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1952 Clam Cleansing Studies (Mya) -

Combined Reports

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I. INTRODUCTION

by

J. C. Medcof

In 1951 several experiments on self-cleansing of soft-shelled clams (*Mya arenaria*) were conducted co-operatively at St. Andrews, N.B., by the Fish Inspection Laboratory, Department of Fisheries and the Fisheries Research Board of Canada. These are described in Manuscript Report of the Biological Stations No. 440 (Fish. Res. Bd. Can.). The results showed that under certain conditions heavily sewage-polluted clams cleanse themselves. This encouraged belief that inexpensive handling methods could be developed that would permit cleansing of large volumes of clams to such an extent that they would be fit for consumption.

There are considerable stocks of polluted clams in the Maritimes and it would be doubly advantageous if these could be cleansed on an industrial scale and marketed without risk to the public health.

(1) The income to the clam industry would be increased.

(2) The removal of dense stocks now growing in contaminated areas would to a great extent eliminate the health hazard that clams "bootlegged" from these sources now represent.

After a review of Report No. 440 in March 1952, the Interdepartmental Shellfish Committee recommended further co-operative investigation designed to improve our understanding of the cleansing process, and to develop a suitable system for treating commercial-size lots of clams. This recommendation has been acted on with participation by four different agencies as the title page shows. The work was all done in the St. Andrews, N.B., area.

Investigations by the Fish Inspection Laboratory covered the periods April 4 to 29, July 1 to August 6, September 15 to 30 and October 31 to November 3; by the Laboratory of Hygiene, April 28 to June 30 and by the Public Health Engineering Division, September 8 to 10. The Fisheries Research Board investigators were active throughout this period not only with the work they did and reported on more or less independently but also in assisting the investigators of the co-operating agencies wherever possible, by supplying boats and equipment, purchasing materials and by reviewing and co-ordinating the results of the several programs as they were assembled. Their final chore was the compilation, editing, production and distribution of this report.

In editing the report the pagination and table and figure numbering has been made sequential throughout. It is believed that this will avoid the confusion a reader might encounter if there were, say, three different table 1's in the report. For the same reason, the numbers of figures have been made to conform with those of the tables whose data they illustrate. In following this convention some figure and table numbers are skipped over, e.g. there are no figures numbered 8 to 11 (inclusive).

It is believed that the efforts of the several persons involved in these studies have improved our understanding of the cleansing process in an important way and shown the feasibility of industrial application of the principles involved.

It would be wrong to close this section without a statement of the editor's appreciation of the amicable relations he has had with all those who participated in the work and of his praise for their interest and self-sacrificingly industrious efforts in conducting their programs.

II. DESIGN AND OPERATION OF A CLAM-CLEANSING CAR

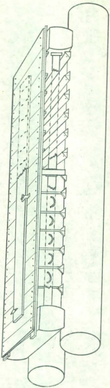
If successive commercial-size lots of polluted clams are to be exposed for several-day periods in clean water for self cleansing, some holding device is required to maintain them there and some regular scheme of handling must be followed to make use of it. Last year's work (Mullan et al 1952) showed that half-bushel wooden hods of the type used in Halifax County, N.S. (Medcof and MacPhail 1952) afford good conditions for cleansing. It also showed that a floating car for holding groups of hods is preferable to fixed shore installations. From this beginning the floating car (See text figure) was developed in the spring of 1952 by the Fisheries Research Board.

This 26-hod model with which we worked was cheaply made of seasoned wood to avoid any possible adverse effects that sap or resin leeching from green wood might have on clams held in it. It was large and sturdy enough to be stable in small seas, to bear a man's weight even when it was fully loaded with clams and to withstand the strain that comes with "grounding-out" in the intertidal zone in choppy seas as the tide drops. At the same time it was not too large to be towed by one man in an oared boat as need required.

The hods were held at such a level that the clams in them were always submerged when the car was afloat. They were loaded or unloaded in two tiers from opposite sides by sliding them into or out of spaces provided for them. Guides at the corners kept the hods in position. A retaining screen (¼" mesh) above prevented clams from being washed out and a locking bar over the free ends of the hods prevented them from working out. A special locking device was added later. Pins dropped through holes in the plank cover engaged with the inner ends and prevented removal of the hods. The pins were covered and kept in place by a hinged board that could be locked in position.

With the great amplitude of the tide in the St. Andrews area it was found possible to moor the car in such a position over a firm part of the beach that a motor truck could be backed down to it at low tide for loading and discharging. This position, however, involved a long inter-tidal, air-exposure period for the clams being treated and it was agreed that it was preferable to moor the float further out so that the air-exposure period during treatment would be short, then move the car shoreward at high tide just before it was planned to load or discharge it.

The car was moored from either or both ends so that the long axis was parallel to the tidal currents. This meant that the water percolated through the hods sideways providing what was considered to be good conditions for bringing clean water to the animals



being cleansed and for clearing away the products of cleansing. The provision of spaces between hods and the complete openness of the bottom of the car, except for the main timbers and hod guides, seemed to provide the same facilities. The only obstruction to water passage into or out of the tops of the hods was the screen cover. The gentle up-and-down motion of the car in the waves seemed to produce a regular sluicing of the clams without disturbing them sufficiently to interfere with their siphoning of water.

Basically the design of the car proved satisfactory but some difficulties were encountered and modifications have been suggested to circumvent them. The hods sometimes seized in their grooves. This difficulty could be overcome by more precise carpentering of hods and guide bars or by changing the design of the car to enable loading and unloading from above. The planked top could easily be divided into doors hinged down the mid-line or thereabouts. The hods could be set into place and held there, as they are now, by screening but it would have to be fixed to the inside of the door and the door would have to be locked. It is questionable whether such a change would cure more ills than it would create.

A good device for barring cars against thieves might or might not be needed in a commercial operation because even the best-constructed cars would require constant supervision by a watchman.

An improved system of mooring seems desirable and a "running line" with pulley attachments would seem the simplest solution. This device allows someone on land to haul the car shoreward or seaward so long as it is afloat.

The use of a car of this kind permitting the cleansing of clams in the same containers in which they were dug has great advantages in keeping breakage losses low (Medcof and MacPhail 1952). It would require, however, some modification of the present digging practices in Charlotte Country where sacks are in general use. It is desirable that this practice be abandoned on economic grounds so if cleansing ever comes into vogue the introduction of the use of hods should not be disturbing to those affected.

References

Medcof, J. C. and J. S. MacPhail. Breakage - the bug bear in clam handling. Fish. Res. Bd. Can., Prog. Rep. (Atl.) 54:19-25, 1952.

Mullan, M. W., A. B. Williams and D. R. Colwell. Preliminary experiments in the self-cleansing of clams (Mya arenaria). Fish. Res. Bd. Can., MS. Rep. Biol. Stns. #440, 1952.

