F I S H E R I E S R E S E A R C H B O A R D
O F C A N A D A

MANUSCRIPT REPORTS OF THE BIOLOGICAL STATIONS

No. 440

Title

Preliminary Experiments in the Self-Cleansing of Clams (Mya arenaria L.)

by

M. W. Mullan, A. B. Williams and D. R. Colwell

Fish Inspection Laboratory, Halifax, N.S.

In co-operation with

J. C. Medcof

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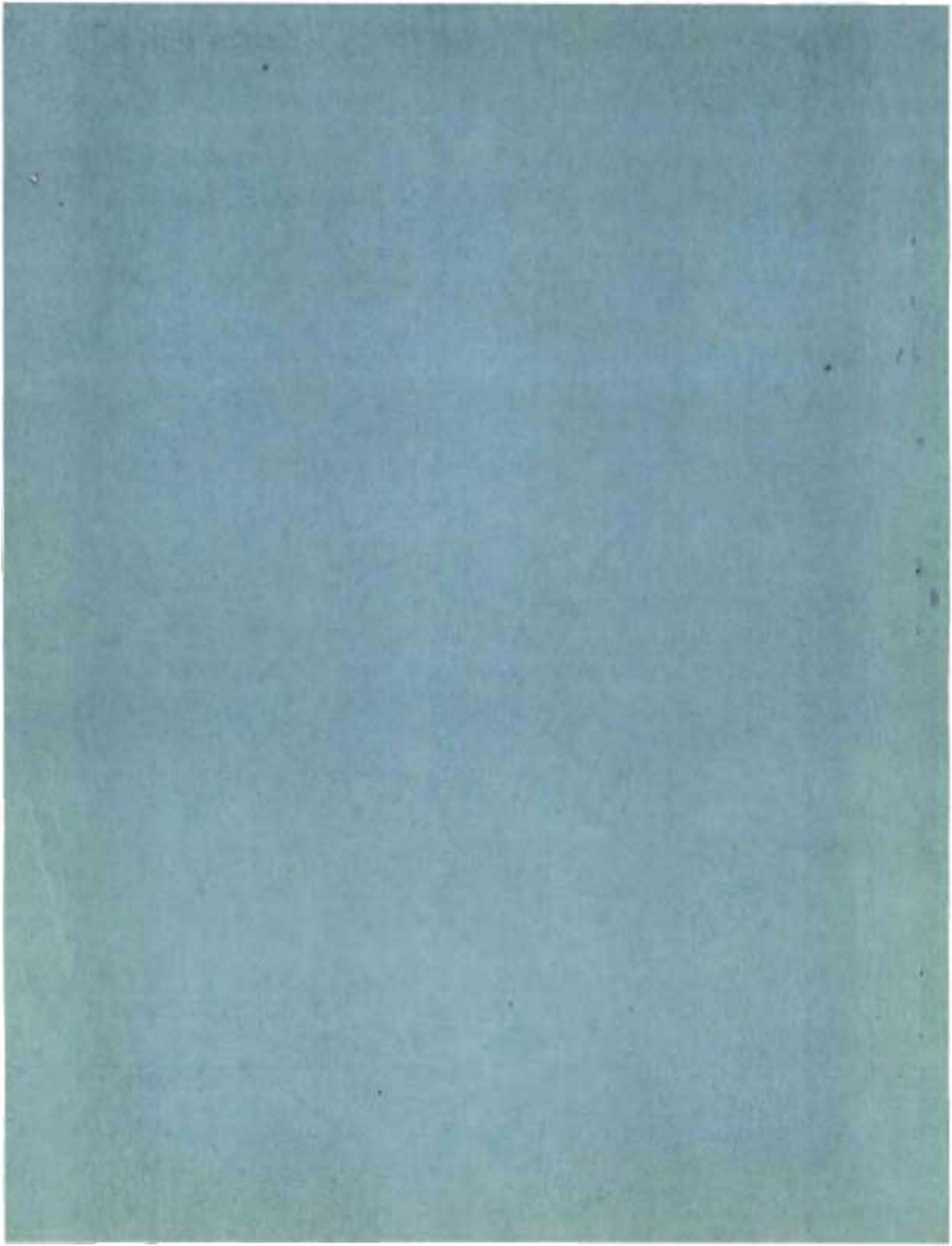

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SECTION I. INTRODUCTION

1. In the summer of 1951, the Canadian Department of Fisheries in co-operation with the Atlantic Biological Station of the Fisheries Research Board of Canada, conducted preliminary experiments in the self-cleansing of soft-shell clams (Mya arenaria L.). Such studies were deemed timely first, because of the importance of the clam industry. The clam is the Maritimes' commercially most important mollusc. Its marketed value is 1950 was $1,400,000. The study is timely for a second reason. Increasing numbers of clam beds are being closed because of sewage pollution. Not only has the number of closures increased, but, with the depletion of the stocks in the unrestricted beds by intensive exploitation, the quarantined stocks have come to represent a greater and greater proportion of the total clam population on the coast.

If the production from these closed beds was made available to the fishery the Department of Fisheries would be in a better position in working out management programs that might involve area-to-area rotation of digging with rest periods between. Such a device would have a long-term beneficial effect on the industry.

2. It is apparent then that the development of a practicable cleansing system would have distinct economic and administrative advantages, however, it would also be advantageous from the point of view of public health. The safe use of polluted stocks after cleansing would greatly reduce the risk that arises from the small quantities of polluted clams that are marketed in spite of the best efforts of the fishery officers to prevent fishing in the closed areas.

Diggers participating in this illegal trade have much to gain. From the relatively well stocked closed beds, they can dig more clams in a day than they can from the depleted open areas. They represent the polluted clams as being from open areas and sell them at the price prevailing for legally-procured clams. From this practice they gain enough to make it worth running the risk of being caught and prosecuted. If by virtue of an approved cleansing system, the polluted stocks could be rendered marketable, the diggers could achieve the same gains legally and without jeopardy to the public health. Besides this the exploitation thus made possible would reduce the stocks in closed areas to about the same level as in the open and the temptation to market polluted clams directly would to a large extent disappear.

3. In the past shellfish purification problems have received considerable attention both in Europe and North America where sewage-contaminated shellfish are recognized as a serious health and economic problem. The classical work in this field was done by Dodgson (1928) who reviewed the problems with great care and described experiments on mussel purification conducted in England.

The first of these was simply the taking of shellstock from polluted water and relaying them in clean areas. The mussels were

found to cleanse themselves but several factors make this system unsatisfactory in England. Few areas could be found near the producing grounds which were not polluted. The alternative, the use of clean areas on the open coast, was ruled out because the distances involved were too great and mussels relaid at such exposed spots were frequently washed away or covered with sand and mud.

As an alternative to the relaying process attempts were made to purify mussels by placing them in tanks of water which contained some active chlorine. It was thought that the disinfecting effect of the chlorine plus the natural eliminative activities of the mussels would be effective, providing of course that the chlorine did not interfere with the normal functioning of the mussels.

Early work seemed to indicate that purification could be effected in 24 hours using a chlorine concentration of from 5 to 10 p.p.m. Dodgson, however, determined that active chlorine in excess of 1 p.p.m. inhibited the muscels, excretory processes making purification impossible. Tests showed that the presence of chlorine in concentrations below 1 p.p.m. did not inhibit the biological processes of the mussels but its use was unnecessary because mussels cleansed themselves in clean water just as well when there was no chlorine present at all.

Dodgson showed that purification of mussels could be assured by holding them in clean water for a period of 72 hours and this led to the adoption on a commercial scale of the Conway purification process for mussels and oysters. This system has been in use now for thirty years and has amply confirmed Dodgson's conclusions.

4. Of much greater importance to the coastal regions of Canada is the work of Renn, (1947) and Dallas and McCarthy (1947) who studied clam pollution problems in the State of Massachusetts. The method favoured by Renn is rather similar to the operation described by Dodgson. He found that clams readily cleanse themselves in clean sea water but that in Massachusetts clean water is so hard to find close to the centres of production of contaminated clams that it is cheaper to provide tanks, as in England, of artificially purified water.

Renn found that clams held in clean chlorine-free water can be depended upon to purify themselves enough for conformity with the Public Health regulations of the State of Massachusetts. The best results of the series of experiments reduced the coliform bacterial count from 350,000 to 23 per 100 ml. in 40 hours.

In addition to the tank purification system described above, Renn also attempted to purify clams by holding them in crates on the beach below low-water mark. His experiment was similar to those conducted at St. Andrews and to be described.

Tennant (1948) describes the results of a clam flushing experiment using a concrete tank of circulating sea water at the

Atlantic Biological Station. He was able to demonstrate cleansing so long as the water was free of contamination but the uncertainty of the quality of the sea water pumped through the Biological Station's tanks made it impossible to conduct a careful study there.

5. A realization of the need in Canada for a cleansing system applicable to soft-shell clams has been growing for some time in the minds of those Fisheries and Public Health officials concerned with the Industry. The Department of Fisheries is interested in developing an economically practicable cleansing system applicable to soft-shell clams and local conditions as we have already pointed out. The shores of the Maritimes are indented with many accessible inlets, many of which are free from sewage pollution. A cleansing system making use of these natural waters should be more economical than one involving high installation and maintenance costs of tanks and pumps.

At the 1951 meeting of the Interdepartmental Shellfish Committee, the realization came to a head. The Committee, including representatives of the Department of National Health and Welfare, and the Department of Fisheries, adopted a motion recommending that the Atlantic Biological Station and the Fish Inspection Laboratory co-operate in 1951 to carry out preliminary experiments in this field. The Fish Inspection Laboratory was further requested to make a bacteriological survey of the region about the Atlantic Biological Station with a view to discovering supplies of contaminated clams for use as experimental stock and of a convenient clean-water area that might serve as a site for the cleansing experiments. Dr. J. C. Medcof of the Biological Station was asked to meet with Mr. W. J. Brownlee and Mr. M. W. Mullan of the Fish Inspection Laboratory and plan and conduct the investigation since they had been advocating it for some time.

During the course of the work which followed, M. W. Mullan was in charge of the Department of Fisheries' Mobile Bacteriological Laboratory which was assigned to the investigation and was assisted by A. B. Williams who was relieved part of the time by D. R. Colwell both Bacteriologists of the Fish Inspection Laboratory. Dr. A. W. H. Needler, Director of the Atlantic Biological Station, acted as a consultant and Dr. J. C. Medcof was directly responsible for the Station's share of the effort which involved several members of the Fisheries Research Board's staff.

SECTION II. LOCALE

A bar joining Minister's Island in Passamaquoddy Bay to the mainland at St. Andrews, N.B. (fig. 1) was selected as the experimental cleansing site. Waters of this area form part of the Bay of Fundy system and are therefore characterized by high tides and low summer water temperatures. The bar is exposed at half-ebb tide, and is regularly used as a private roadway to and from the Island. Strong tidal current sweep the bar when it is submerged except for a brief period at high slack tide. The following characteristics recommend the area as an experimental cleansing site.

