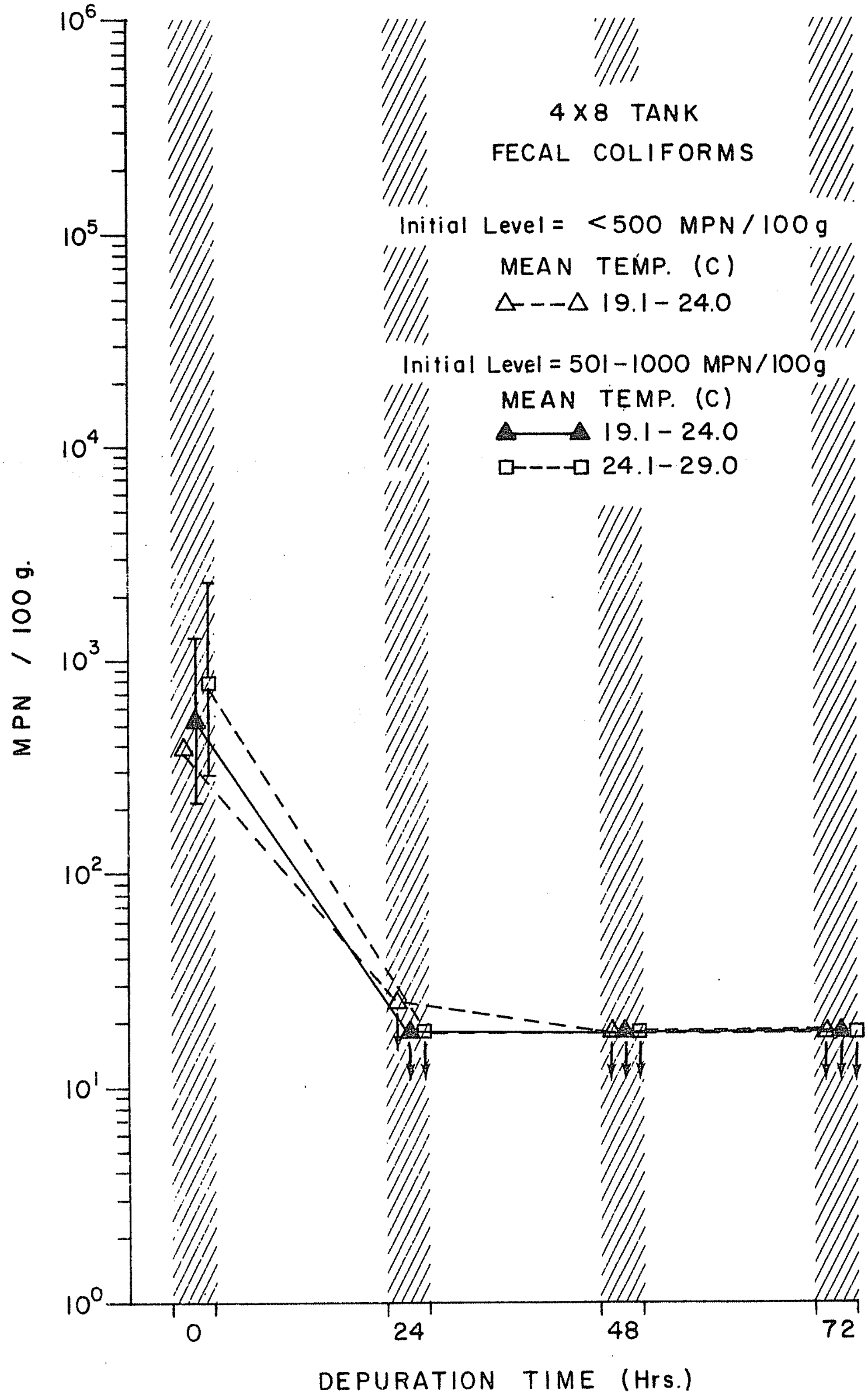

Figure 44. Mean fecal coliform levels in oysters depurated in the 4 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 45

Legend	Experiment Numbers
▲	48 (3, 7)
■	62
□	51

Figures in parentheses indicate tray numbers.

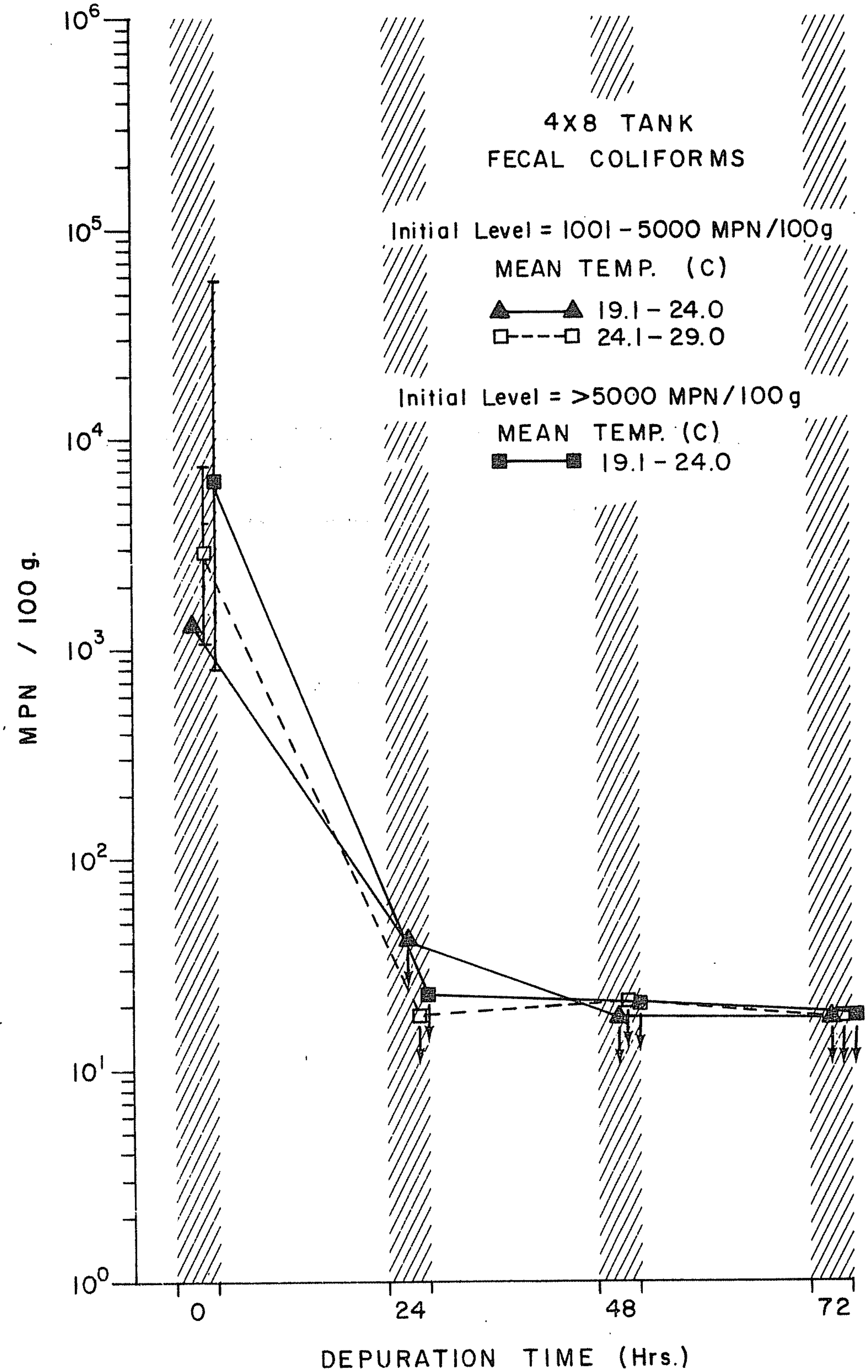

Figure 45. Mean fecal coliform levels in oysters depurated in the 4 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 46