III. EXPLORATORY EXPERIMENTS IN THE SELF-CLEANSING OF CLAMS (Mya arēnaria L.)

by

M. W. Mullan and A. B. Williams

SECTION I INTRODUCTION

1. Objectives

That portion of the 1952 clam investigation carried out by the Fish Inspection Laboratory in co-operation with the Atlantic Biological Station had three principal objectives. The first was to discover whether clams taken from sewage polluted flats and transferred to non-polluted waters, under both early spring and mid-summer conditions, could be relied upon to cleanse themselves of coliform bacteria, as such clams did under fall conditions in 1951 (1). The second was to test again for the effect of "dug-age" on the self-cleansing of clams. The third was to assess again the effect, on the rate of self-cleansing, of hose-washing the polluted stocks prior to transferring them to the cleansing station.

In addition to these, the objectives included the gaining of information on the mechanism of self-cleansing. The practical importance of such information was felt to be the light it could throw on the sanitary significance of the process. The term self-cleansing, as ordinarily used, has meant the process by which polluted clams reduce their coliform bacterial count to 2400 per 100 ml. or less, a level considered to indicate, in clams taken directly from the ground, edibility without serious danger from sewage borne pathogenic bacteria. In setting this 2400 level for directly fished clams it was assumed that there was a maximum probable ratio between the numbers of coliform bacteria and intestinal pathogens found in clams, and that the ratio was such that a meal of clams having 2400 coliform bacteria per 100 ml. of shell contents would never carry intestinal pathogens in sufficient numbers to produce disease. If the mechanism of self-cleansing of polluted clams were shown to be such that it would reduce the numbers of intestinal pathogens to the same extent as it does coliforms, there could be no doubt that the pathogen coliform ratio and the 2400 standard based on this ratio are as applicable to self-cleansed as to directly fished clams.

In this connection it may be well to acknowledge that there are justifiable doubts as to the soundness of the bacteriological basis used in setting the 2400 level. These are capably dealt with by Renn et al (2) and need not be discussed here. Examination of the basis for selection of acceptable bacteriological standards for directly fished clams is not a purpose of this work but examination of the mechanism of self-cleansing for the purpose of discovering whether it is such that the edibility of both self-cleansed and directly-fished clams can rightly be judged by the same standards, is among the purposes of this work.

It is true that such comparability is already assumed by those governments whose public health regulations permit the marketing of self-cleansed shellfish. Moreover there have been no reported cases of disease traceable to the consumption of such shellfish in the regions so governed. The Canadian health regulations have for some years permitted the marketing of oysters cleansed by the re-laying method and the consumption of these has never been reported to have caused disease. According to Dodgson (5) and Sherwood (10) the British records show no cases of intestinal disease traceable to the consumption of self-cleansed mussels. Similarly the American records show no evidence of disease having been borne by self-cleansed clams. Nevertheless experimental evidence either for or against the bacteriological comparability of self-cleansed and directly-dug clams would assist in removing some of the uncertainty characteristic of present day shellfish bacteriology.

In seeking such evidence there are certain approaches which suggest themselves. One is the quantitative testing for intestinal pathogens during self-cleansing of polluted clams. Another is laboratory comparison studies of multiplication and survival of different sewage bacteria under environmental conditions existing in clams. Yet another is the carrying out of experiments designed to reveal information on the relationship between the clam's physiological mechanisms and self-cleansing. Because of the time-consuming techniques required for the identification and counting of intestinal pathogens the first two approaches are impracticable in field experiments. The third approach is the one most easily incorporated in a program of field work, and is the one used in the experiments herein reported.

It may be well to consider at this point the possible significance certain kinds of bacteriological information that might be sought, would have in our thinking about the general problem of protecting the public health.

(1) If the reduction in coliform bacterial counts were shown to be brought about by the action of bacteriophages or some other bacteriocidal agent or agents specific for members of the coliform group of bacteria, there would be no guarantee that a parallel reduction of sewage borne pathogenic bacteria takes place.

(2) On the other hand, if the reduction of coliform bacteria were shown to be brought about by the inability of these bacteria to compete with other species of the flora under the conditions of temperature and salinity existing in the clams at the time of

self-cleansing it would be reasonable to assume parallel reductions in the counts of other bacteria such as intestinal pathogens having the same environmental requirement as bacteria of the coliforms group.

(3) Similarly if the mechanism of coliform bacterial reduction were shown to be one of physical removal of these bacteria through the action of water and food passing through the clam, the mechanism could be relied upon to carry off equal fractions of all sections of the bacterial population.

(4) Physiological information as to the clams feeding, digesting and excreting processes could not fail to throw some light on the mechanisms responsible for self-cleansing. Some information of this sort can be obtained from biological literature.

In the time available for the 1952 clam cleansing investigation only a portion of the desired information could be sought. Enough was learned to justify advancing an hypothesis as to the mechanism of self-cleansing. The hypothesis is dealt with in the final section of this report.

2. Allotment of Time

The month of April was devoted chiefly to investigating the effects of early spring conditions on clam cleansing, and most of July to studying clam cleansing under mid-summer conditions. It proved necessary to spend considerable time ascertaining the suitability of the Holt's Point area for the location of a clam cleansing station. Periods in early August and late September were employed for the necessary bacteriological surveys of this area.

3. Methods

It is worth while to review the methods with the purpose of ascertaining their efficacy in yielding conclusive results. Since most of the results are concerned with differences between different lots of clams as regards their contained number of coliform bacteria, and are expressed in terms of Hoskin's Most Probable Number tables, (3), the review should be directed at the clam sampling program, the Most Probable Number method, and its applicability to bacteriological analyses of shellfish.

(a) Sampling methods - Each clam sample analysed consisted of twelve clams ranging in overall length from two to four inches. In the work carried out prior to July 1952, sufficient clams were used from each of these samples to make 100 ml. of shell contents. This 100 ml. was diluted with 100 ml. of sterile tap water, the mixture blended in a Waring Blendor and so on according to the practice recommended by the U. S. Public Health Service (4). After

July 1st, 1952, the twelve clams of each sample were shucked into a sterile Blendor, and the tap water dilution, at this stage of the procedure, eliminated. The resulting suspension was as easily manipulated as its diluted predecessor had been, a possible source of error (in the diluting) was eliminated, and the procedure simplified. The number of individual clams contributing directly their effects on the results, was kept constant. Considering the likelihood of individual variation among the clams, this uniformity in numbers of clams was felt to be more important than uniformity in volume of shell contents. Moreover the change in method obviated the frequent necessity of using only part of the shell contents of a clam in order to obtain a volume of exactly 100 ml. In view of the likelihood of uneven distribution of the clam's bacterial load, arbitrary division of the shell contents seemed unsound.

No attempt was made to discover the extent of variations as to coliform bacterial load among individual clams at different stages of self-cleansing. The number of micro-blenders which could be made available for such work was insufficient, and more urgent and readily carried out lines of investigation were followed.

(b) Bacteriological methods - The Most Probable Number Method for the ennumeration of coliform bacteria is based on a probability curve representing the number of coliform bacteria in a water sample as determined by the geometric series dilution and lactose fermentation method, (3).

It has been shown by Halvorson & Ziegler, (2) that, assuming completely uniform suspension of bacteria in the sample (after blending in case of clams) and perfect technique on the part of the analyst, any observed M.P.N., may vary between 30% and 360% of the true M.P.N., when the five-tube-per-dilution method is used. These investigators state that their calculated range of error applies in 97% of cases, and that in the other 3% the observed M.P.N., lies beyond the limits of this 30% to 360% range.