1. It is reasonably close (2 to 3 miles) to the Biological Station and St. Andrews, and accessible either by automobile or boat.

2. It is centrally located in relation to considerably well stocked areas of polluted clams. (Oak Bay, St. Andrews Harbour and Magaguadavic River) so a variety of stocks is available for experimentation.

3. There is a vigorous circulation of clear, highly saline seawater over a mud-free bottom.

4. The water shows only traces of sewage contamination.

The bacteriological survey which served as the basis for selection of the bar involved an analysis of water samples from the general region and from nine strategically located sampling stations (see fig. 1). Besides this samples of clams from four of the stations were tested. The results are presented in tables 1 and 2.

The records show that the coliform bacterial content of the water (Table 1) was almost consistently low and might be expected to provide conditions suitable for the proposed experiments.

In all cases the coliform bacterial content of the clams tested was low (Table 2) confirming the conclusion drawn from the analysis of Table 1.

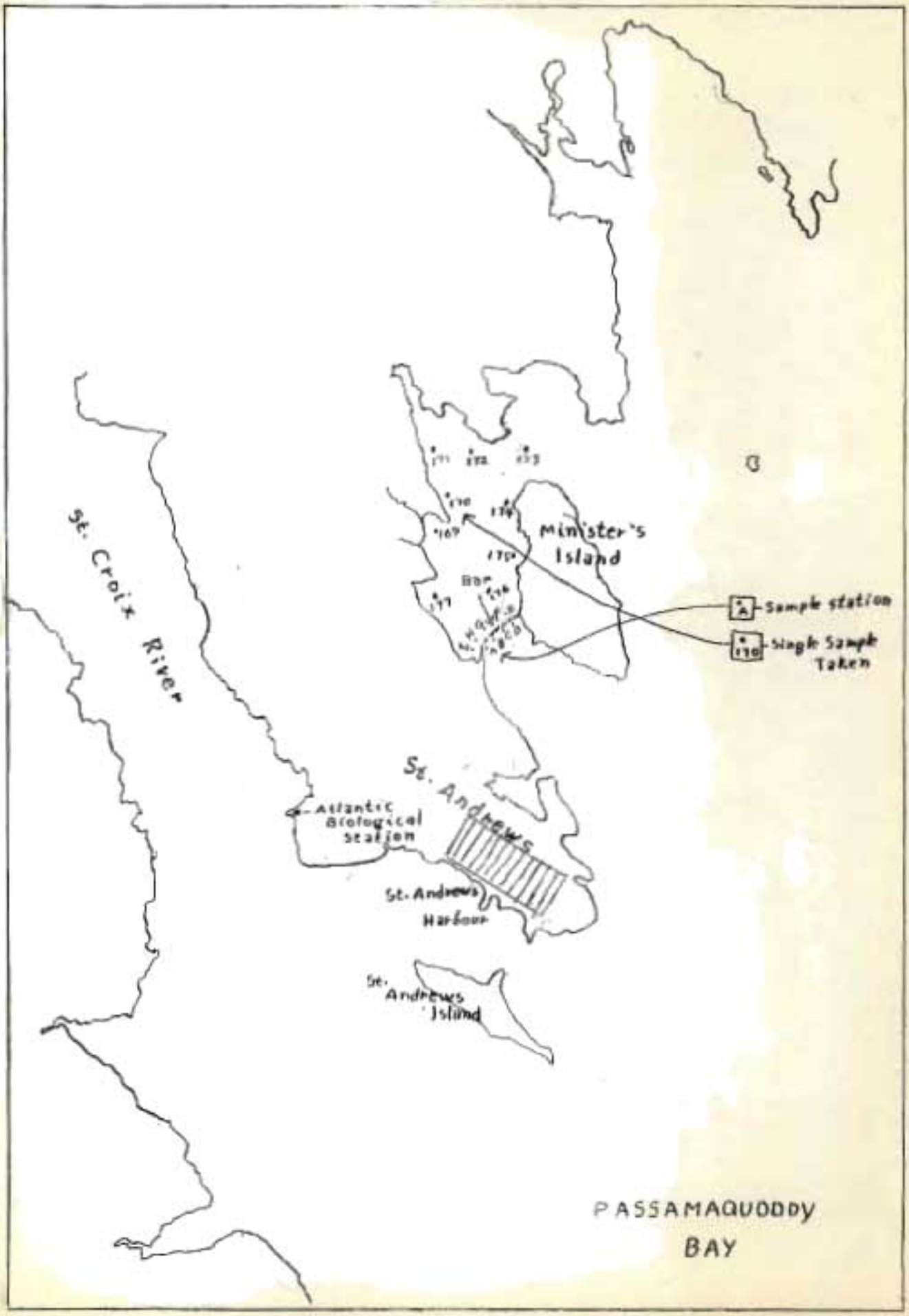

St. Croix River
Minister's Island
Sample station
Single Sample Taken
St. Andrews
Atlantic Biological station
St. Andrews Harbour
St. Andrews Island
PASSAMAQUODDY BAY

Table 1. Results of quadruplicate measurements of coliform bacterial content per 100 ml. (M.P.N.) of water samples taken under different tidal conditions during the bacteriological survey of Minister's Island Bar area, June 27 to July 7, 1951.

Regular Sampling Stations	Half-ebb June 29 M.P.N.	Low-ebb June 27 M.P.N.	Half-flood July 6 M.P.N.	High-flood July 3 M.P.N.
A	neg*	9.1	3.6	neg
	43	23	neg	"
	23	9.1	3.6	"
	3.6	9.1	neg	"
Average	17.4	12.5	1.8	neg
B	23	43	3.6	neg
	9.1	93	neg	"
	9.1	23	"	"
	3.6	23	"	"
Average	11.2	45.5	.9	neg
C	9.1	9.1	neg	neg
	3.6	9.1	3.6	"
	neg	3.6	3.6	"
	"	23	neg	"
Average	3.2	11.2	1.8	neg
D	3.6	neg	neg	neg
	3.6	"	9.1	"
	3.6	"	neg	"
	9.1	"	3.6	"
Average	4.9	neg	3.2	neg
E	neg	neg	3.6	3.6
	"	3.6	neg	neg
	"	9.1	9.1	"
	3.6	neg	9.1	3.6
Average	.9	3.2	5.4	1.8
F	neg	neg	neg	9.1
	"	9.1	"	neg
	3.6	9.1	"	"
	3.6	3.6	"	"
Average	1.8	5.4	neg	2.3
G	3.6	460	neg	neg
	3.6	460	"	"
	3.6	460	"	"
	3.6	460	"	"
Average	3.6	460	neg	neg
H	neg	3.6	3.6	3.6
	"	9.1	neg	neg
	"	9.1	3.6	3.6
	"	9.1	3.6	neg
Average	neg	7.7	2.7	1.8
I	neg	23	3.6	3.0
	"	9.1	neg	neg
	"	9.1	3.6	3.6
	"	3.6	3.6	neg
Average	neg	11.2	2.7	1.7

Miscellaneous Sampling Points (see fig. 1) sampled at half-ebb July 4.

Sample No.	M.P.N.
169	3.6
170	neg
171	"
172	"
173	"
174	"
175	"
176	"
177	"

* This sample taken June 27 - all others in this column on date indicated.

Table 2. Results of triplicate measurements of the coliform bacterial content per 100 ml. clam samples taken from four stations on Minister's Island bar, June 28, 1951.

Sampling Station	Meats and Shell-liquor combined M.P.N.	Meats alone M.P.N.
A	91	2400(?)
	91	750
	230	930
Average	137	1360(?)
B	230	430
	73	230
	91	230
Average	131	297
C	930	930
	930	930
	230	2400
Average	697	1420
D	91	230
	91	230
	neg	91
Average	61	183

SECTION III. DESIGN OF EXPERIMENTS.

Because of the limited facilities and time available, and the empirical nature of the Department's interest in the problem of cleansing at this stage of the study, the experiments had to be few in number and of a simple sort. It was hoped that these would yield information to serve as a guide in the planning of future studies of self-cleansing of clams.

The schedule of the series of experiments was so arranged that the first tests provided the basis for selection of a type of container and a location (submerged or intertidal) that might be used in the subsequent tests to show how rate of cleansing is affected by various factors such as crowding in containers.

The series planned included eight experiments as described by the titles listed in the table of contents of this report.

As a basis for interpretation of the experimental results regular observations on the purity and hydrography of the water at the cleansing station were made throughout the experimental period.

SECTION IV. EQUIPMENT.

1. Experimental Equipment.

Hods. The half-bushel hod (fig. 2) is a container used by clam diggers in Halifax Co., N.S. Its inside dimensions are 24" x 8" x 8". The ends are of solid wood, the sides and bottoms of wooden laths (1¼" x ¼", in cross section) with ¼" spaces between laths. A wire-cloth cover (mesh ½") was added to prevent the loss of clams during the purification period.

Boxes. These containers (fig. 3) were patterned on those used in the Clam Purification Plant at Newburyport, Massachusetts. They are 2' x 12" x 12" (inside dimensions). The sides, bottom and cover are of wire cloth (½" mesh) and the ends of solid wood.

Floating tray. This container (fig. 4) in a larger size is in use on the Canadian east coast for rearing young oysters. Our model measured 3' x 2' x 5". The sides, ends and cover were of wood and the bottom of wire cloth (½" mesh).

Car. This device (fig. 5) was constructed for our experiments from no pattern at all. It was a floating structure 8' x 6' x 2½' with ends and sides of logs and a wooden platform bottom. The top was incompletely covered by planks with wide spaces between. The hods and boxes were made fast in the car with ropes and held firm against the topping planks. The structure floated with its uppermost logs above the surface of the water and it was steady and buoyant enough to allow a man to stand and work on it except when there was a sea running.

Skiff. An oared boat was used as a means of conveyance between the beach and the car anchorage.

2. Laboratory Facilities.

The laboratory used was mobile being a converted house trailler 18' long. A ½-ton panel model truck was used to haul it. This unit is ordinarily used by the Department of Fisheries for "on the spot" bacteriological tests of Shellfish plant water supplies and plant products and on such work it is crewed by a bacteriologist and a technician. The working unit is admirably suited for such work.