Legend	Experiment Numbers
●	72, 73
○	69
▲	63, 65

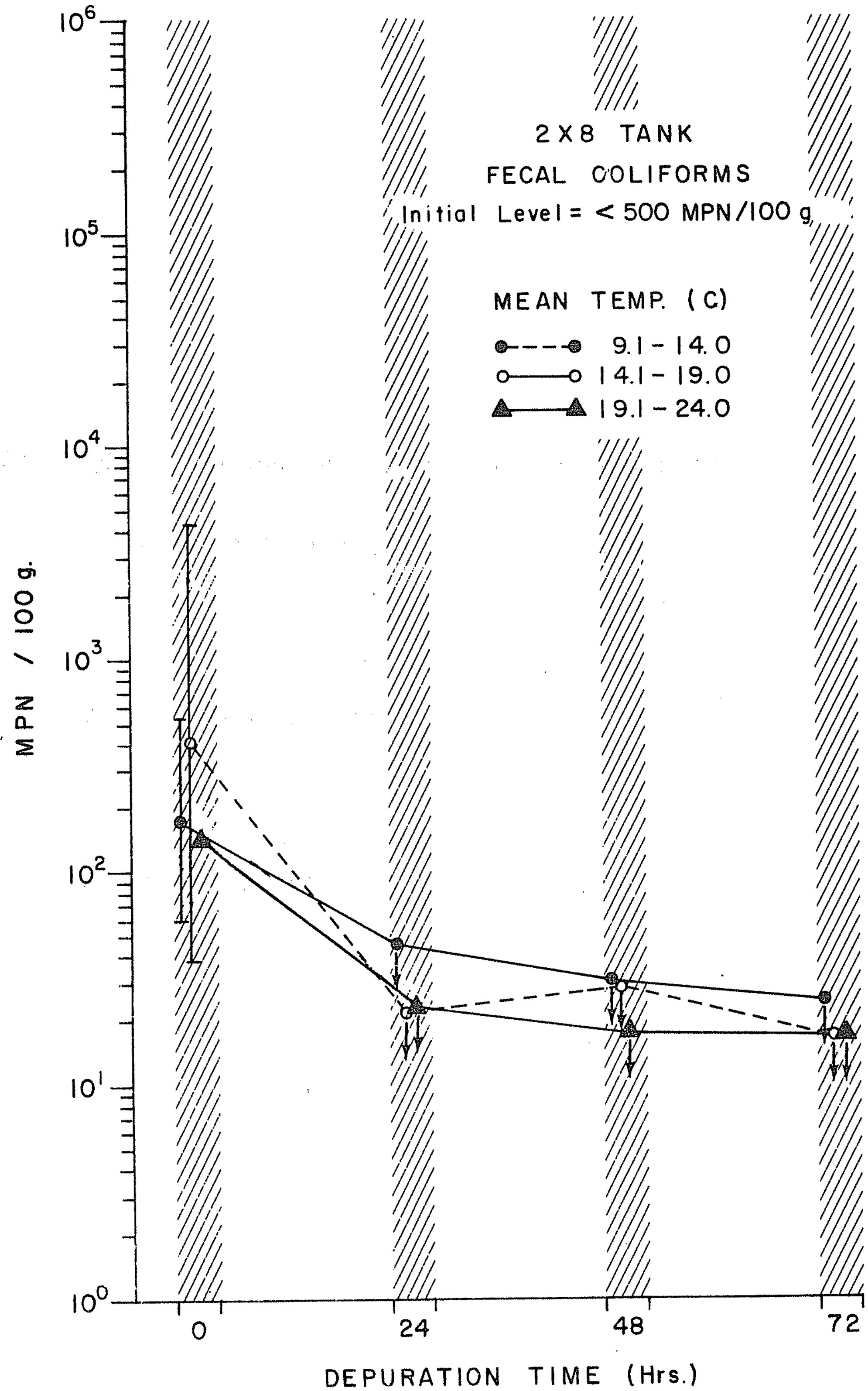

Figure 46. Mean fecal coliform levels in oysters depurated in the 2 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 47

Legend	Experiment Numbers
○	71
▲	67, 68
□	53 (2, 4, 5, 7), 60

Figures in parentheses indicate tray numbers.

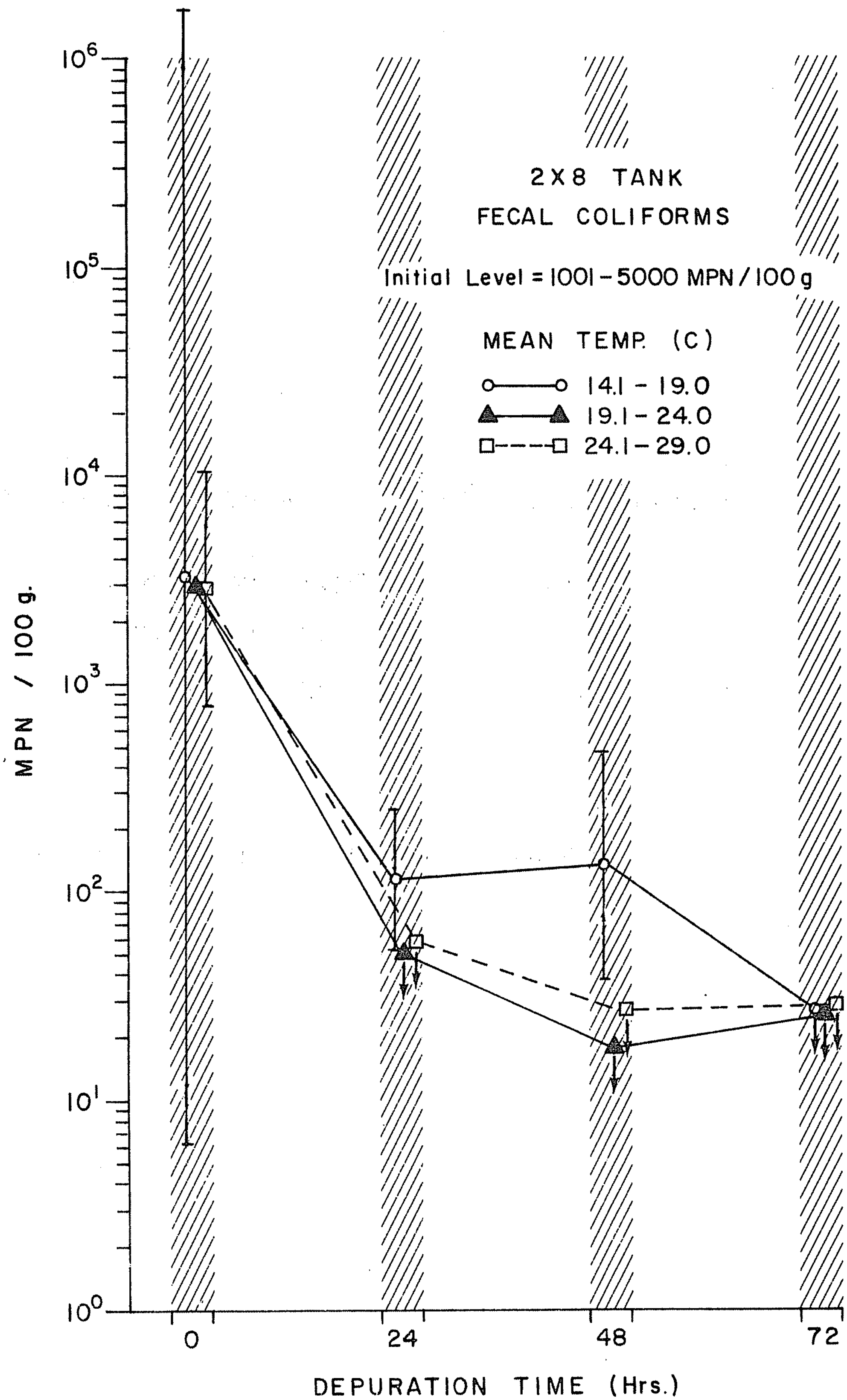

Figure 47. Mean fecal coliform levels in oysters depurated in the 2 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 48

Legend	Experiment Numbers
□	53 (1, 8), 54 (3, 6, 7, 8)
■	54 (1, 2), 61

Figures in parentheses indicate tray numbers.