Renn et al (2) considered that other unavoidable errors in the preparation of shellfish samples further widens the range of eventual error, for instance, differences in the true M.P.N., of different samples from the same lot of clams, resulting from variation in the actual concentration of coliform bacteria in individual clams. Unfortunately no better method has been developed for estimating the number of coliform bacteria in low coliform count materials such as cleansed clams. The use of differential plating media is impracticable, since an inoculum large enough to produce thirty colonies per plate (the minimum for statistical accuracy) contains enough solid material to mask much of the bacterial growth. It may also contain enough organic material to destroy the differential properties of the media.

In view of the inaccuracies inherent in the methods, analyses carried out in the work herein reported were replicated when the frequency of sampling did not necessitate so much work as to render such replication impracticable. A single value which differs widely from its replicates must be ignored completely, as must also the result of a non-replicated analysis, if the result lies far off the line of progression marked by other pertinent data.

Total plate counts on nutrient agar incubated at 37° C. were used for comparing the concentrations of non-marine bacteria in different lots of clams. While undoubtedly some true marine bacteria were able to grow on this media at 37° C., their numbers must have been insignificant.

(c) General - The stocks of polluted clams were procured by digging from an area close to sewer outfall in St. Andrews Harbour. Following digging these clams were dip-washed in the harbour and sorted, the undersized, the oversized and the injured being rejected. The sorted clams were placed in hods and trucked to the cleansing station, dip-washed again in the non-polluted water, and placed, still in hods, in the cleansing car. The car was moored near the low water mark in the intertidal zone. This location of the car permitted a 3 to 4 hour air exposure of the clams each low tide.

SECTION II - EXPERIMENT I (April 7-16)

To explore the self-cleansing of clams under spring conditions, and to test for the effect of "dug-age" on self-cleansing.

Procedure: Four hods of polluted clams were procured as in Section I - Methods. One hod was transferred immediately to the cleansing station and the other three stored in a dark room at 13° C. - 15° C., and covered with damp clean potato bags. At the end of 24 hours of such storage one hod was transferred to the cleansing station, at the end of 48 hours another hod was transferred and at the end of 72 hours the last was transferred. Samples were taken at the beginning of the experiment, at the time of each transfer, and at various times during the exposure of the clams to the waters at the cleansing station. The samples were analysed as in Section 1 - Methods. The results appear in Table 1 and are illustrated in Figure 1. They support the following conclusions.

Conclusions:

1. During the first 48 hours after transfer, little change occurred in the number of coliform bacteria in clams with a dug-age of 0 hours. During the next 12 hours, however, unusually rapid cleansing took place. Between 60 and 72 hours there was a definite increase of coliform bacteria, however by 109 hours their numbers had again fallen.

2. Clams with a dug-age of 24 hours demonstrated no self-cleansing during the first 24 hours in water but from that time onwards followed

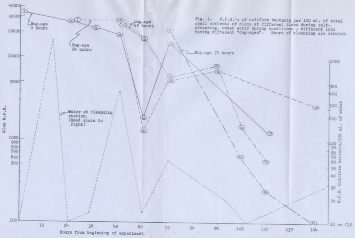


Fig. 1. M.P.N.'s of coliform bacteria per 100 ml. of total shell contents of clams at different times during self-cleansing, under early spring conditions - different lots having different "dug-ages". Hours of cleansing are circled.
Dug-age 0 hours
Dug-age 24 hours
Dug-age 48 hours
Dug-age 72 hours
Water at cleansing station. (Read scale to right)
Clam M.P.N.
M.P.N. Coliform bacteria/100 ml. of water
Hours from beginning of experiment
10 or <10

a cleansing pattern very similar to that begun at the 48 hour point by the 0 "dug-age" clams.

3. The self-cleansing of the 48 hour "dug-age" clams followed the stages observed during the 1951 cleansing experiments. (1) That is, rapid cleansing during the first 24 hours after transfer, little, if any, during the next 24 hours, and a final cleansing beginning at about 48 hours which brought the coliform bacterial count down by the

Table 1. The Most Probable Number of Coliform Bacteria per 100 ml. of shell contents of clams at different times during self-cleansing, under early spring conditions. The four lots tested had different "dug-ages".

Dug-age of Clams	Initial M.P.N.	M.P.N. 12 hrs.	M.P.N. 24 hrs.	M.P.N. 36 hrs.	M.P.N. 48 hrs.	M.P.N. 60 hrs.	M.P.N. 72 hrs.	M.P.N. 109 hrs.
0-2 hrs.	4,700*		22,000	24,000	24,000	2,400	16,600	790
	18,000		24,000	22,000	17,000	2,200	17,000	2,400
	54,000		35,000	24,000	17,000	790	1,300	790
	35,000		28,000	1,100*	17,000	2,200	17,000	-
	Av. 35,000		27,000	23,000	19,000	1,900	16,600	1,300
24 hrs.	35,000		24,000	790	2,400		230	2,400
	33,000		35,000	1,100	9,200		5,400	2,400
	22,000		24,000	1,100	5,400		9,200	3,500
	22,000		1,100*	2,200				
	Av. 28,000		27,000	1,300	5,600		7,300	2,700
48 hrs.	24,000	17,000	5,400		9,200	790	490	
	35,000	17,000	9,200		9,200	2,200	790	
	17,000		2,400		5,400	54,000*	490	
	3,100*							
	Av. 25,000	17,000	5,600		7,900	1,500	590	
72 hrs.	24,000			1,100	270		110	5,400*
	24,000			490	230		92	110
	17,000			330	3,500*		92	78
	3,800*							40
	Av. 21,600			640	250		98	76

* This value discarded in calculating average.

72 hour mark to that level considered acceptable in clams taken directly from the flats. However, in these spring clams, the initial rate was somewhat slower, and the degree of cleansing reached, before the

24 hour levelling-out, somewhat less than in summer clams studied last year (1).

4. After 36 hours' exposure to water, the 72 hour "dug-age" clams had reduced their coliform bacterial count to an extent that indicated a rate of self-cleansing comparable with that observed in the 1951 studies (1). The results show little lessening of this rate until after 72 hours.

5. While the coliform bacterial count of the water at the cleansing station fluctuated, occasionally reaching very high levels which were maintained for short periods, there was no clear correlation between these fluctuations and the self-cleansing patterns exhibited by the clams. However, it may have been that undetected upward fluctuations interfered with normal self-cleansing. In view of this possibility, the results of experiment 1 must be most cautiously interpreted.

6. The 48 hour and 72 hour "dug-age" clams exhibited more rapid and extensive self-cleansing than did the 0 hour and 24 hour "dug-age" lots.

7. During winter and early spring clams in the cold mud of the beaches may live at a low metabolic rate - perhaps in a state of semi-hibernation. It is possible that when these are subjected to 48 or more hours of air storage at temperatures somewhat higher than that of the mud flats, they may emerge from this semi-hibernation and cleanse themselves more rapidly than those that are more freshly dug rather than returning immediately to hibernation. If this were the case the findings just described could be readily understood.