For the work described in this report, the mobile laboratory has both advantages and disadvantages. Being mobile it can be moved to the scene of the experiments which cuts down travel time-wastage and reduces the chances for changes which may affect samples between collection and analysis. It enables laboratory personnel to do much of the field work. This greatly reduces liaisson and permits all involved a clearer view of the problems treated. It is

Figure 2. - Hod

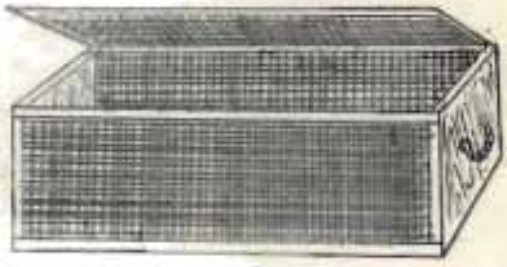


Figure 3. - Box

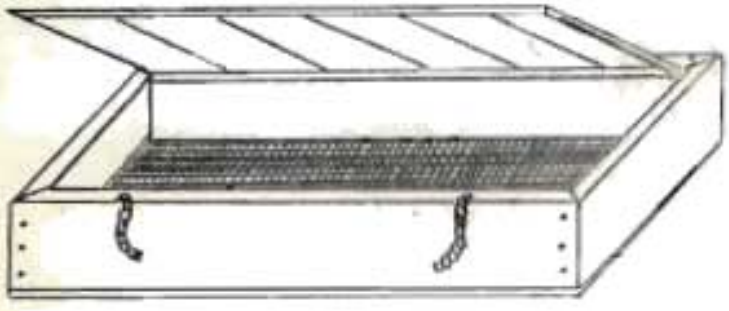


Figure 4. - Oyster Float

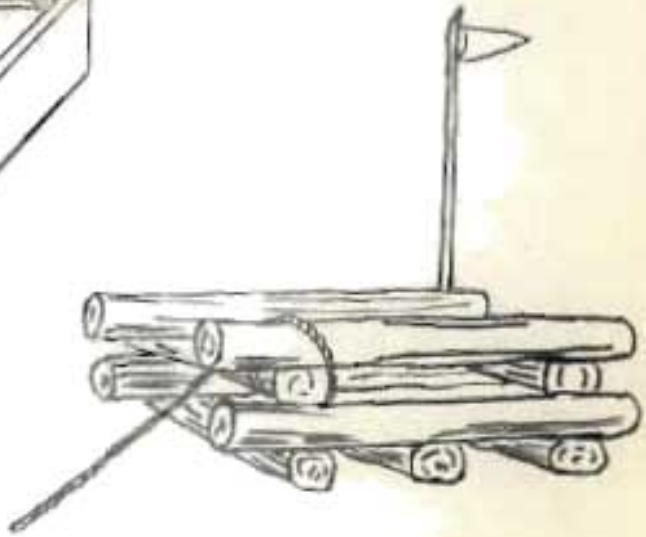


Figure 5. - Car

efficient in other ways. The truck was used not only for hauling the laboratory but also for collecting samples and moving experimental stocks and equipment.

The chief disadvantage of the unit was its small size which limited the size of the staff and the amount of equipment - in other words - the amount of work which could be done in the time available. Fresh supplies of sterile media had to be prepared almost daily and frequent sterilizations of the limited numbers of culture tubes, "blendors" were required. The size of the incubator was another limiting factor. More equipment and a second technician would have greatly increased the work capacity of the unit.

SECTION V. METHODS.

1. Selection of Experimental Stock.

A well-stocked section of beach in St. Andrews Harbour, in close proximity to a sewer outfall was selected as a source of polluted clam stock and all experimental lots of clams were dug from this area by a commercial digger under supervision, and gathered in wire-mesh baskets. The full baskets were immersed and agitated in sea water to remove loose sand and dirt particles adhering to the shells. Broken and dead clams were culled out and only clams ranging in length from 2" to 3½" were used in the tests. To insure comparability of results from samples that might be taken the experimental stocks were thoroughly mixed before they were subjected to treatments. Only a brief interval (2 hours maximum) elapsed between the digging of the clams and the commencement of treatments.

2. Hydrographic Procedures.

The temperature of the sea water at the cleansing station was taken with an ordinary surface thermometer calibrated in °C. Surface water samples for salinity were also taken there at intervals throughout the experimental period and stored in stoppered bottles until the end when salinities were measured by standard hydrometric methods.

3. Bacteriological Procedures.

a. Sample collection. To be sure that the samples taken for bacteriological analysis were representative, the clams constituting them were withdrawn individually and randomly from all parts of the lot being tested. Each sample was placed in a labelled fine-meshed wire basket for transport to the laboratory.

Samples of sea water to check coliform bacterial content were taken aseptically from the cleansing site at more or less regular intervals throughout the experimental period.

b. Analysis of samples. The analytical procedures were essentially those recommended by the American Public Health Association as outlined in "Recommended Methods of Precedure for Bacteriological Examinations of Shellfish and Shellfish Waters". The only departure was in the method adopted for sterilization of the outsides of the shellfish tested.

Because clams are unable to close their shells completely, it was feared that if they were immersed in a bath some of the solution might enter the shell cavity and affect the bacterial content. Accordingly the clams were scrubbed clean in running tap water (chlorinated) from the St. Andrews town supply then rinsed in the same water and placed on paper towels to dry. The lips of the shell were flamed prior to opening the clams. The

adductor muscles were cut with a flamed scalpel and the shell liquor and clam body extracted into a sterile graduated jar. To insure uniformity of treatment the separate steps in the procedure, shucking, blending, innoculating, etc. were performed, wherever possible, by the same person throughout the experiments.

The limitations of the M.P.N. method have been pointed out by Renn, McCarthy and Wright (1945) and the need for large numbers of samples for averaging out its inherent statistical error has been stressed. In the work reported here triplicate samples were analysed in almost all cases. In a few instances, all of which are noted, a reading that departed widely from the average of the other two (ten times or more) has been discarded.

SECTION VI. EXPERIMENT I (Sept. 11-14).

To discover if clams cleanse themselves and if so how method of exposure and type of container affect cleansing rate.

Procedure: Sewage polluted clams were procured and their coliform count determined as described in Section V. They were then exposed without crowding (3 deep) in:

(a) Box in intertidal zone.
(b) Box in car (always submerged).
(c) Hod in intertidal zone.
(d) Hod in car (always submerged).
(e) Floating oyster tray (always submerged).

Samples were withdrawn and tested at 24, 48 and 72 hours. The results are reported in Table 3 and illustrated in Figure 6 together with data from Table 12 which describe the changing M.P.N.'s of the water.

Conclusions:

1. Clams do cleanse themselves and there appear to be three phases in the cleansing process:

A. An initial phase of rapid cleansing marked by a sharp drop in bacterial counts;

B. A phase of no cleansing during which the counts may or may not rise;

C. A final phase characterized by a second rapid cleansing in which the count drops to almost negligible levels. Since samples were withdrawn only at 24, 48 and 72 hours, it is impossible to judge the exact times of the changes of phase.

Suggestions of phases in the cleansing process appear in the data presented by Dallas and McCarthy (1947) but with nothing like the clarity or consistency with which they appear here and in the experiments which follow. Their significance is not clear.

2. The rate of self-cleansing is remarkable in phase A and C, being approximately the same for all treatments. The cleansing rate during phase B is low compared with that in phase A or C and may be in a positive or negative direction. It is not certain whether this is determined by environmental factors such as light conditions or tidal phase or by the physiological characteristics of the shellfish themselves.

3. The extent of the change is as remarkable as the rate. The number of coliform bacteria fell from approximately 8,000 to 1,000 or less (well below the level required for direct marketing of clams from approved areas) within 24 hours (phase A); there was little further change by 48 hours but by 72 hours it had dropped very low, averaging less than 150.

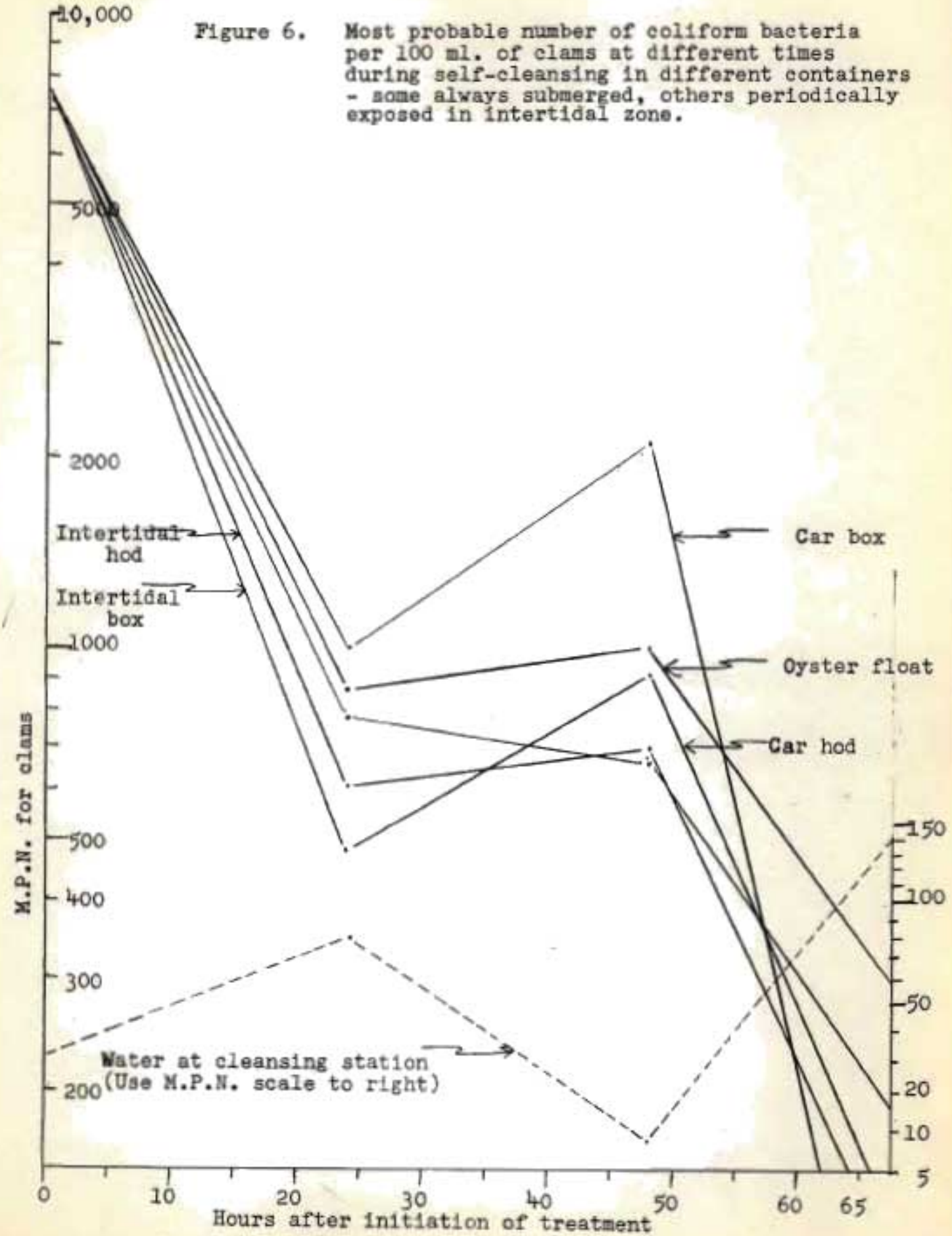
4. The type of container had relatively little effect on the rate or extent of self-cleansing.

Table 3. Coliform bacterial content of clams at various times during self-cleansing in different types of containers - some always submerged, some periodically exposed.