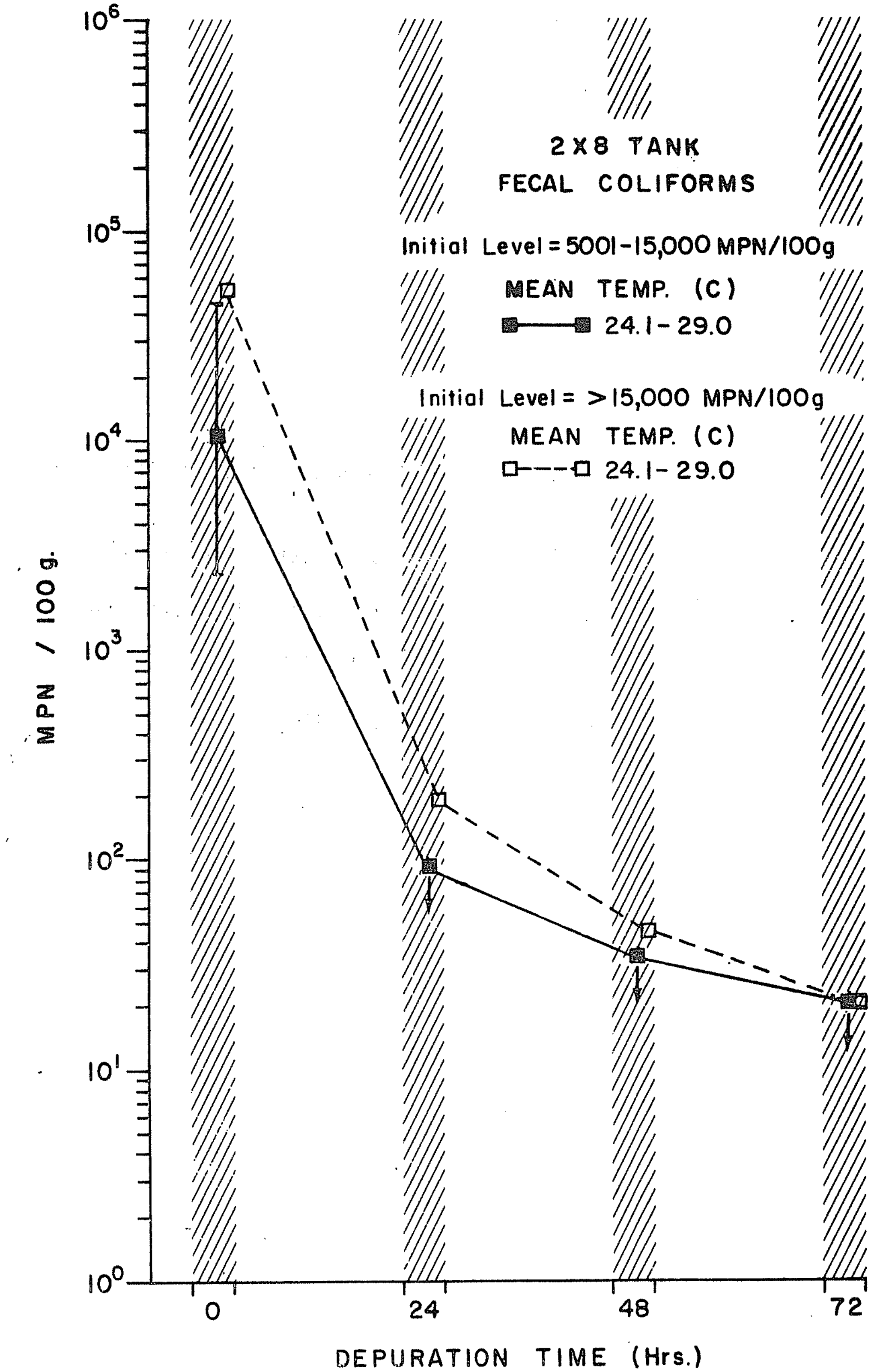

Figure 48. Mean fecal coliform levels in oysters depurated in the 2 x 8 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 49

Legend	Experiment Numbers
■	70, 170, 83, 183, 84, 184, 171, 271
●	172, 272, 173, 273
o	82, 182

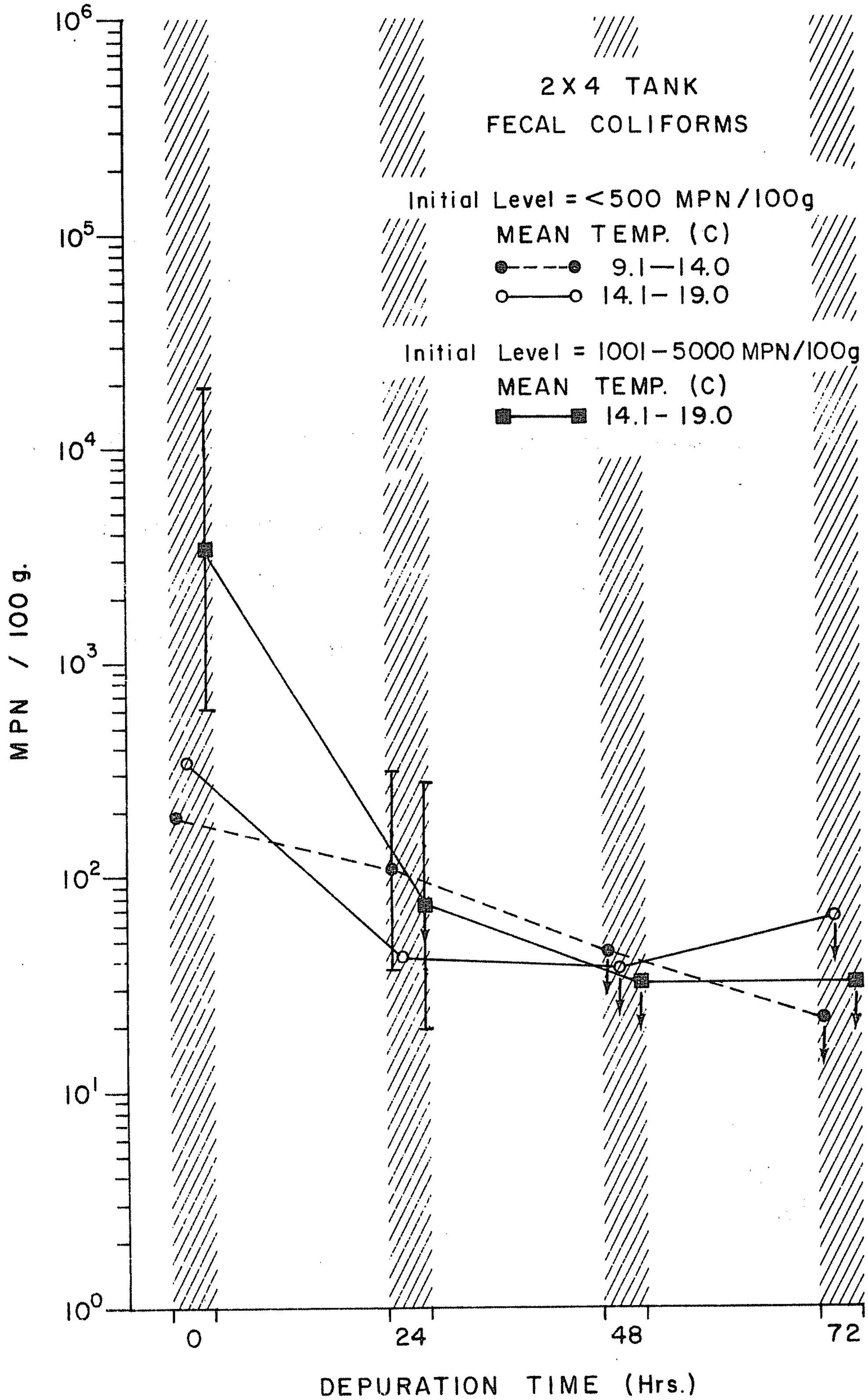

Figure 49. Mean fecal coliform levels in oysters depurated in the 2 x 4 tank with respect to initial MPN level and mean temperature. Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 50

Legend	Experiment Numbers
○	49, 149, 55, 155
●	56, 156, 59, 159, 259

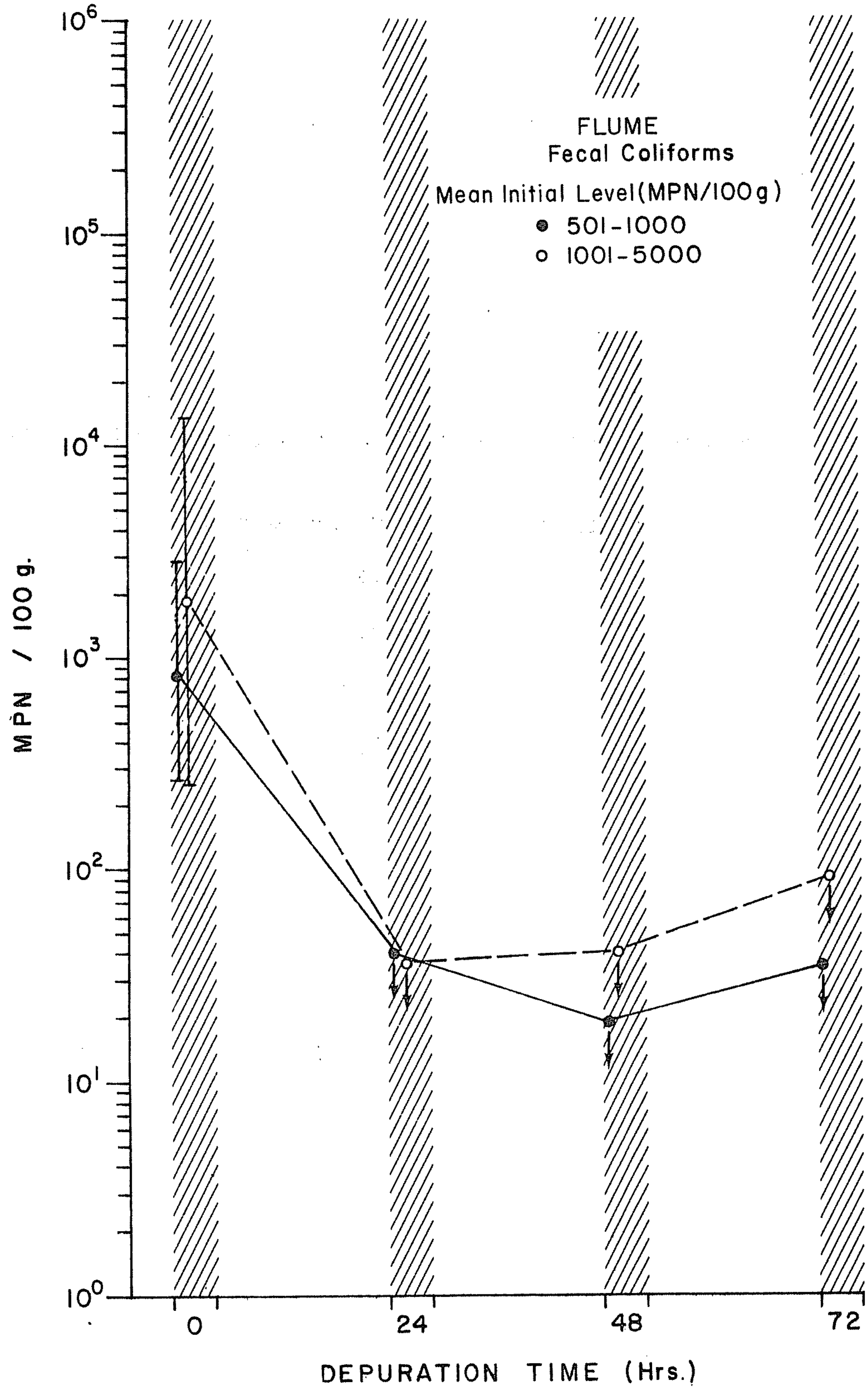

Figure 50. Mean fecal coliform levels in oysters depurated in the flume with respect to initial MPN level (mean temp. = 24.1-29.0 C). Vertical lines represent the 90% confidence intervals for the mean. Oysters contaminated in nature.