8. Further early spring trials should reveal whether air storage could be useful in conditioning spring clams for self-cleansing.

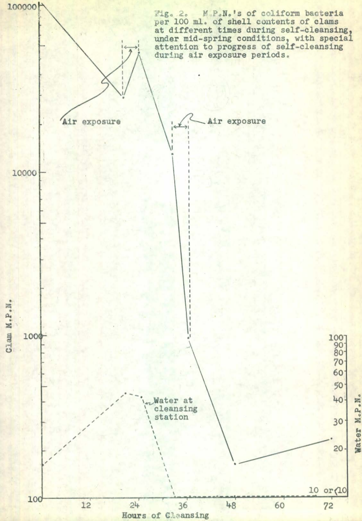
SECTION III - EXPERIMENT 2 (April 19-22)

To explore the progress of self-cleansing during air exposure in the intertidal zone and to further study the progress of self-cleansing under spring conditions.

Method: Clams were procured and handled as described in Section 1 - Methods. Samples were taken at the time of transfer to the cleansing station and again at 20 hours, the beginning of an air-exposure period, and four hours later when the flooding tide ended air-exposure. The clams were sampled again at 32 hrs. and at 36 hrs., the beginning and the end, respectively, of another air exposure, and finally at 72 hours.

The results of the analyses of the samples appear in Table 2 and are illustrated in Figure 2.

Fig. 2. M.P.N.'s of coliform bacteria per 100 ml. of shell contents of clams at different times during self-cleansing, under mid-spring conditions, with special attention to progress of self-cleansing during air exposure periods.



Conclusions:

1. Self-cleansing progressed little if any during the first air exposure period, but continued unhampered during the later exposure. There has not yet been revealed sufficient information on the mechanism of self-cleansing to explain this difference.

2. These clams appeared to commence self-cleansing at the time of transfer to the cleansing station. This may imply that during the two week period between the commencement of Experiment 1 and that of Experiment 2, the metabolism of clams, in their natural habitat, had gained momentum with the progressing spring.

Table 2. The Most Probable Number of Coliform Bacteria per 100 ml. of shell contents of clams held in the intertidal zone at different times during self-cleansing, especially before and after air exposure periods.

Initial M.P.N.	Beginning of air exposure M.P.N. 21 hrs.	End of air exposure M.P.N. 25 hrs.	Beginning of air exposure M.P.N. 33 hrs.	End of air exposure M.P.N. 37 hrs.	M.P.N. 48 hrs.	M.P.N. 72 hrs.
92,000	54,000	54,000	16,000	480	170	130
92,000	24,000	54,000	9,200	280	150	330
160,000	9,200	54,000	140*	2,200		
Av. 115,000	29,000	54,000	13,000	986	160	230

* This value discarded in calculating average.

SECTION IV - EXPERIMENT 3 (April 25-27)

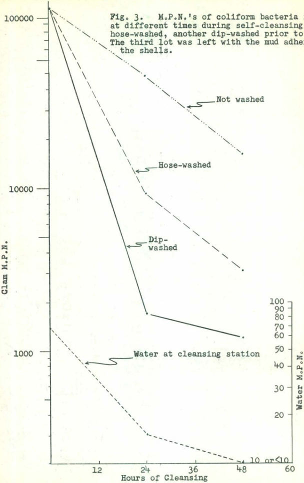
To assess the value of hose-washing the polluted clams prior to their transfer to the cleansing station and to further explore the effects of the advancing spring on self-cleansing.

Procedure: 3 hods of clams were procured as in Section 1 - Methods. One hodful was left completely unwashed, another was given the usual dip-wash (Section 1, Methods, general), and the third was thoroughly hose-washed.

The clams were sampled at the time of transfer to the cleansing station and all three of the hods at 24 and 48 hours.

The results of bacteriological analysis of the samples are tabulated in Table 3 and appear in graph form in Figure 3.

Fig. 3. M.P.N.'s of coliform bacteria in clams at different times during self-cleansing, one lot hose-washed, another dip-washed prior to treatment. The third lot was left with the mud adhering to the shells.



Conclusions:

1. Some form of pre-washing seems to hasten the initiation of self-cleansing. This conclusion accords with that from last year's test (Mullan et al 1952, p. 30).

2. The dip-wash method seems to be as effective as the hose-wash method.

3. The most likely explanation of the tardiness of the unwashed clams in self-cleansing would seem to be that as the adhering polluted mud gradually loosened from the shells some of it was ingested by the clams. The accompanying intake of coliform bacteria would offset to some extent the reduction in the numbers of these organisms resulting from the self-cleansing forces.

Table 3. The Most Probable Number of Coliform Bacteria in clams at different times during self-cleansing, one lot having been hose-washed prior to their transfer to the cleansing station, another having been dip-washed, and a third left with the mud adhering to the shells.

Conditions of test		Original M.P.N.	M.P.N. 24 hrs.	M.P.N. 48 hrs.
Not washed		160,000	54,000	16,000
		90,000	35,000	16,000
		160,000	54,000	16,000
	Av.	137,000	48,000	16,000
Dip-washed			1,700	490
			1,100	790
			2,400	2,400
	Av.	137,000	1,700	1,200
Hose-washed			9,200	3,500
			9,200	2,400
			9,200	3,500
	Av.	137,000	9,200	3,100

SECTION V - EXPERIMENT 4 (July 5-8)

To explore the self-cleansing of clams under mid-summer conditions and to compare bacteriological changes in clams transferred to non-polluted waters with those occurring in clams dug from the flats but left in a hod anchored in the polluted waters.

Procedure: Two hods of clams were procured as in Section 1 - Method. One hodful was transferred as usual to the cleansing station; the other was anchored to the intertidal flat in the polluted area. It was planned that both hods be sampled once every six hours for 72 hours. Such a program proved too heavy for the personnel engaged and was not rigidly followed.

The results of the bacteriological analyses appear in Table 4 and are illustrated in Figure 4.

Conclusions:

1. There was little change in the count of coliform bacteria in the clams held in polluted water, while a substantial decrease in the number of these organisms occurred in the clams transferred to non-polluted water. This demonstrates that it is not the change from life in a burrow to life in free-flowing water that causes clams to reduce their load of coliform bacteria. It also shows that a clam's bacterial flora is closely related to that of the water in which it lives.

2. Self-cleansing at this season appeared to proceed at a slower rate than that observed during September 1951. This may reflect a mid-summer slump in the clams' metabolic rate; on the other hand it may reflect increased bacterial multiplication which offsets the clams' self-cleansing activities.

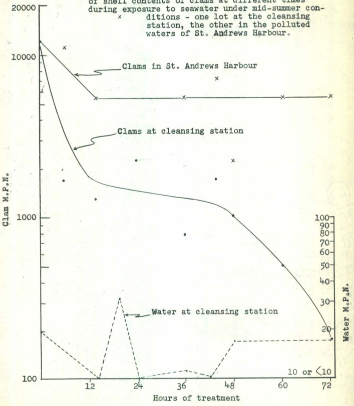


Fig. 4. M.P.N.'s of coliform bacteria per 100 ml. of shell contents of clams at different times during exposure to seawater under mid-summer conditions - one lot at the cleansing station, the other in the polluted waters of St. Andrews Harbour.