Conditions of test	Initial M.P.N.	M.P.N. at 24 hrs.	M.P.N. at 48 hrs.	M.P.N. at 72 hrs.
Always submerged				
	2200	950	1400	
Hod	16000	230	1100	
	5400	230	230	
Av.	7866	483	910	78
		400	5400	
Box		*16000+	490	
		640	490	
Av.	7866	1020	2126	20
		490	490	
Oyster Float		790	1400	
		1300	*16000+	
Av.	7866	863	945	220
Periodically Exposed in Intertidal Zone.				
		1700	790	
Hod		330	700	
		330	490	
Av.	7866	786	660	68
		230	1300	
Box		490	460	
		1100	330	
	7866	606	696	140

* This result discarded in calculating the average

Figure 6. Most probable number of coliform bacteria per 100 ml. of clams at different times during self-cleansing in different containers - some always submerged, others periodically exposed in intertidal zone.



5. The location of the containers (always-submerged or periodically exposed) had no effect on the rate or extent of cleansing so far as these data reveal.

SECTION VII. EXPERIMENT II (Sept. 19-21).

To determine more precisely the shape of the cleansing curve for clams that are always submerged and those that are periodically exposed to air in the intertidal zone.

Procedure: Experiment I showed that type-of-container has little effect on the rate or extent of cleansing, so the hod (fig. 2), the most convenient container, was chosen for use in all the later experiments. It is the cheapest to build and the easiest to handle of the several types tested.

Polluted clams were procured (as in Section V) and placed three deep in each of two hods. One was secured in the floating car, and the other fastened to driven stakes which held it one foot off the bottom in the intertidal zone.

Before the results of Experiment I were available the sampling programme was planned in anticipation of a regular logarithmic decrease in coliform counts such as that represented by Dallas and McCarthy (1947). Samplings at 2, 4, 8, 16, 24 and 48 hours after exposure were thought adequate and these were carried out for the always-submerged hod because the car holding it was accessible by boat at all times. The hod staked in the intertidal zone, however, was accessible only at times in the tidal cycle when the water level was below the location of the stakes. For this reason the intertidal hod was sampled only at 6, 12, 18, 24 and 48 hours.

The results of the tests appear in table 4 and are illustrated in figure 7 where water conditions at the cleansing station (Table 12) are also described.

Conclusions:

1. As far as they go the results for the always-submerged clams resemble those of Experiment I as regards rate and extent of cleansing except that phase C is not represented because no 72-hour sample was taken.

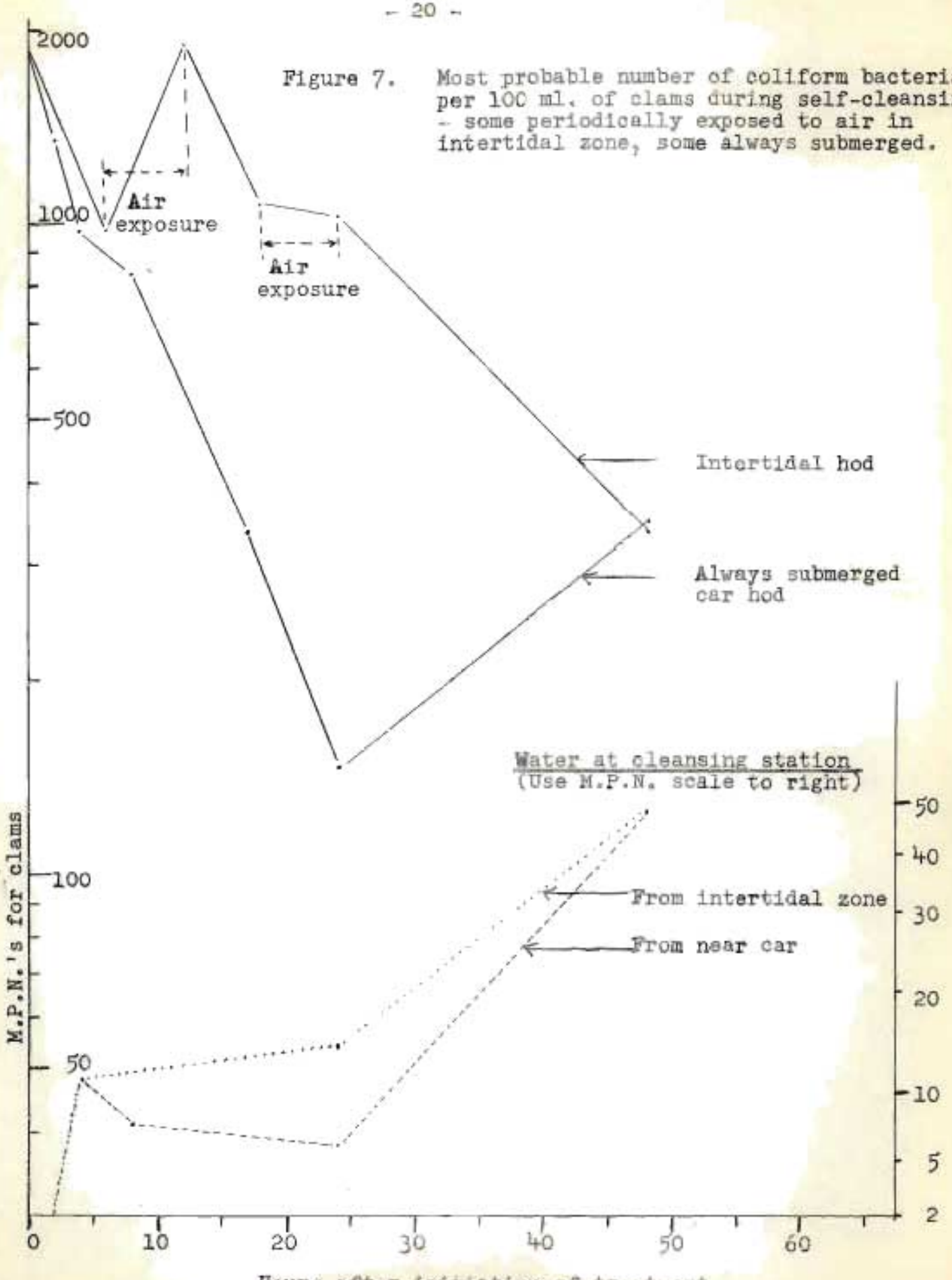
2. Results for the periodically air-exposed clams are not in accord with those for the periodically exposed in Experiment I or for the always-submerged in Experiments I and II. The initial decline seems to have been reversed during the first air exposure and halted during the second. Between 24 and 48 hours the counts show an over-all drop but the data are insufficient to show whether similar changes occurred in subsequent air exposures or whether phase B was eliminated.

3. By the end of the 48 hour cleansing period the counts in both the always-submerged and the periodically air-exposed stocks had reached approximately the same low level. This finding which accords with that of Experiment I in which the initial pollution of the stocks

Table 4. Coliform bacterial content per 100 ml. of shell contents of clams at various times during self-cleansing - some clams always submerged, others periodically exposed in the intertidal zone.

Conditions of test	Initial M.P.N.	2 hrs.	4 hrs.	6 hrs.	8 hrs.	12 hrs.	17 hrs.	18 hrs.	24 hrs.	48 hrs.
Always submerged clams	2100	1700	330		1100		330		130	490
	1700	640	1300		1100		330		78	230
	1700	1700	1300		330		330		230	330
Av.	1833	1346	976		843		330		146	350
Periodically exposed in intertidal zone				330		1700		2200	1400	210
				1300		2200		790	490	460
				1300		1700		220	1300	330
Av.	1833			976		1866		1070	1030	333

Figure 7. Most probable number of coliform bacteria per 100 ml. of clams during self-cleansing - some periodically exposed to air in intertidal zone, some always submerged.



was much higher and in which there were replications of the test, justifies the selection of the intertidal zone as the site for the following experiments in spite of the disparity mentioned in conclusion 2 above.

4. The disagreement just noted may have resulted from characteristics of the sampling schedules of Experiments I and II. In Experiment I no samples were withdrawn either at the beginnings or the ends of any of the air-exposure periods. Had such samples been taken the curves for the two intertidal cleansing tests might have had more in common.

On the other hand the disagreement may be fundamental and attributable to other factors which affect the cleansing process, e.g. intensity of light (Dodgson 1928).

SECTION VIII. EXPERIMENT III. (Sept. 28-Oct. 1 and Oct. 5-7).

To discover the effects of crowding clams in containers on the rate and extent of self-cleansing.

The intertidal area was chosen for this and subsequent experiments on the basis of results from Experiments I and II. The maintenance of staked hods here was so difficult that the car, which provided better holding facilities, was shifted from deep water, moored in the intertidal zone and used throughout to contain the hods. Two kinds of tests were used to show how crowding affects cleansing.

A. Procedure: Polluted clams were obtained as in Section V on Sept. 28. One hod was filled, another half-filled and a third a-quarter-filled and placed in the car.

Samples were withdrawn from each hod at 12, 24, 36, 48 and 72 hours according to the system described in Section V.

The results of this part of the experiment appear in table 5 and are illustrated in figure 8 which also shows the condition of the water (table 12) during the test.

B. Procedure: Polluted stocks were obtained on October 5 as described in Section V and three hods were filled with polluted clams and secured in the car as in (A) above. Sampling was carried out in the following manner. At 24 hours all samples were withdrawn from Hod No. 1. The "top" samples were removed randomly then clams were thrown out until the middle layer was reached. The "middle" samples were then withdrawn and clams thrown out again until the bottom layer was exposed. The three "bottom" samples were withdrawn from this. At 36 hours Hod No. 2 was similarly treated and at 48 hours Hod No. 3.

The results of this part of the experiment appear in table 6 and are illustrated in figure 9.

Conclusions from parts A and B of Experiment III:

1. The degree of crowding seems to have relatively little effect on cleansing except, perhaps, to alter the timing of phase change.

2. All the curves show the three phases in the cleansing process.

3. There is a suggestion, from a comparison of results from parts A and B, that in the heavily contaminated stocks (Part A) the initiation of phase B is delayed about 12 hours. For mildly polluted stocks (Part B) this begins 24 hours after exposure.

4. In all cases but one (A - half-full hod) the coliform count dropped from high levels (9000 or 16,000) to well below the 2400 standard within 24 hours.

Table 5. Most probable number of coliform bacteria per 100 ml. of shell contents of clams at different times during self-cleansing with different degrees of crowding.

Conditions of tests	Initial M.P.N.	M.P.N. at 12 hrs.	M.P.N. at 24 hrs.	M.P.N. at 36 hrs.	M.P.N. at 48 hrs.	M.P.N. at 72 hrs.
Hod ¼ full	16,000	16,000	*16,000+	330	5,400	230
	16,000	16,000	700	700	1,700	490
	16,000	9,200	1,700	230	2,800	*16,000
Av.	16,000	13,733	1,200	420	3,300	360
Hod ½ full		3,500	3,500	2,200	330	230
		16,000	1,700	1,300	9,200	790
		16,000	3,500	700	5,400	1,100
Av.	16,000	11,833	2,900	1,400	4,976	706
Hod full		16,000	1,700	490	700	330
		5,400	1,300	2,800	950	230
		2,400	1,100	5,400	2,400	230
Av.	16,000	7,933	1,366	2,896	1,350	263

* This result discarded in calculating the average.