Experiments included in construction of Figure 51

Legend	Experiment Numbers
○	78
●	74
▲	80
△	81
■	79

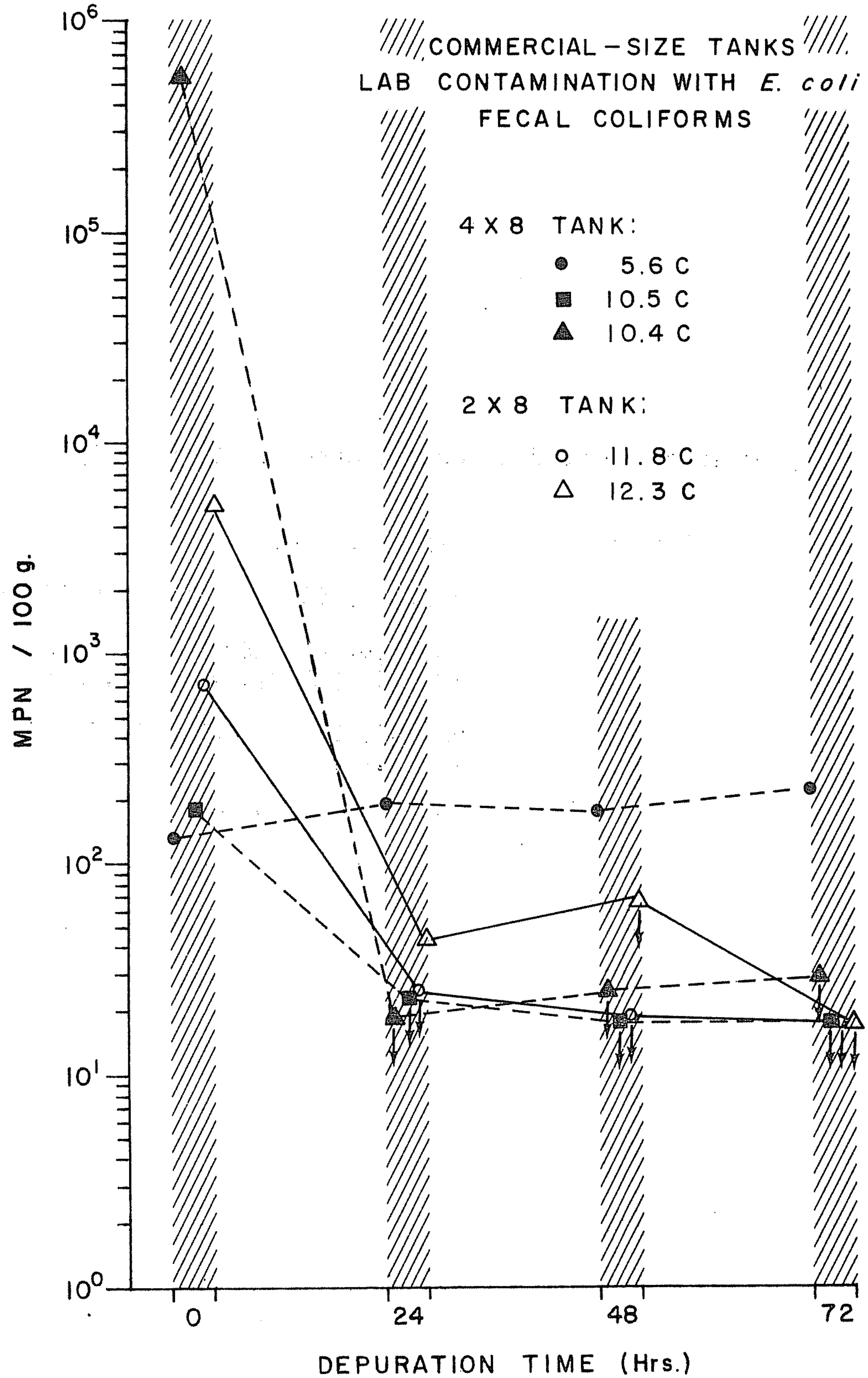

Figure 51. Mean fecal coliform levels in oysters depurated in commercial-size tanks with respect to mean temperature. Contaminated with E. coli in the laboratory.

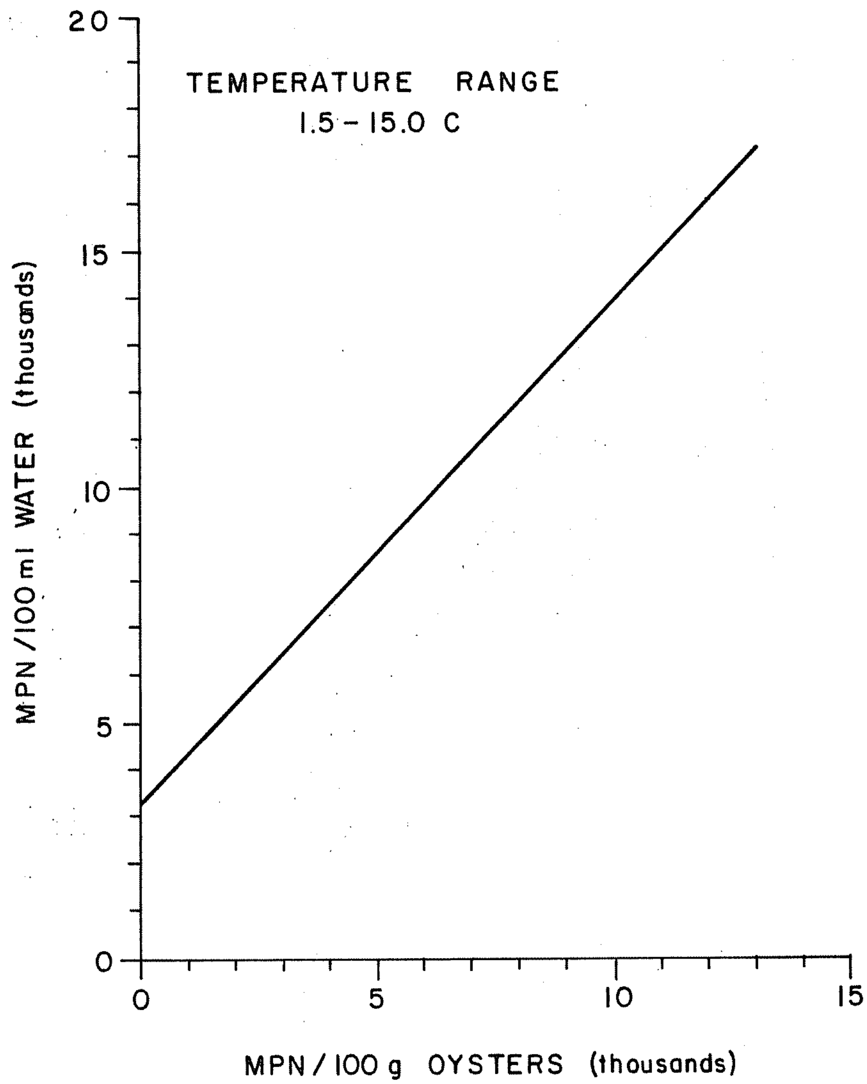

Figure 54. Correlation of total coliform concentration in oysters and shellfish growing waters.