Table 4. The Most Probable Number of Coliform Bacteria per 100 ml. of shell contents in clams, one lot being exposed to the waters at the cleansing station and a second lot being exposed to the polluted waters of St. Andrews Harbour.

	Exposed to waters of cleansing station	Exposed to polluted waters of St. Andrews Harbour
Initial M.P.N.	12,000	12,000
M.P.N. 6 hrs.	1,700	11,000
M.P.N. 14 hrs.	1,300	5,400
M.P.N. 20 hrs.	22,000	17,000
M.P.N. 24 hrs.	2,200	54,000
M.P.N. 36 hrs.	790	5,400
M.P.N. 42 hrs.	1,700	7,000
M.P.N. 48 hrs.	1,000	2,400
M.P.N. 60 hrs.	500	5,400
M.P.N. 72 hrs.	175	5,400

<u>Note</u>. The above M.P.N. values are the results of single analyses and the accuracy of any single result must be judged by its proximity to the curve established by the greater number of results.

<u>SECTION VI - EXPERIMENT 5 (July 15-21)</u>

<u>To check again the efficacy of self-cleansing under mid-summer conditions and by very frequent sampling to follow the stages of self-cleansing</u>.

<u>Procedure</u>: One hod of clams was procured as in Section 1 - Methods and transferred to the cleansing station. It was sampled at that

time and every two hours thereafter for 18 hours. Two-hour sampling began again at 22 hours and continued until 32 hours. At 42 hours 3-hour sampling began and continued until 51 hours. Further samples were taken at 65 and 72 hours.

All samples were tested for numbers of coliform bacteria and many of the early samples for other non-marine bacteria, see Section 1 - Methods - Bacteriological.

The results appear in Tables 5 and 6 and are illustrated in Figure 6.

Conclusions:

1. The results of frequent sampling uphold the conclusion drawn last year (1) that there are phases in self-cleansing.

2. The observations support conclusion 1, experiment 4 of this report, in suggesting that in mid-summer self-cleansing proceeds at a slower rate than in late spring and early autumn.

3. The data of Table 6 indicate an early qualitative change in the clams' bacterial flora and an early decrease in the total of non-marine bacteria in the shell contents.

Table 5. Most Probable Number of Coliform Bacteria per 100 ml. of shell contents in clams sampled at frequent intervals during self-cleansing.

Time from beginning of experiment	M.P.N.
0 hrs.	7,500 & 6,800
2 "	3,500
4 "	5,300
6 "	2,400
8 "	2,400
10 "	54,000
12 "	3,500
14 "	3,100
16 "	8,300
18 "	1,600
22 "	1,100
24 "	2,500
26 "	4,600
28 "	3,500
30 "	8,300
32 "	1,300
42 "	3,500
45 "	52,000
48 "	5,400
51 "	2,400
65 "	2,400
	1,500
72 "	820
	700

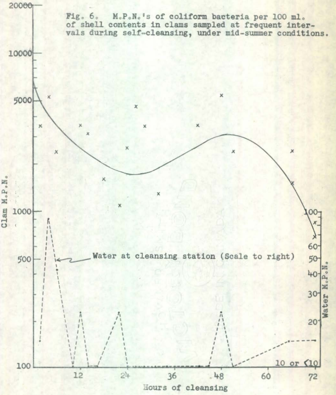


Fig. 6. M.P.N.'s of coliform bacteria per 100 ml. of shell contents in clams sampled at frequent intervals during self-cleansing, under mid-summer conditions.
20000
10000
5000
1000
500
100
Clam M.P.N.
Water at cleansing station (Scale to right)
100
60
50
40
30
20
10 or <10
Water M.P.N.
12
24
36
48
60
72
Hours of cleansing

SECTION VII - EXPERIMENT 6 (July 29 - Aug. 2)

To explore the general bacteriology of polluted clams during self-cleansing.

Procedure: One hod of polluted clams was procured as in Section 1 - Methods and transferred to cleansing station.

Samples were withdrawn at the beginning of the experiment and again at 24, 48 and 72 hours.

All samples were tested for numbers of coliform bacteria, as well as for total numbers of non-marine bacteria.

Bacteria from colonies taken at random from the agar plates were Gram stained and examined microscopically.

Similar tests were carried out on the waters from both the polluted area and the cleansing station.

The results appear in Tables 7, 8 and 9 and are illustrated in Figure 9.

Results:

1. During the early hours of self-cleansing there was a decrease in the relative frequency of G+ bacteria and yellow-colony bacteria and an increase in that of brown-star-shaped-colony bacteria in the flora of the clams.

2. Several G+ and several yellow-colony bacteria were isolated from St. Andrews Harbour water. No G+ and few yellow-colony bacteria were isolated from the cleansing station waters.

3. See Tables 7, 8 and 9 and Figure 9.

Conclusions:

1. The decrease in numbers of coliform bacteria in the clams followed the usual pattern (See Experiment 5 of this report and reference (1)).

2. The total numbers of non-marine bacteria in the total shell contents dropped more gradually than the coliform count and the overall decrease was less extensive.

3. Counts taken of the total number of non-marine bacteria in various parts of the clam showed the following changes in numbers of these bacteria.

(a) On the gills these bacteria decreased in numbers at about the same rate and to the same extent as in the total shell contents.

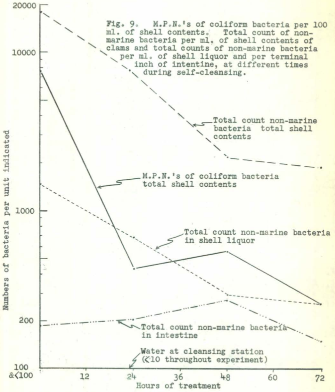


Fig. 9. M.P.N.'s of coliform bacteria per 100 ml. of shell contents. Total count of non-marine bacteria per ml. of shell contents of clams and total counts of non-marine bacteria per ml. of shell liquor and per terminal inch of intentine, at different times during self-cleansing.

Table 6. Some changes in the bacteriological flora of clams during the early hours of self-cleansing.

Time from beginning of experiment	Total count per ml. on nutrient agar aerobic plates incubated at 37° C.	Colony types	Gram stain reaction of colonies chosen at random
0 hrs.	7,000	Many tiny yellow " " white	Some G+ Chiefly G-
2 "	4,500	As above	Few G+ Chiefly G-
6 "	4,500	Fewer yellow Many white	Odd G+ Chiefly G-
8 "	3,800	Few yellow, some brown star-shaped, many white	As above
12 "	3,500	Odd yellow, some brown, many white	No G+ all G-
24 "	2,800	No yellow, several brown star-shaped, many white	all G-

Time from beginning of experiment	at 17° C.	Colony types	Gram stain reaction
0 hrs.	14,000	Changes in colony types about as above	Changes in gram stain reaction about as above
2 "	9,000		
6 "	8,500		
8 "	5,000		
12 "	7,500		
24 "	5,000		

Note: Gram reaction in column four does not refer to the colony type listed opposite in column three but describes the plate growth as a whole and is estimated from results obtained on colonies picked at random.