Figure 8. Most probable number of coliform bacteria per 100 ml. of clams during self-cleansing - some lots more crowded than others.

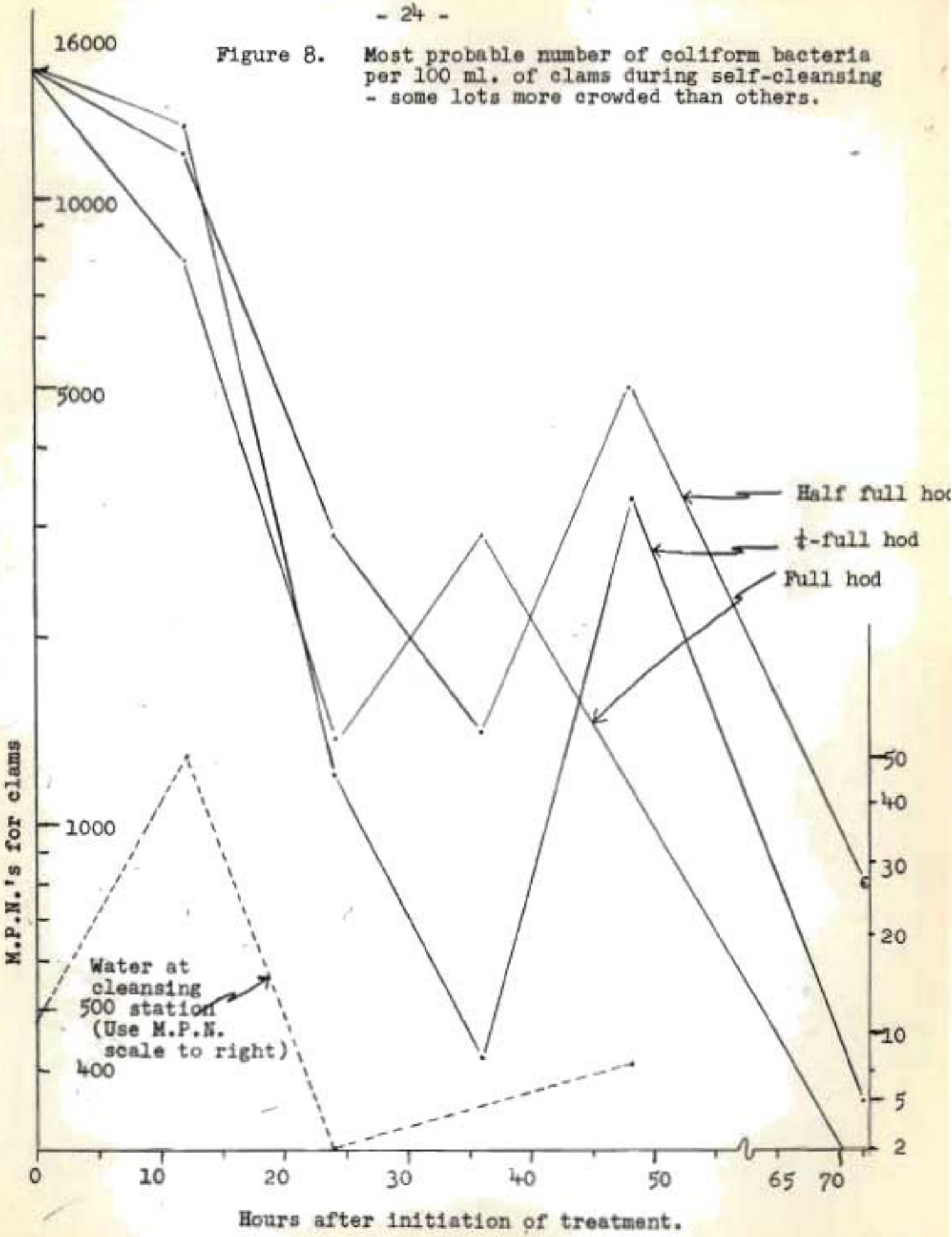
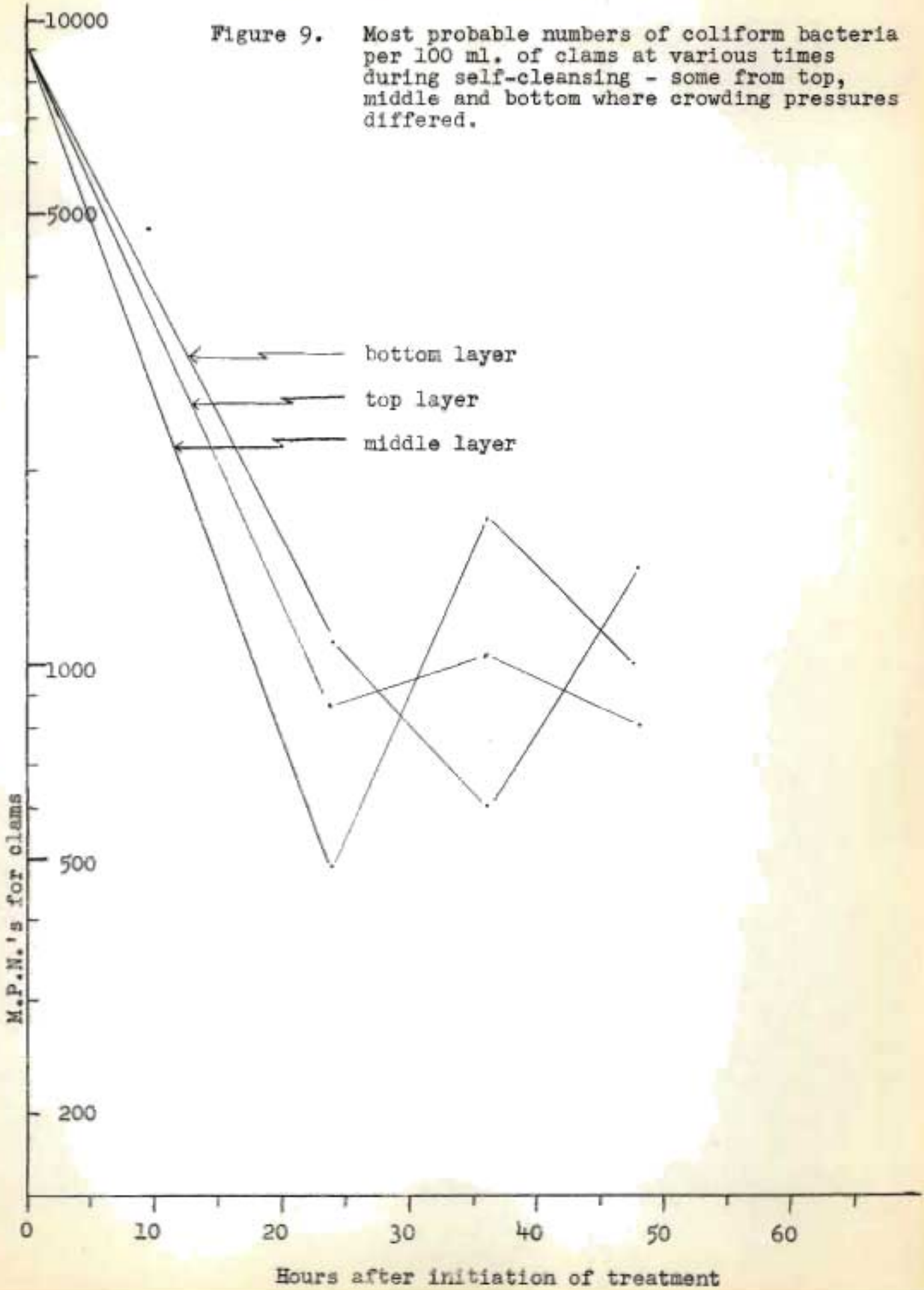


Table 6. Most probable number of coliform bacteria per 100 ml. of shell contents of clams at different times during self-cleansing and at different levels in full hod.

Description of samples	Initial M.P.N.	M.P.N. at 24 hrs.	M.P.N. at 36 hrs.	M.P.N. at 48 hrs.
Top layer	9,200	1,300	1,100	1,700
	9,200	490	1,800	230
	9,200	790	170	490
Av.	9,200	860	1,023	806
Middle layer		310	3,500	330
		460	790	2,200
		700	1,100	490
Av.	9,200	490	1,796	1,006
Bottom layer		790	790	1,700
		2,200	790	2,100
		490	330	460
Av.	9,200	1,093	603	1,420

Figure 9. Most probable numbers of coliform bacteria per 100 ml. of clams at various times during self-cleansing - some from top, middle and bottom where crowding pressures differed.



SECTION IX. EXPERIMENT IV (October 27-30).

To discover the effect of "dug-age" on self-cleansing.

Procedure: Three hodfuls of polluted clams were procured as in section V. One hodful was placed at once in the car moored in the intertidal zone. The other two were stored in a shaded location subject to outdoor temperatures and temperature changes. After 12 hours' storage one of these was placed in the car and after 24 hours' storage the third hod was placed in the car.

The polluted stock was sampled right after it was fished. Hod 1 was sampled after 24 and 48 hours of cleansing. Hod 2 was sampled after 12 hours air exposure and after 12, 36 and 60 hours of cleansing. Hod 3 was sampled after 24 hours air exposure and after 24 and 48 hours of cleansing. The results appear in table 7 and are illustrated in figure 10.

Conclusions:

1. During air storage there was a significant increase in coliform count.

2. Dug-age up to 24 hours did not seem to affect the cleansing rate. The rate during phase A was almost the same in all lots. In the case of the sample with a dug-age of 24 hour phase B was eliminated or its initiation postponed.