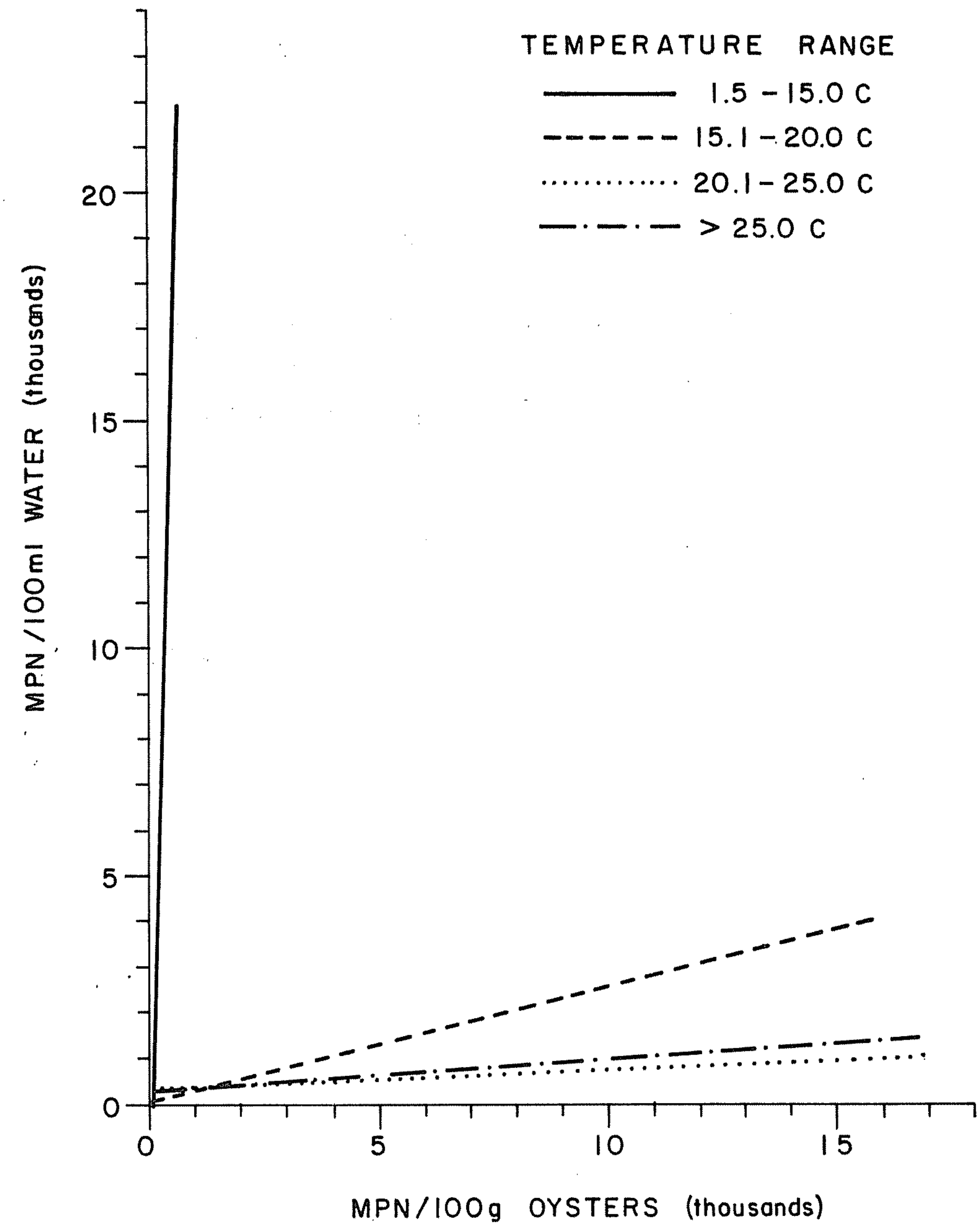

Figure 55. Correlation of fecal coliform concentration in oysters and shellfish growing waters.

Experiments included in construction of Figure 56

Legend	Experiment Numbers
▲	60, 63, 65
●	69
△	67
○	53 (Trays 2, 4, 5, 7)

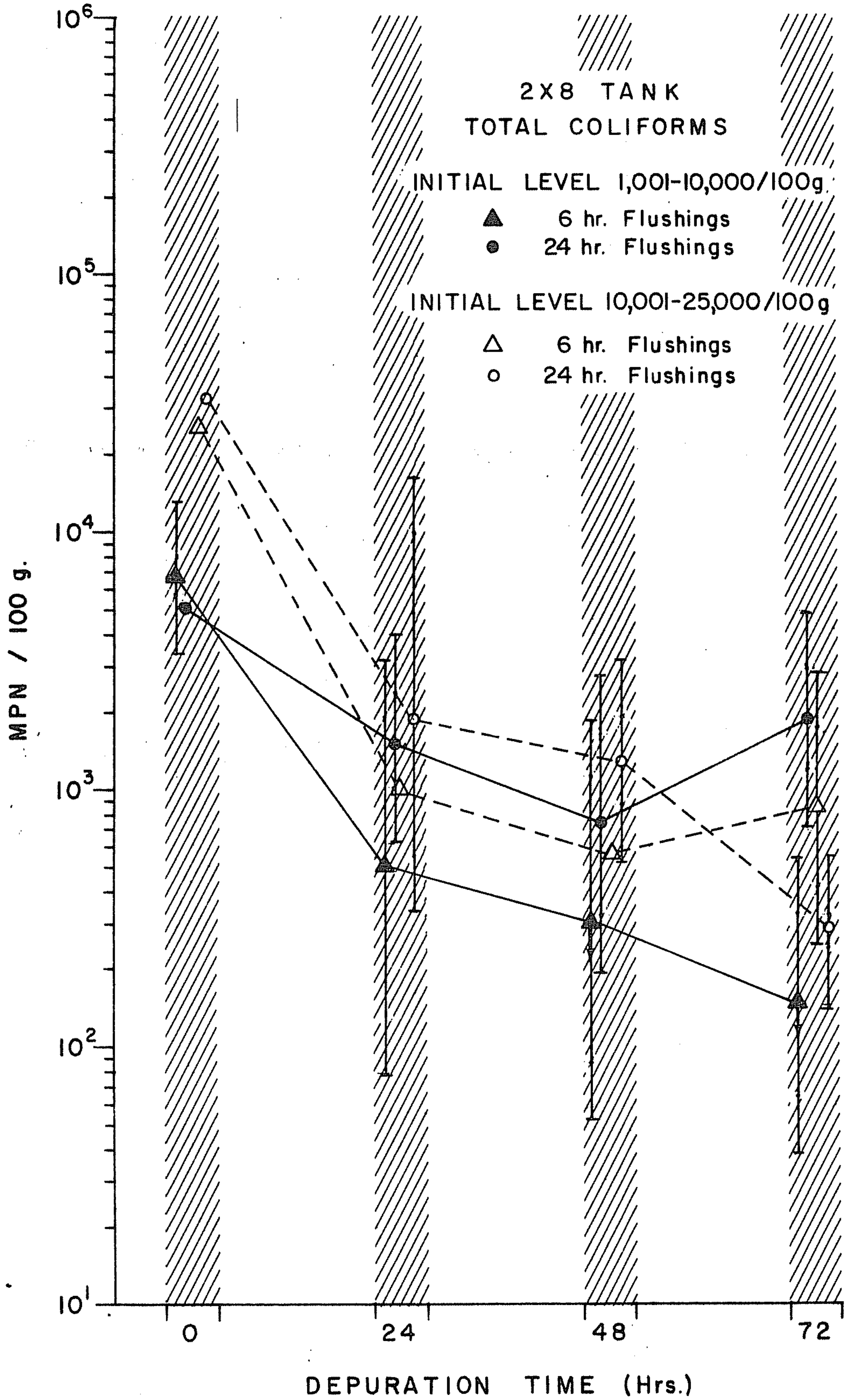

Figure 56. Mean total coliform levels in oysters depurated in the 2 x 8 tank with respect to frequency of tank flushing (6 hr versus 24 hr intervals) and initial MPN level. Oysters contaminated in nature.

Experiments included in construction of Figure 57

Legend	Experiment Numbers
▲	63, 65
●	69
△	60, 67
○	53 (Trays 2, 4, 5, 7)