Table 7. Most Probable Number of Coliform Bacteria per 100 ml. of shell contents of clams at different times during self-cleansing.

	Initial M.P.N.	M.P.N. 24 hrs.	M.P.N. 48 hrs.	M.P.N. 72 hrs.
	5,400	540	490	220
	9,200	390	490	330
	9,200	400	700	230
Av.	7,900	440	560	260

Table 8. Some changes in the bacteriological flora of clams during self-cleansing.

Time from beginning of experiment		Total count on nutrient agar aerobic plates (incubation at 37°C.) per ml. of shell contents	per ml. of shell liquor	per gill	per terminal inch of intestine	per style
0 hrs.		16,900		50	176	73
		15,600		15	200	130
		22,600		25	200	
	Av.	18,400	1500	30	190	100
24 hrs.		8,700		50	360	34
		9,600		40	200	14
		4,300		30	80	
	Av.	7,500	700	40	210	24
48 hrs.		2,500		9	330	9
		2,200		20	240	6
		2,000				2
	Av.	2,200	300	14	280	6
72 hrs.		1,350		15	150	7
		2,250		11		15
		2,000				
	Av.	1,900	260	13	150	11

Table 9. Quantitative differences in the non-marine or adventitious bacteria in the waters of St. Andrews Harbour and the waters of the cleansing station, as measured by the nutrient agar aerobic plate method (See Section - Methods - Bacteriological) and the M.P.N. coliform bacteria method.

	St. Andrews Harbour water		Cleansing station water	
Time from beginning of experiment	M.P.N. coliforms per 100 ml.	Total count per ml.	M.P.N. coliforms per 100 ml.	Total count per ml.
0 hrs.	>1,500	310	<1.8	13
24 "	240	38	<1.8	30
48 "	>1,500	210	9.1	30
72 "	>1,500	400	9.1	15

(b) in the shell liquor as in (a) above.

(c) on the style as in (a) above.

(d) in the intestine these bacteria showed no overall decrease in numbers until after 48 hours of self-cleansing.

4. The same sort of qualitative changes were observed as reported in conclusion 3 of experiment 5.

5. The water of St. Andrews Harbour contained, during this period, approximately three times as many adventitious bacteria as the water at the cleansing station.

6. There was some difference as to the kinds of bacteria making up the adventitious floras of the two waters.

7. The water of St. Andrews Harbour during this period contained over 800 times as many coliform bacteria as the water at the cleansing station.

8. Quantitatively and qualitatively the changes in the clams' flora of coliform and other non-marine bacteria during self-cleansing seems to represent a substitution of the flora of the cleansing station water for the flora of the polluted water.

9. Those portions of the clam's body which were most exposed to the stream of water pumped through the mantle cavity were the first to show a decrease in the number of non-marine bacteria.

10. The intestine, which is known to be by-passed by this stream, was the last to show a decrease in the number of non-marine bacteria.

11. The decrease in number of non-marine bacteria in the intestine commenced at about the same time as phase III (second period of sudden decrease) of the cleansing process.

12. Replication of this experiment is required to show whether the above changes were coincidental or causally related. If we hypothesize that they are causally related then the results obtained in both the 1952 and 1951 clam purification studies (1) become more intelligible. More particularly the hypothesis is that the non-polluted water pumped by the transferred polluted clam through the incurrent siphon, over the gills, through the mantle cavity and out the excurrent siphon, washes the bacteria and other particles from these parts. It replaces the old shell liquor and brings into the mantle cavity new bacteria characteristic of the waters of the cleansing station. The intestine, however, retains its contents for a longer period, then releases it rapidly at defication, a process not directly related to the water flow just discussed.

The above hypothesis gains creditability from the fact that the washing of the exposed parts could hardly fail to occur. It is well known that an individual mollusc normally pumps several gallons of water through its mantle cavity each day (5). Flowing water is a cleansing agent and could hardly fail to remove all but the most firmly adhering particles. Provided that the washing water does not carry the same quantity and kind of material as is carried by the water from which the clams were taken, the load on the washed parts should show a change.

It is known also that the rate of movement of materials through the digestive tract is only indirectly dependent on the rate of water flow through the mantle cavity. Indeed the mollusc can pump water without at the same time taking any of it or its suspended matter into his digestive tract (5). The rate of movement of materials through the digestive tract is known to be influenced by different factors. According to Renn (6) temperature and the amount of suspended material in the water are both influential. It is known that with some molluscs the kind and quantity of available food influence the rate of feeding and intestinal flow. It is not surprising then to find, in the results of these clam-purification studies, evidence that the clam, after being moved from his burrow beneath the harbour waters to floating structures in different waters, experiences some irregularity in the rate of movement of materials through its digestive tract. A low feeding rate would almost certainly result in a low evacuation rate. A failure of the intestine to evacuate before 48 hours after transfer could result from the intestines requiring this length of time to fill to a point at which intestinal evacuation is stimulated.

The experimental data of this report and those of the 1952 report (1) agree well with the above hypothesis. The finding of this experiment demonstrate that the parts exposed to the water currents in the mantle cavity experience early quantitative and qualitative changes in their floras of adventitious bacteria and that these changes are related to differences between the bacterial content of the water in which the clams previously lived and that of the water to which they were transferred. The same results demonstrate that the intestine, which is known to be by-passed by the water pumped through the mantle cavity, experiences no early changes. There was no decrease in the number of bacteria carried in the intestine until after forty-eight hours.

The frequently observed pattern of decrease in numbers of coliform bacteria in the entire shell contents of clams during self-cleansing (Experiments 1 and 3-6) agrees well with the above hypothesis. The early decline in coliform numbers (Phase 1) is what would be expected from the washing of voluminous portions of the clam body with coliform-free water. The later long halt in coliform decrease (Phase 2) would be expected to occur between the completion of the cleansing of exposed parts and the commencement of gut evacuation. The time of recommencement of coliform decrease (Phase 3) corresponds with the time at which the total count of adventitious bacteria in the gut begins to diminish and can be explained as being a result of the gut's emptying its contents at this time into the excurrent stream.

The fact that quantitative decreases of coliform bacteria in the clams were more extensive than those of other adventitious bacteria is explainable on the basis of the water analyses results. While the cleansing station water showed lower counts of both coliform and adventitious (non-coliform) bacteria than harbour water, the difference with respect to the former was much the greater. In the case of non-coliform bacteria the loss of the old flora would be masked in part by replacement by a new flora of adventitious bacteria carried by the cleansing waters. On the other hand the cleansing waters, being practically free of coliform bacteria, would bring into the clam very few bacteria of this group but would carry many out.

SECTION VIII - EXPERIMENT 7 (Apr. 7-10; Sept. 22-26, and Oct. 31 - Nov. 3)

Quantitative changes in the population of coliform bacteria in polluted clams held under different conditions.