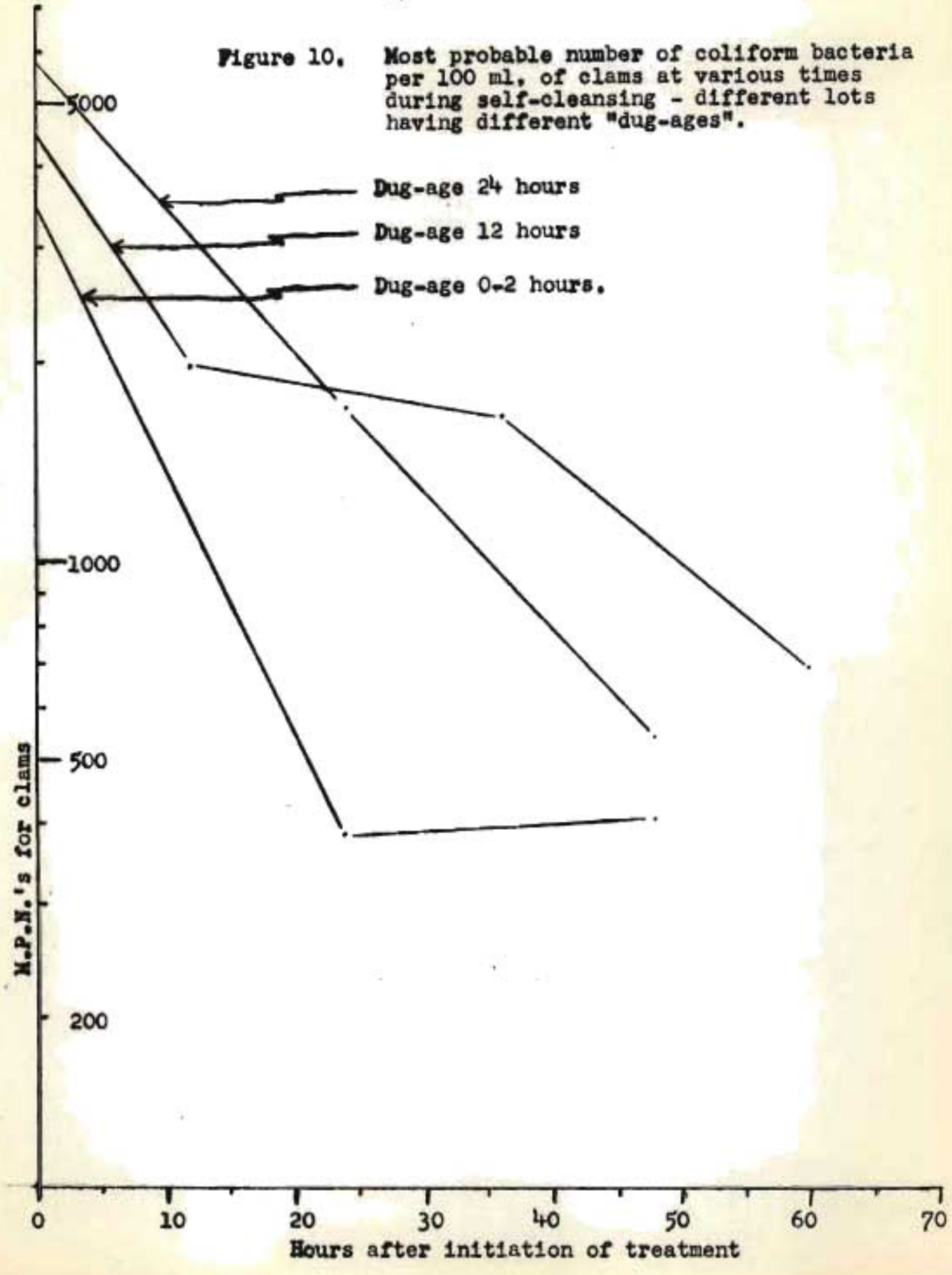
3. The lack of a sample from the stock with a 12-hour dug-age after 24 hours of cleansing is regretable because it prevents a comparison of the initiation times for phase B in the three stocks.

4. Within 24 hours of the beginning of the cleansing treatment the coliform counts fell from as high as 6000 to well below the 2400 standard.

Table 7. Most probable number of coliform bacteria per 100 ml. of shell contents of clams at different times during self-cleansing. The three lots tested had different "dug-ages".

Dug-age of clams	Initial M.P.N.	M.P.N. at 12 hrs. dug-age	M.P.N. at 24 hrs. dug-age	M.P.N. at 12 hrs. cleansing	M.P.N. at 24 hrs. cleansing	M.P.N. at 36 hrs. cleansing	M.P.N. at 48 hrs. cleansing	M.P.N. at 60 hrs. cleansing
0-2 hrs.	3,500				490		490	
	3,500				540		490	
	3,500				110		230	
Av.	3,500				380		403	
12 hrs.		3,500		3,500		3,500		490
		5,400		1,200		460		1,300
		-		950		1,100		230
Av.	3,500	4,450		1,883		1,686		673
24 hrs.			2,200		3,500		490	
			9,200		790		330	
			-		790		790	
	3,500		5,700		1,693		536	

Figure 10. Most probable number of coliform bacteria per 100 ml. of clams at various times during self-cleansing - different lots having different "dug-ages".



SECTION X. EXPERIMENT V (October 22-24).

To discover the effect of preliminary hosing on self-cleansing.

Precedure: Two hodfuls of polluted clams were obtained as described in Section V. One hodful was evenly spread one-layer-deep on a concrete floor sloped to drain and hosed with sufficient pressure to turn the clams over. The other hodful was taken as it came from the beach. It was even denied the usual "wash" in sea water diggers usually give clams. Both lots were placed in the car moored in the intertidal zone. Samples were withdrawn after 24 and 48 hours of cleansing.

The results appear in table 8 and are illustrated in figure 11.

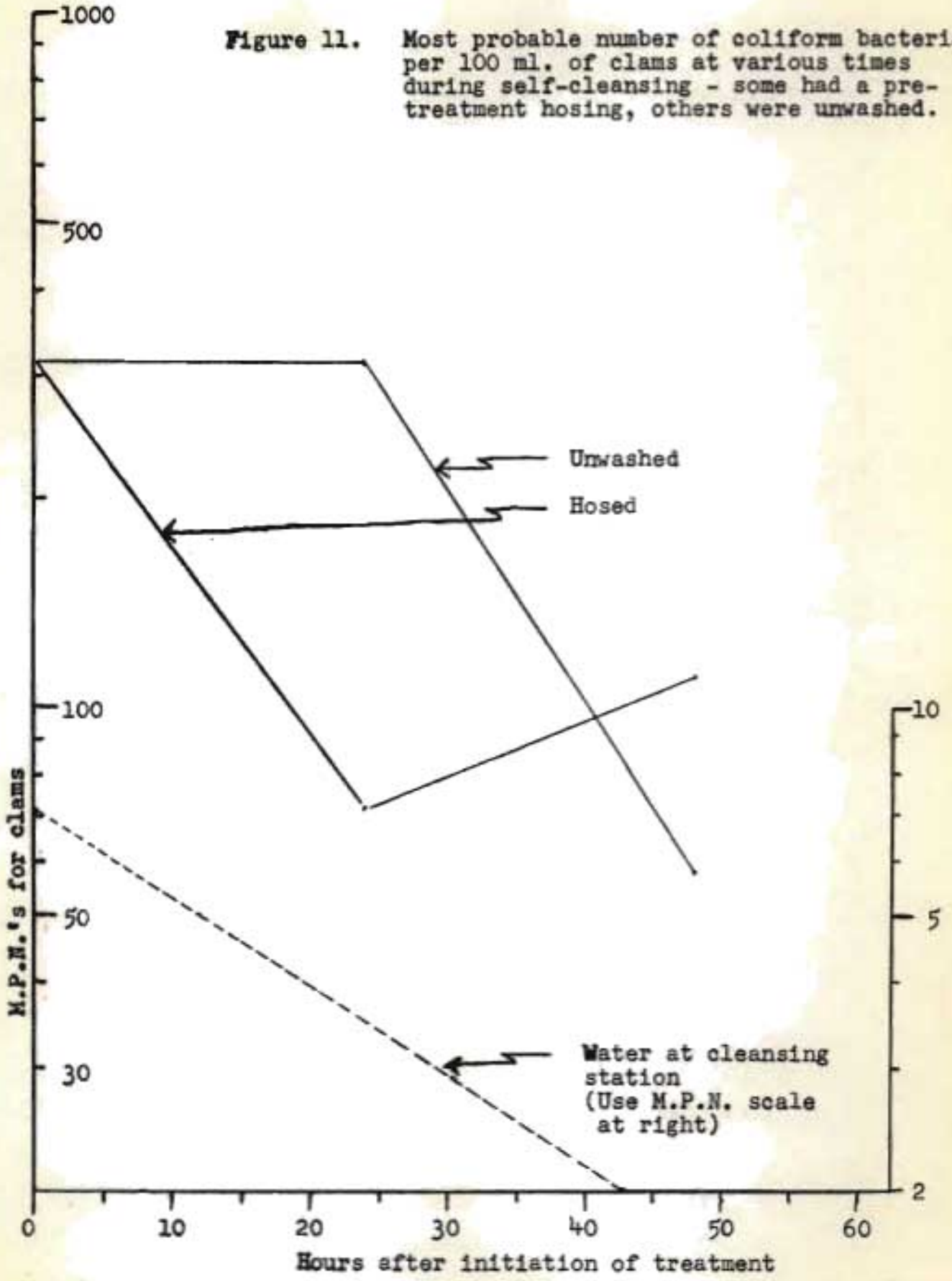
Conclusions:

1. This experiment showed that preliminary hosing hastened the cleansing process. Apparently dirt on the outside of the shells delays the initiation of the cleansing process but once it is initiated it goes on at the same rate as in clams whose shells were "clean" to start with. This finding accords with that reported by Medcof and Campbell (1942) for oysters.

Table 8. Most probable numbers of coliform bacteria per 100 ml. of shell contents of clams at different times during self-cleansing - some clams having been hosed prior to cleansing and others left with mud adhering to shell exteriors.

Description of sample	Initial M.P.N.	M.P.N. at 24 hrs.	M.P.N. at 48 hrs.
Muddy clams	230	230	78
	490	230	20
	230	490	78
Av.	316	316	58
Hosed clams		78	68
		61	140
		78	130
Av.	316	72	112

Figure 11. Most probable number of coliform bacteria per 100 ml. of clams at various times during self-cleansing - some had a pre-treatment hosing, others were unwashed.



SECTION XI. EXPERIMENT VI (October 22-24).

To discover the effect of shell-breakage on self-cleansing.

Procedure: A hodful of polluted clams obtained as described in Section V. Half these were intentionally cracked by knocking them on a small stone. These injured clams were then mixed with the unbroken stock, replaced in the hod and the hod was placed in the car with the two used in Experiment V which was started at the same time and sampled at the same times.

During the analysis of the 24-hour samples it was noticed that the "broken" clams had lost all their shell liquor and the bacterial counts derived were from the "dry" meats. The "unbroken" clams were assayed in the regular way and the counts apply to the whole shell contents - shell liquor as well as meats. At the 48-hour samplings the "broken" stock was treated as before but the shell liquor was deliberately drained off the meats of the unbroken samples and its volume measured. The bacterial counts were made on the "dry" meat samples.

Obviously all the results of the experiment are not comparable as they stand but they can be adjusted so as to justify comparison among themselves and plotting in the same form as other data presented in this report. The basis for this adjustment is our knowledge that the volume of the shell liquor regularly constitutes between 1/2 and 2/3 of the volume of the total shell contents (meat and shell liquor combined) and Tennant's demonstration (1948) that the coliform bacterial count of shell liquor is equal to that of the water from which the clams are taken.

To obtain a value for the relative volume of the shell liquor that would be applicable to the particular stock of clams used in this experiment the volume of the total shell contents of the 48-hour sample of unbroken clams was measured first and then the volume of the shell liquor alone. From this it was found that the shell liquor comprised 9/16 and the meats alone 7/16 of the volume of the total shell contents.