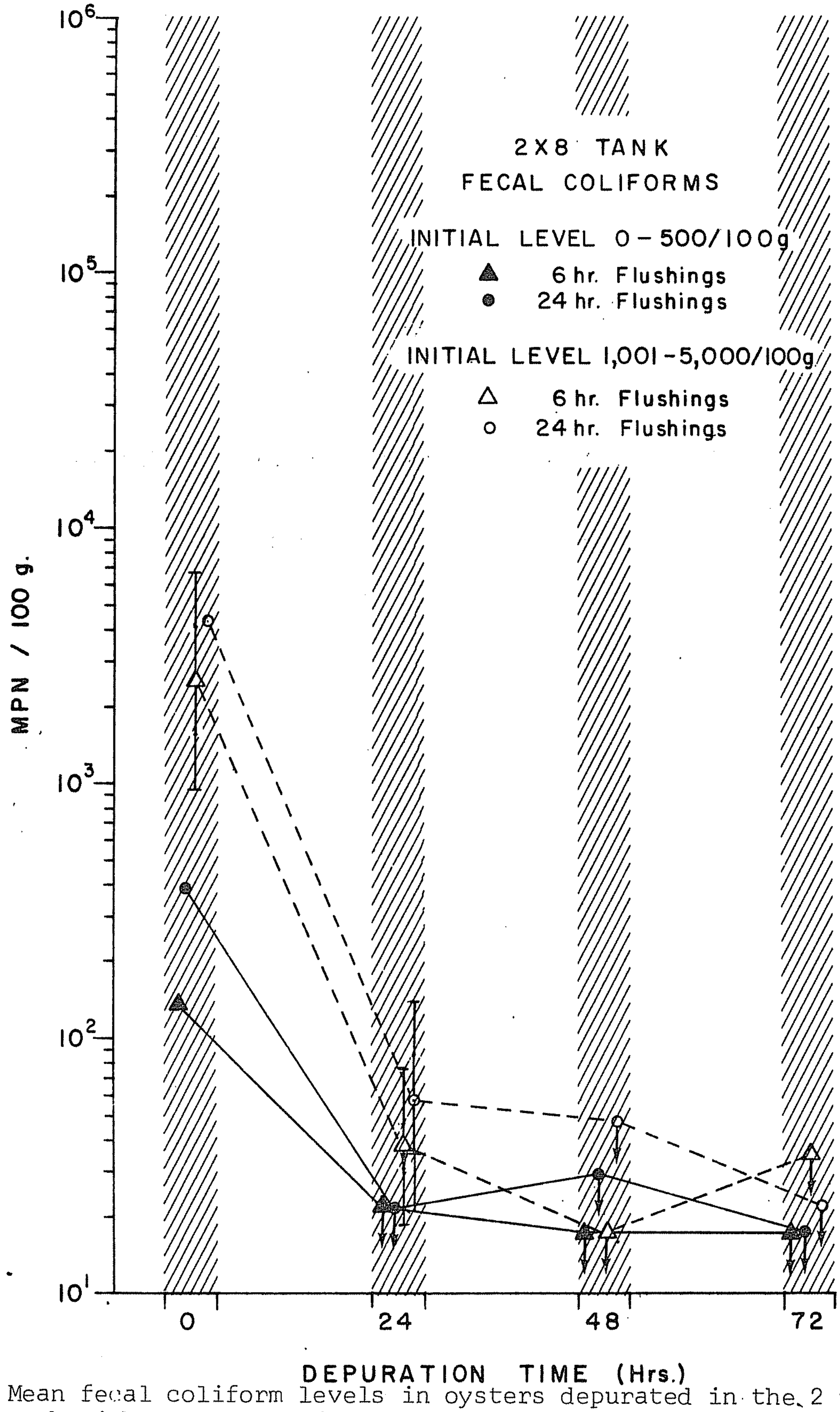

Figure 57. Mean fecal coliform levels in oysters depurated in the 2 x 8 tank with respect to frequency of tank flushing (6 hr versus 24 hr intervals) and initial MPN level. Oysters contaminated in nature.

Experiments included in construction of Figure 58

Legend	Experiment Numbers
▲	68
●	61

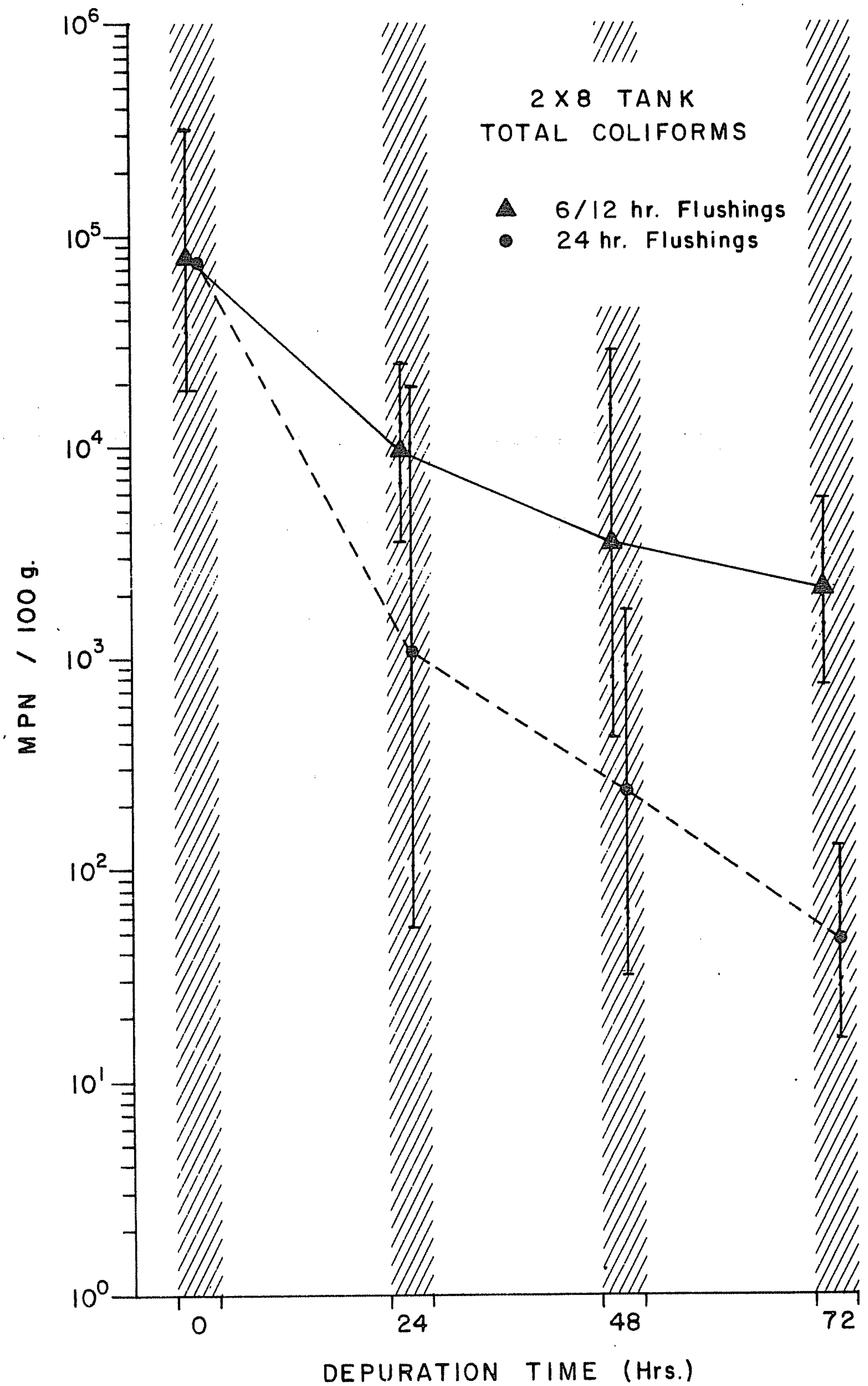

Figure 58. Mean total coliform levels in oysters depurated in the 2 x 8 tank with respect to frequency of tank flushing (alternating 6 hr and 12 hr invervals versus 24 hr intervals). Oysters contaminated in nature.

Experiments included in construction of Figure 59

Legend	Experiment Numbers
▲	68
●	53 (Trays 2, 4, 5, 7)