Method:

On April 7, 1952, polluted clams were procured as in Section 1 - Methods and stored in hods in the dark, covered with damp potato bags in a room where the temperature ranged between

13 and 15° C. The clams were sampled at the beginning of the experiment and again 24, 48 and 72 hours later. The samples were tested for numbers of coliform bacteria.

On September 10, 1952, three hods of polluted clams were similarly procured; hod #1 was stored at 15° C. in the basement of the Atlantic Biological ~~Biological~~ Station, St. Andrews, N.B.; hods #2 and #3 were transferred immediately to the Holt's Point cleansing station. After 13 hours' exposure there, hod #2 was taken from the waters and stored in the A.B.S. basement. Hod #3 was left at Holt's Point. Hod #1 was sampled at 0, 48 and 72 hrs.; hod #2 at 0, 13, 37 (13 water and 24 air hours) and 72 (13 water and 59 air) hours; hod #3 at 0, 13, 48 and 72 hours. The samples were tested for numbers of coliform bacteria.

On October 31, 1952, two lots of polluted clams were procured as above, and taken by car to the University of New Brunswick, Fredericton, N.B., where they were stored in air - one at 25° C. for 72 hours and another at 4° for 48 hours. The 25° lot was sampled at the beginning of storage and again 48 and 72 hours later. The 4° lot was sampled at the beginning of storage and again at 24 and 48 hours. The samples were tested for numbers of coliform bacteria.

The results appear in Table 10 and are illustrated in Figure 10.

Conclusions:

1. During 72 hours of air storage at 13° C. to 15° C. (April and September) there was a very gradual decrease in the number of coliform bacteria in polluted clams. This decrease was negligable as compared with that taking place in water at comparable temperatures.

2. During air storage at 25° C. there was a considerable increase in the numbers of coliform bacteria.

3. During 48 hours of air storage at 4° C. there was no significant change in the numbers of coliform bacteria in the clams.

4. In partially cleansed clams removed from the cleansing station after 13 hours of self-cleansing and placed in air storage at 15° C. for 59 hours, the coliform count increased greatly. The clams remaining in the cleansing waters continued self-cleansing.

5. The pattern of self-cleansing in the clams that were exposed continually at Holt's Point (Hod #3) was not the usual one, in that the third phase did not appear. However, the 72 hours result is doubtful, as noted on the graph, and a 24 hours assay was not made. These short-comings in the data could be responsible for the apparent departure from the usual pattern.

Figure 10. M.P.N.'s of coliform bacteria per 100 ml. of total shell contents of clams at various times during different storage and cleansing treatments in 1952.

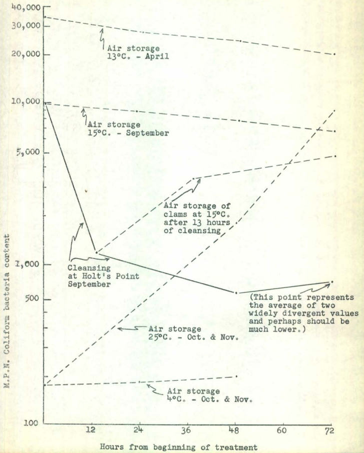


Table 10. Most Probable Number of Coliform Bacteria per 100 ml. of shell contents of clams at various times during different storage treatments.

Treatment	Original M.P.N. 0 hours of treatment	M.P.N. after 13 hours of treatment	M.P.N. after 24 hours of treatment	M.P.N. after 37 hours of treatment	M.P.N. after 48 hours of treatment	M.P.N. after 72 hours of treatment
Air storage	4,700*		35,000		24,000	24,000
at 13° C.	18,000		33,000		35,000	24,000
April 7-10,	54,000		22,000		17,000	17,000
1952	35,000		22,000		3,100*	3,800*
Av.	35,000		28,000		25,000	21,600
Air storage	12,000				8,400	5,800
at 15° C.	9,000				7,900	6,400
Sept. 22-25, 1952	8,400				7,600	5,800
Hod #1 Av.	10,000				8,000	6,000
Exposed to		790			790	1,380
cleansing		1,100			500	330
water at 13°C.		1,700			780	
Sept. 10,1952						
Hod #3 Av.	10,000	1,200			693	800
Exposed to cleansing water at 13°C. for 13 hrs. Sept. 10, then						
transferred to				3,500		4,300
air storage at				3,500		5,600
15° C. Sept. 10-13.				3,500		4,400
Hod #2 Av.	10,000	1,200		3,500		4,900
Air storage						
at 25° C.	230				1,300	
Oct. 31 -	140				2,400	9,200
Nov. 3, 1952 Av.	185				1,850	9,200
Air storage						
at 4° C. Oct.			170		240	
31 - Nov. 3,			210		170	
1952 Av.	185		190		205	

* This value discarded in calculating the average.

6. The fact that partially cleansed clams do not continue self-cleansing when they are removed from the cleansing waters and placed in air storage at a temperature approximating that of the cleansing water, demonstrates that if the primary factor in self-cleansing is a bactericidal agent acquired from the cleansing water by the clams, then this agent to be effective requires continuous renewal.

7. The fact that polluted clams held in air storage decrease their coliform count only slightly as compared with similar clams exposed to non-polluted water, at temperatures approximating that of the air storage, demonstrates that the primary factor in self-cleansing is not a bactericidal agent retained by the clams after their transfer from polluted waters.

8. During air storage at 15° C. the number of coliform bacteria in partially cleansed clams increased but decreased in clams commencing storage with a higher original coliform bacterial count. The coliform count of the two different lots of clams seemed to approach the same value. The suggestion is that for a given set of environmental conditions there is opportunity of life for a more or less fixed number of individuals of a given group of bacteria. Whether the number of coliform bacteria in clams in air storage at constant environmental conditions increases or decreases seems to depend on whether the number of these bacteria in the clams at the commencement of storage is higher or lower than that for which opportunity for life exists under prevailing environmental conditions.

9. That temperature is one factor which greatly influences the environment's capacity for supporting the life of coliform bacteria is demonstrated by the fact that in duplicate lots of clams, one in storage at 25° C. and the other in storage at 4° C., the coliforms multiplied in the lot stored at 25° C. but their numbers remained constant in the lot stored at 4° C. Undoubtedly other factors besides temperature, such as kind and quantity of available food, nutritional requirements and by-product output of other microorganisms present exert a strong influence on the coliforms' multiplication rate in the clam body as these factors are known to do in other environments(7).

10. In view of conclusion 8 of this experiment and the probability of the clam's intestine evacuating rather infrequently under self-cleansing conditions, it seems possible that, under these conditions a small population of coliforms would maintain itself indefinitely in the clam's intestine. However, judging from the numbers of coliforms found in these experiments, to remain in the clam after 72 hr. of self-cleansing, it can be predicted that the numerical value of any lingering coliform population would be far below the allowed 2400/ 100 ml. level.

SECTION IV - WATER CONDITIONS AT THE CLEANSING STATION DURING PERIODS OF EXPERIMENTATION

The hydrographic and bacteriological conditions of the cleansing waters over the different experimental periods are described in Table 11.

Any apparent relationships between variations in these conditions and the experimental observations are discussed with the appropriate experiments in the body of this report.