The logic of the adjustment can best be described by assigning symbols to the different values involved, as follows:

a = M.P.N. coliform bacteria per 100 ml. of shell liquor.
b = " " " " " " " meat.
c = " " " " " " " total shell contents.

Because the relative volumes of these three materials are known the following formula should apply: -

$$\frac{9}{16} a + \frac{7}{16} b = c$$

The aptness of this formula was checked by substituting observed values for a and b and obtaining a calculated value for c which was then compared with corresponding observed values of c. Three examples of this comparison are given below using values for a, b and c obtained for the same samples and described in tables 1 and 2.

Example I. Data for water and clam samples taken at Station B (tables 1 and 2) provide the following observed values:

a = 28
b = 297
c = 131

The value 28 for "a" in this case is the average M.P.N. of the low-ebb and half-ebb water samples. As indicated above we are justified by Tennant's findings in regarding this as equivalent to "a" the M.P.N. of the shell liquor.

The calculated value for "c" obtained by substituting in the formula is:

$$\frac{9}{16} \times 28 + \frac{7}{16} \times 297 = \underline{146}$$

The disparity between this and the observed value for "c", 131, is approximately 11%.

Example II. Data for water and clam samples taken at Station C (tables 1 and 2) provide the following observed values:

a = 8
b = 1420
c = 697

The calculated value of "c" is:

$$\frac{9}{16} \times 8 + \frac{7}{16} \times 1420 = \underline{626}$$

The disparity between the observed and calculated value of "c" in this case is 10%.

Example III. Data for clam and water samples at Station D (tables 1 and 2) provide the following observed values:

$$\begin{aligned} a &= 2 \\ b &= 183 \\ c &= 61 \end{aligned}$$

The calculated value for "c" is:

$$\frac{9}{16} \times 2 + \frac{7}{16} \times 183 = \underline{81}$$

The disparity here between the calculated and observed value of "c" is 25%.

The approximation of calculated and observed values for "c" in the three examples cited is well within the limits of the statistical error inherent in the most probable number method itself (Renn, McCarthy and Wright 1945) and justifies the application of the device for the adjustment of the data from Experiment VI which is necessary before they can be compared either among themselves or with the results of other experiments. The calculations have been made and the observed and adjusted values of "b" and "c" appear in table 9 and the adjusted values are illustrated in figure 12.

The data support the following conclusions:

1. It appears that clams with broken shells cannot cleanse themselves. This deduction is justified by the data in either their original or adjusted form.

2. The unbroken clams cleansed themselves in a normal fashion in spite of the presence of broken clams in the same hod with them.

Table 9. The most probable number of coliform bacteria per 100 ml. at various times in the self-cleansing of clams - some with broken and some with unbroken shells.

Condition of clams	Initial M.P.N. (c)	M.P.N. at 24 hrs. (b)	M.P.N. at 24 hrs. (c)	M.P.N. at 48 hrs. (b)	M.P.N. at 48 hrs. (c)
Unbroken shells	230		130	5,400**	
	490		78	78	
	230		130	130	
Av.	316		112	104	47*
Broken shells	(count before damaging)	950		5,400	
		1,400		2,800	
Av.	316	1,175	517*	4,100	1,795*

* These are adjusted values by use of formula as described.
** This value was discarded in calculating the average.
"b" is the M.P.N. for drained meats.
"c" " " " " total shell contents.

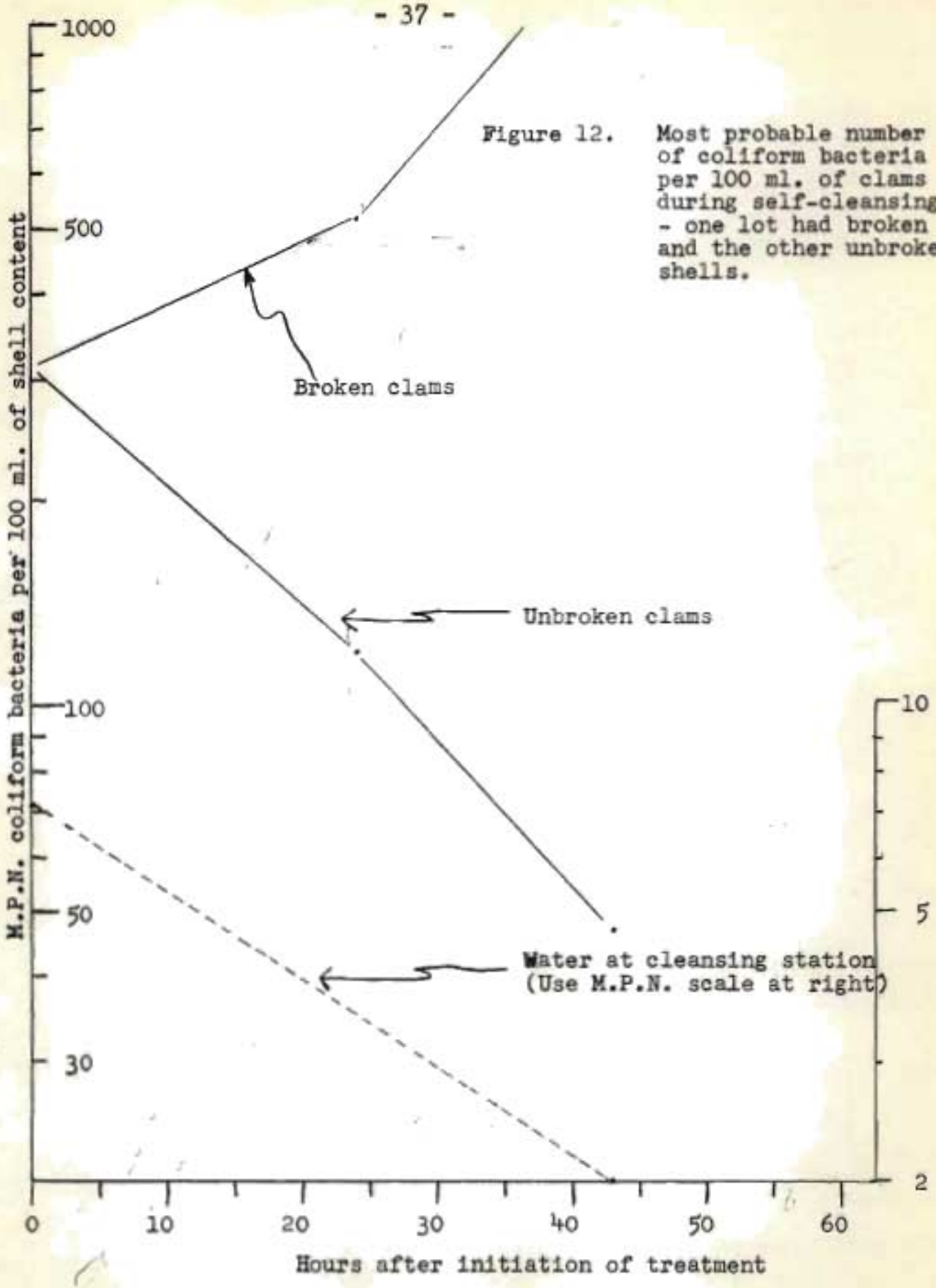


Figure 12. Most probable number of coliform bacteria per 100 ml. of clams during self-cleansing - one lot had broken and the other unbroken shells.

SECTION XII. EXPERIMENT VII

To discover the effect of severity of contamination of polluted stocks on their self-cleansing rate.

Source of data: The original plan called for a separate experiment with the above aim but time did not permit the execution of this part of the plan. However, the desired information may be derived from a comparison of the results of some of the experiments reported above which involved stocks with considerable differences in the initial level of contamination.

The pertinent data and their sources appear in table 10 and are illustrated in figure 13.

Conclusions:

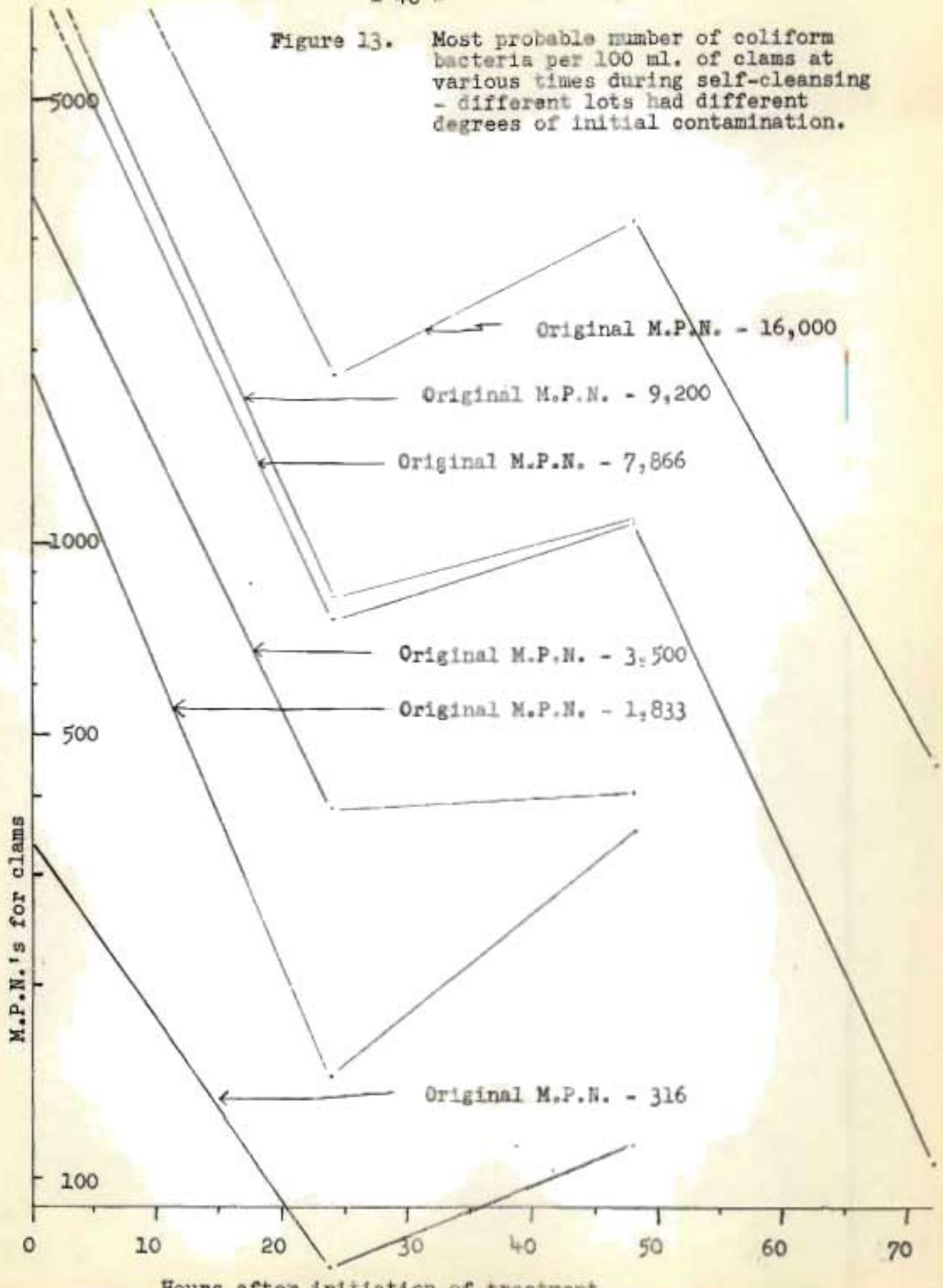
1. The severity of initial contamination has little or no effect on the rate of cleansing but has an obvious influence on the M.P.N. at the end of phase **A** in the self-cleansing process - the less heavily contaminated the stock the lower is the level of pollution reached by the end of phase **A**.