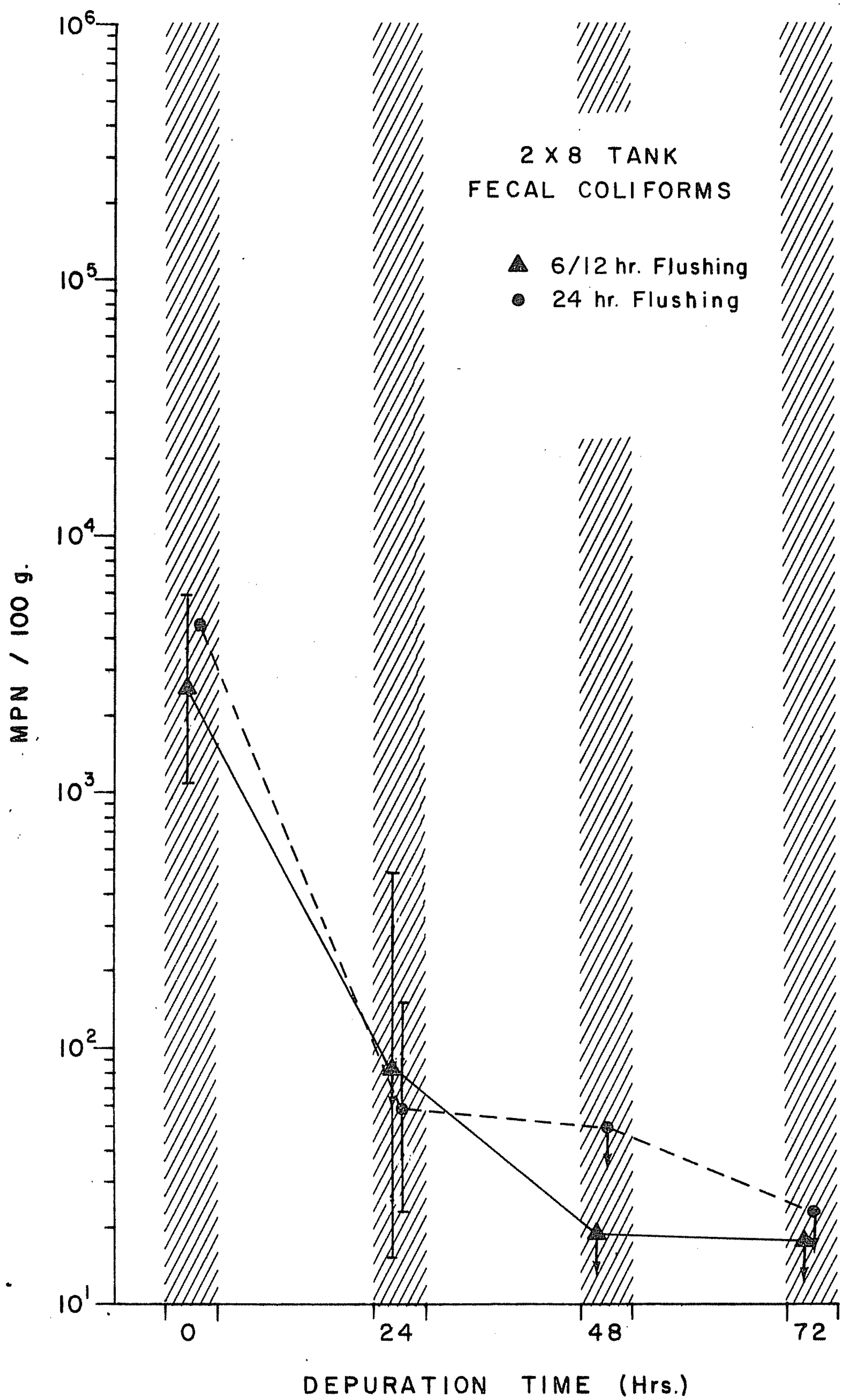

Figure 59. Mean fecal coliform levels in oysters depurated in the 2 x 8 tank with respect to frequency of tank flushing (alternating 6 hr and 12 hr intervals versus 24 hr intervals). Oysters contaminated in nature.

Experiments included in construction of Figure 60

Legend	Experiment Numbers
○	82, 83, 84
△	82, 83, 84
●	182, 183, 184

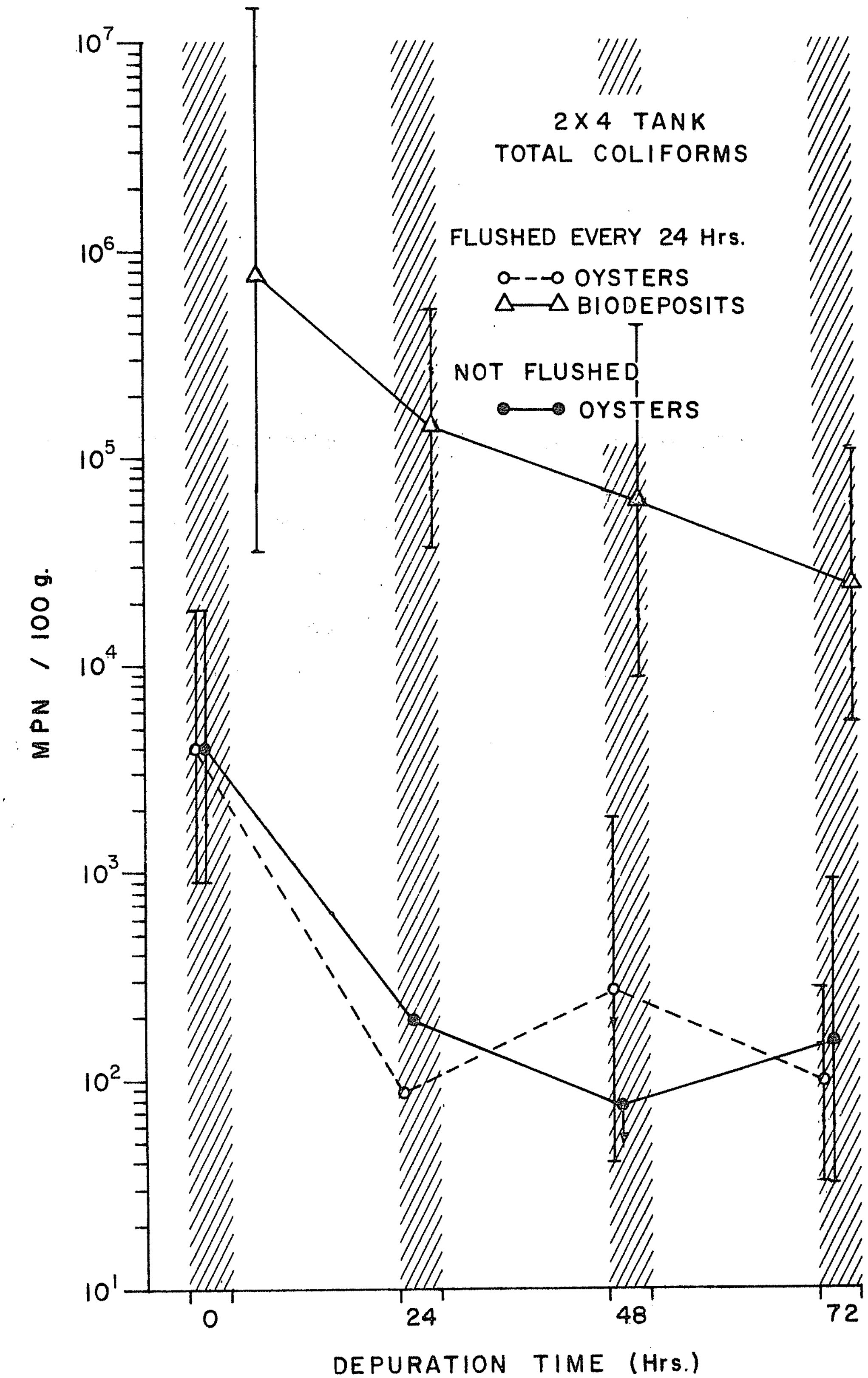

Figure 60. Mean total coliform levels in biodeposits and oysters depurated in the 2 x 4 tank with respect to inclusion or elimination of tank flushing. Oysters contaminated in nature.

Experiments included in construction of Figure 61

Legend	Experiment Numbers
o	82, 83, 84
△	82, 83, 84
●	182, 183, 184

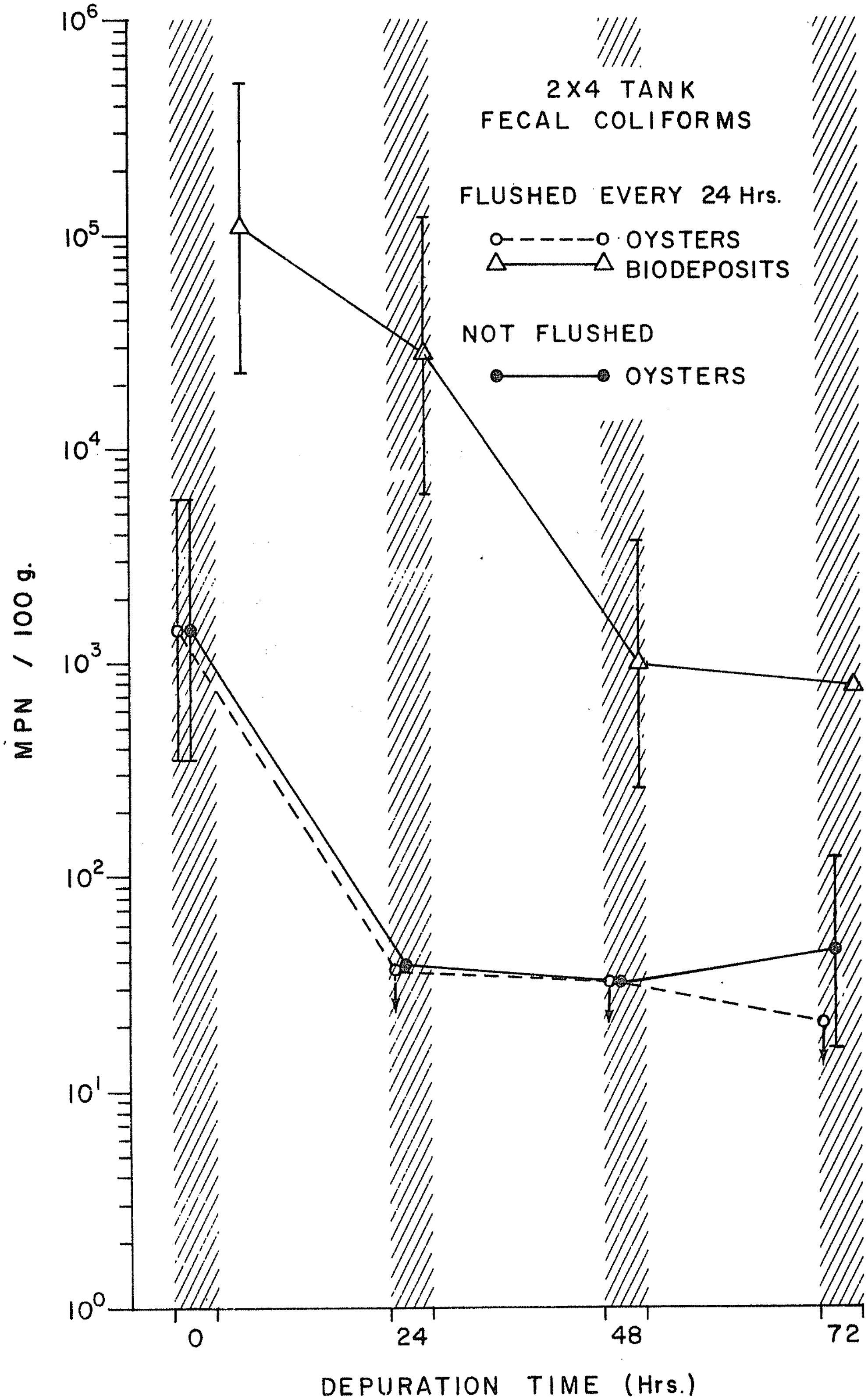

Figure 61. Mean fecal coliform levels in biodeposits and oysters depurated in the 2 x 4 tank with respect to inclusion or elimination of tank flushing. Oysters contaminated in nature.