2. Even the most heavily contaminated stocks cleansed themselves of coliform bacteria to a level below the 2400 per 100 mls. within 24 hours.

Table 10. Most probable number of coliform bacteria per 100 ml. of shell contents of clams at various times during self-cleansing - the different lots having different initial levels of pollution.

Source of data	Initial M.P.N.	M.P.N. 24 hrs.	M.P.N. 48 hrs.	M.P.N. 72 hrs.
Experiment IIIA Table 5	16,000	1,200	3,300	360
	16,000	2,900	4,976	706
	16,000	1,360	1,350	263
Av.	16,000	1,820	3,208	443
Experiment IIIB Table 6	9,200	860	806	
	9,200	490	1,006	
	9,200	1,093	1,420	
Av.	9,200	814	1,077	
Experiment I Table 3	2,200	483	910	78
	16,000	1,020	2,126	20
	5,400	863	945	220
	-	786	660	68
	-	606	696	140
Av.	7,866	751	1,067	105
Experiment II Table 4	2,100	130	490	
	1,700	78	230	
	1,700	230	330	
Av.	1,833	146	350	
Experiment IV Table 7	3,500	490	490	
	3,500	540	490	
	3,500	110	230	
Av.	3,500	380	403	
Experiment V Table 8	230	78	68	
	490	61	140	
	230	78	130	
Av.	316	72	112	

Figure 13. Most probable number of coliform bacteria per 100 ml. of clams at various times during self-cleansing - different lots had different degrees of initial contamination.



SECTION XIII. EXPERIMENT VIII.

To discover the effects of different methods of preliminary storage of polluted clams on their self-cleansing.

Time and weather conditions did not permit the carrying out of this experiment.

SECTION XIV. DISCUSSION AND GENERAL CONCLUSIONS

Experimental conditions.

The conditions under which these experiments were conducted may have conditioned the results and it is worth examining them from this point of view.

Hydrographic conditions:

The results of the hydrographic observations (See Section V) are listed in table 11.

Table 11. Surface hydrographic observations at cleansing station (Bar Road).

Date 1951	Time in hours	Water level & phase of tide	Water temperature °C.	Salinity p.p.m.
Sept. 11	16.45	½R	17.9	31.8
12	13.50	L.F.	16.9	31.6
13	10.15	H.F.	17.6	30.1
	14.15	L.F.	17.5	31.3
14	15.30	L.F.	17.8	31.7
19	11.00	½R	--	32.3
	15.00	H.F.	14.9	31.7
	19.00	L.F.	--	32.3
20	04.00	H.F.	--	31.4
	11.00	½R	15.5	31.0
	12.00	H.R.	16.0	31.9
21	11.00	L.R.	14.8	31.7
25	15.45	½R	15.9	32.2
25	22.30	½F	14.2	32.1
28	17.00		14.5	32.3
29	17.00	½R	12.2	31.9
30	08.00	½R	10.0	32.5
	16.45	½F	13.0	32.3
Oct. 22	--	½F	15.0	33.3
24	--	--	12.2	32.2
27	13.30	½F	11.1	32.9
29	15.45	½F	10.0	33.3
30	18.00	L.R.	11.0	32.9
31	18.00	L.F.	10.0	31.6

L = low: H = high: R = rising: F = falling: the fractions refer to the water level H = 1, L = 0, being the limits.

Table 11 shows that the salinity of the water fluctuated very little during the course of the experiments and it may be

assumed that it had no significant effect on the results.

The water temperature, on the other hand, did drop more or less steadily from the beginning to the end from about 18 to 10°C. Associated with this temperature drop there was a more or less regular drop in the level of initial pollution of the experimental stock but it must not be assumed that the two are causally related. It might be expected that the temperature change would affect the cleansing rates but the slopes of the cleansing curves for the first- and last-performed experiments (figures 6 to 13) are essentially the same. From this we may conclude that temperature differences encountered had relatively minor effects. This deduction accords with that of Dallas and McCarthy (1947) who report that "best results were obtained with temperatures slightly over 60°F. (15.5°C.) and that when the temperature dropped to 50°F. (10.0°C.) purification suffered". The lowest temperatures encountered in our tests were, therefore, at about the level where, according to Dallas and McCarthy, they might have begun to take effect on self-cleansing rates but not lower.

Sanitary condition of water at the cleansing station:

The results of bacteriological analyses of water samples appear in table 12.

Table 12. Observations on pollution of water at cleansing station.

Date 1951	No. of experiment	Hours from beginning of experiment	Water level and phase of tide	M.P.N. of water: Intertidal zone	M.P.N. of water: At car
Sept. 10			L.R.	0	-
" 10			L.R.	0	-
" 10			L.R.	0	-
" 11	1	0	½R	30	-
" 12	1	24	L.F.	79	0
" 13	1	48	L.F.	6.8	0
" 14	1	72	L.F.	140	140
" 19	2	0	L.F.	0	0
" 19	2	4	H.F.	11	11
" 19	2	8	½R	-	7.8
" 20	2	24	L.F.	14	6.1
" 21	2	48	L.R.	49	49 *
" 25	-	-	½R	4.5	
" 28	3-A	0	L.F.	11	
" 28	3-A	12	L.F.	49	
" 29	3-A	24	½R	2	
" 30	3-A	48	½R	7.8	
Oct. 22	5&6	0	½F	7.8	
" 24	5&6	48	L.F.	2	
" 27	4	0	½F	0	
" 29	4	36	½F	9.2	
" 30	4	60	L.R.	0	
" 30	4	72	L.F.	0	

* After this date the car was moved from deep water and moored in the intertidal zone.

For the most part table 12 records show little difference between deep water and water in the intertidal zone. Besides this they agree with the results obtained in the July survey (see Section II) in showing consistently low coliform counts. There are two notable exceptions, the significance of which is discussed below.

1. On September 12, 1951, during Experiment I there was a rise in the coliform count of the water from 32 to 79 that might have affected the shape of the cleansing curves in figure 6 descriptive of Experiment I. It might be argued that the phases in the cleansing process are artifacts introduced by changes in the coliform count of the water either by immediate or delayed action. Such an explanation is not acceptable, however, because the same phases show clearly in other experiments (e.g. Experiment III, table 5, figure 8) even though there were no such accompanying changes in the coliform count of the water.

2. The second important change in the M.P.N. of coliform bacteria in the water was on September 14, 1951, 72 hours after the initiation of Experiment I when the M.P.N. rose to 140. In spite of this change the slopes of the cleansing curves in phase C were not altered and the coliform counts of the clams actually fell to values that were lower than 140.

From this examination it would appear that the variations which occurred in the M.P.N. of coliform bacteria in the water at the cleansing station during the experimental period had no serious effect on the results.

Phases in self-cleansing:

The results of these experiments have demonstrated phases (see Experiment I) in the self-cleansing process which have not been reported by earlier workers in this field although the graphs presented by Dallas and McCarthy (1947) may be interpreted as exhibiting these features. There is nothing to suggest that they are conditioned by the environment from which it might be deduced that they are controlled by the clam's physiological processes that have yet to be investigated. Such an investigation was not attempted in this study which was designed solely to discover empirically what changes take place in the coliform counts in clams during the cleansing process.

Practicability of results.

It is clear from what has been done that even grossly polluted clams that have intact shells are capable, by some means, of ridding themselves of the greater part of their bacterial load. Within 24 hours of exposure to relatively clean sea water their coliform counts dropped to very low levels. In the second period of twenty-four hours the counts usually maintained the twenty-four-hour level or rose slightly. In the third period of twenty-four hours there

was a second drastic drop in the counts so that at 72 hours the pollution was negligible. The results indicate that the cleansing rate is not significantly different in stocks held in the inter-tidal zone and in those kept always submerged. Furthermore, cleansing rates are about the same for clams held in a variety of types of containers. In other words cleansing of grossly polluted clams can be regularly affected by holding them for a relatively short period in clean water. Furthermore the simplicity of the handling methods and of the containers required suggests that this type of cleansing could be carried on economically on an industrial scale.

The likely economic feasibility of application of these findings is only one aspect of the problem. It must also be viewed from the public health standpoint and this raises the whole question of the public health significance of the level of coliform bacterial pollution in self-cleansed clams. Different points of view have been expressed.

In our understanding the choice of the M.P.N. coliform level of 2400 per 100 ml. of shell-contents of clams is based on the assumption that the viability of pathogenic sewage bacteria and coliform bacteria is approximately the same, that the relative abundance of coliform and pathogenic types is roughly the same at all levels of abundance and that the chance of occurrence of dangerous numbers of pathogens in clams that have a coliform count of 2400 or less is so slight that the consumption of such clams is not dangerous. On the basis of these assumptions it is unimportant whether the 2400 level is achieved after a recent buildup from lower counts to this level just before marketing, whether it had maintained itself there for a long period before marketing or whether it had recently declined from higher levels to the 2400 count just before marketing. In other words, "the level is the thing" - If our assumptions are justified clams with a 2400 coliform count should be equally safe whether they are marketed directly from the flats or after they have passed through a cleansing process during which the count has been reduced from higher levels. This view is supported by the British investigators and administrators - to quote Sherwood (1947) who has continued the work of Dodgson (1928). When "mussels were immersed in a supply of uncontaminated sea water, whether by the handful or in bulk, they could be relied on to rid themselves of whatever objectionable material they might contain and become perfectly safe for human food within 48 hours". It should be stressed that the sole basis for judging the purity of these shellfish was the count of coliform bacteria and that the same treatment and assessment methods have been applied by the British for many years in cleansing oysters.

Unless we are mistaken, the demonstrated self-cleansing of highly polluted clams should render them safe for market if their coliform count can be reduced to 2400 or less.

SECTION XV. RECOMMENDATIONS

Provided that self-cleansing as just described is acceptable, then the results of the experiments reported here warrant the following recommendations:

1. That the bacteriological investigations be continued in 1952 to discover (a) How season, light-intensity, hydrographic and other conditions affect self-cleansing.

(b) The basis for the phases observed in the self-cleansing process. A complete understanding of these might admit of improvements in treatment methods.

2. That investigations to discover the best means of industrial application of our findings be carried out by the operation of a semi-commercial pilot plant in 1952.

SECTION XVI. REFERENCES

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