Experiments included in construction of Figure 62

Legend	Experiment Numbers
△	66-1, 66-2
○	66-1, 66-2
●	66-1, 66-2

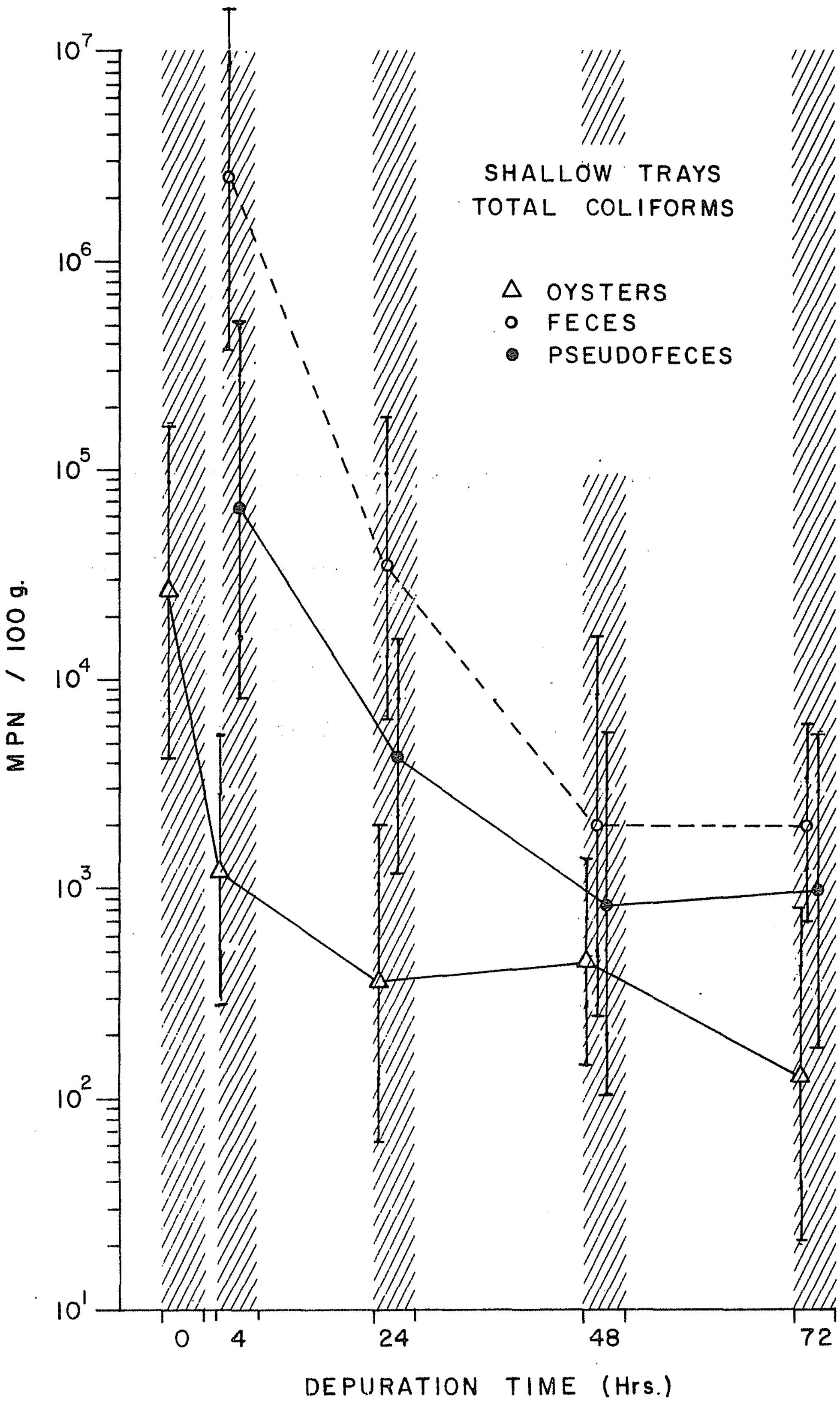

Figure 62. Mean total coliform levels in oysters, feces and pseudofeces depurated in shallow trays. Oysters contaminated in nature.

Experiments included in construction of Figure 63

Legend	Experiment Numbers
△	66-1, 66-2
○	66-1, 66-2
●	66-1, 66-2

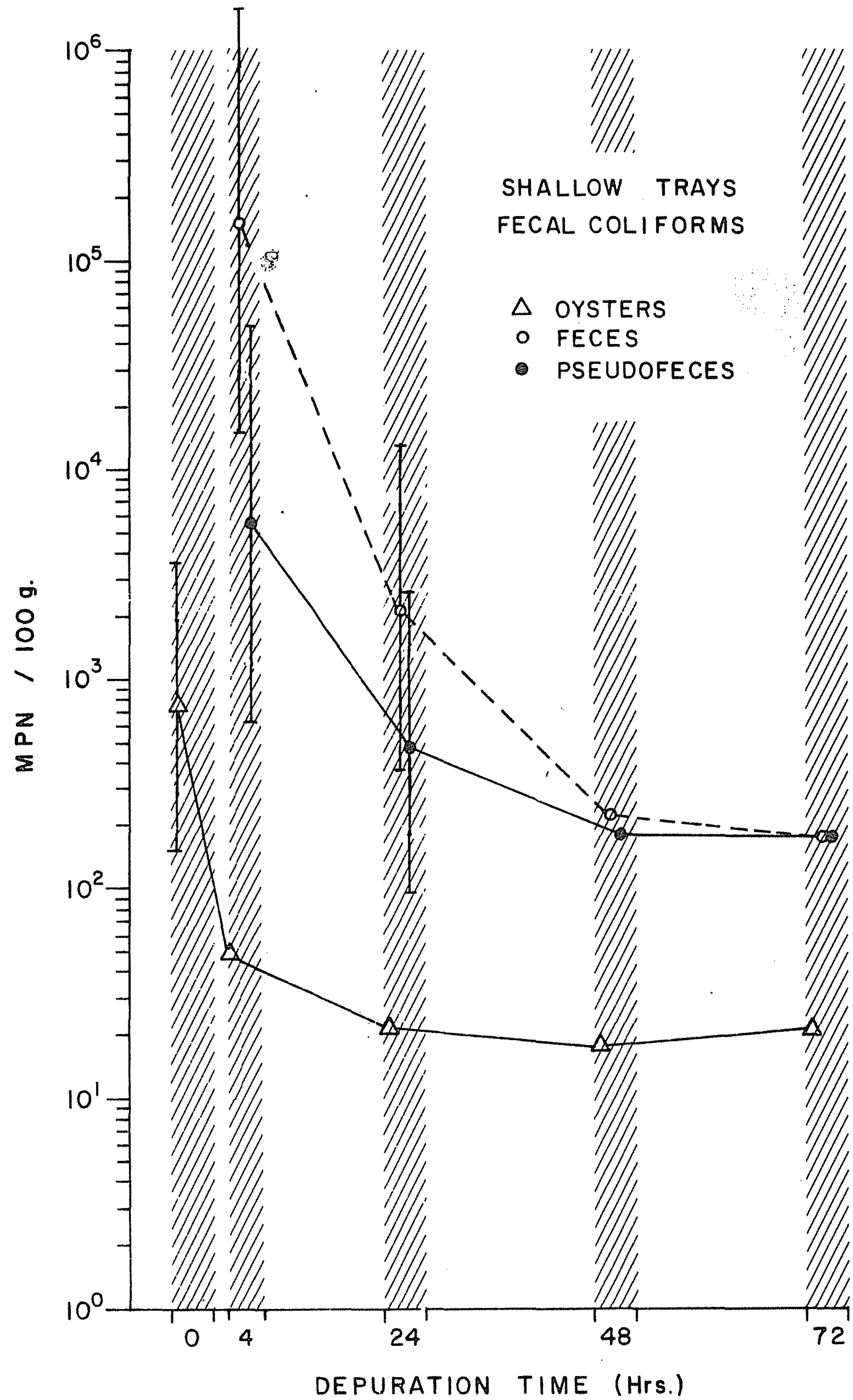

Figure 63. Mean fecal coliform levels in oyster, feces and pseudofeces depurated in shallow trays. Oysters contaminated in nature.