Table 11. Conditions of waters at cleansing stations at intervals during experimental period.

Date 1952	Experiment	Time of day	Hours from beginning of experiment	Surface of water	State of tide	Water temperature °C.	Salinity ‰	M.P.N. coliform bacteria per 100 ml.
April								
7	1	7:00p.m.	0	Calm	½ F.	3	29.6	4.5
8	1	10:00a.m.	15	"	H.E.	4	26.7	1600
8	1	7:00p.m.	24	"	1/3 F.	4	28.3	6.8
9	1	5:00p.m.	34	"	2/3 E.	3	29.2	13
9	1	7:00p.m.	48	"	1/4 F.	4.5	27.9	390
10	1	7:00a.m.	60	"	1/3 F.	4.5		13
10	1	7:00p.m.	72	"	1/3 F.	3.8	28.7	56
11	1	9:00p.m.	100	Swell	½ F.	3	30.3	19
12	1	7:00a.m.	110	"	1/3 F.	0	29.7	3.6
13	1	9:00p.m.	148	Calm	1/4 F.	4		28
16	1	11:00a.m.	208	"	1/4 F.	15	29.5	23
19	2	4:00p.m.	0	Swell	1/3 F.	11	25.9	16
20	2	1:00p.m.	21	Calm	2/3 E.	7	27.5	45
20	2	5:00p.m.	25	Swell	1/3 F.	9	29.1	43
21	2	1:30a.m.	33.5	Calm	2/3 E.	3	30.6	2
21	2	5:00a.m.	37	Swell	1/3 F.	3	30.4	2
21	2	1:30p.m.	43.5	Calm	2/3 E.	5	28.3	2
22	2	2:30p.m.	68.5	"	2/3 E.	7	30.5	3.6
25	3	9:30p.m.	0	Swell	1/3 F.	4.5	29.2	68
26	3	9:30p.m.	24	"	1/3 F.	6	24.3	16
27	3	9:30p.m.	48	"	2/3 E.	7.5	24.5	9.1
July								
5	4	6:00p.m.	0	Calm	1/4 F.	14.1	32.3	19
6	4	0:15a.m.	6	"	1/3 E.	14.1	31.9	14
6	4	8:00a.m.	14	"	1/3 F.	13	31.4	2
6	4	2:00p.m.	20	Swell	L.E.	12	31.0	33
6	4	6:00p.m.	24	Calm	1/3 F.	14.1		2
7	4	6:00a.m.	36	"	L.E.	11	31.4	11

Continued

Table 11 continued

Date 1952	Experi- ment	Time of day	Hours from beginning of experi- ment	Surface of water	State of tide	Water tempera- ture °C.	Salin- ity ‰	M.P.N. coliform bacteria per 100 ml.
July								
7	4	12:00N	42	Calm	1/4 E.	13		7.8
7	4	6:00p.m.	48	Swell	H.E.	15	31.6	17
8	4	6:00p.m.	72	Calm	3/4 E.	14.1	31.3	17
15	5	4:00p.m.	2	"		16.5	31.8	15
15	5	6:00p.m.	4	"		16	31.7	93
15	5	8:00p.m.	6	"		15		43
15	5	11:55p.m.	10	"		14.5	30.9	9.1
16	5	2:00a.m.	12	"		14.5	31.4	23
16	5	4:00a.m.	14	"		14.5	31.2	3.6
16	5	6:00a.m.	16	"		14.6	31.2	7.3
16	5	12:05p.m.	22	"		15.1	31.8	23
16	5	2:00p.m.	24	"		16		3.6
16	5	4:00p.m.	26	"		16	31.7	0
16	5	6:00p.m.	28	"		15.1		9.1
16	5	8:00p.m.	30	"		15	31.4	3.6
16	5	10:00p.m.	32	"		15	31.4	9.1
17	5	8:00a.m.	42	"		15	31.6	9.1
17	5	11:00a.m.	45	"		17	31.3	3.6
17	5	2:00p.m.	48	"		17	31.3	23
17	5	5:00p.m.	51	"		16.5	31.3	0
18	5	7:00a.m.	65	"		14		15
April								
18	5	2:00p.m.	72	Calm		16		15.0
29	6	4:00p.m.	0	"		16		1.8
30	6	4:00p.m.	24	"		16		1.8
31	6	4:00p.m.	48	"		15.5		9.1
Aug.								
2	6	4:00p.m.	72	"		17		1.8

H. high water
F. flood tide
E. ebb tide

SECTION X - SUMMARY

1. The survival of coliform bacteria in polluted clams during air storage at different temperatures shows that the clams carry from polluted waters no active bacteriocidal agent and produce no such agent themselves, at least when living out of water.

2. The failure of the clams to continue self-cleansing when removed to air storage after they have commenced such cleansing shows that the clams carry no active bacteriocidal agent from the cleansing waters.

3. Theories claiming as the principal factor in self-cleansing bacteriocidal agents produced by the clam only when it is living in water or bacteriocidal agents present in the water but effective only when fresh supplies of them are continuously available, would not contradict the experimental results, however, such theories would not explain many of these results.

4. On the other hand a hypothesis advancing (1) the washing action of water pumped by the clam through its mantle cavity, and (2) the clam's evacuation of its intestine, as the primary cleansing agents explains most of the observations and contradicts none of them.

5. The above hypothesis explains well the characteristic pattern of coliform bacterial count reduction during self-cleansing. That pattern consists of (1) an initial phase of rapid reduction lasting about 24 hours followed by (2) a period of little reduction, or no reduction, or in some cases increase, which endures for another 24 hours approximately, after which time (3) rapid reduction recommences and continues, reducing the coliform count to very low levels within another 24 hours. Phase (1) can be explained in terms of the washing of extensive areas of the clam body by the current of sea water pumped through the mantle cavity; phase (2) by the retention and, possibly, multiplication of coliforms in the intestine during this period, as a result of the intestine's failure to evacuate until 48 hours after the transfer of the clams to the cleansing station and phase (3) is explained by the postulated evacuation of the intestine approximately 48 hours after the transfer of polluted clams to the cleansing waters.

6. The multiplication and survival of coliform bacteria in clams under certain conditions shows that coliform multiplication could be a force working against this cleansing effect of some of the clams physiological processes. It would seem that the final extent of self-cleansing under a given set of conditions represents the point of equilibrium between the coliform bacterial multiplication allowed by the environmental conditions and the cleansing effects of the clam's physiological activity allowed by these conditions.

The bacteria-removing effect of these physiological activities should work equally against all groups of bacteria. The anti-cleansing or multiplication force, however, would vary with the nutritional, temperature and other requirements of different species of bacteria, as well as with their tolerances of salinity, biological by-product concentration, etc. In other words, different species of bacteria would differ in their abilities to replace those that are removed by the clam's self-cleansing activities. Thus the relative numbers of different species present in the clam before self-cleansing will change during self-cleansing, except where the species concerned have the same requirements and tolerances. This means that the abundance of the intestinal pathogen group relative to that of the Escherichia and Aerobacter or coliform group will rise or fall depending on which is best fitted for life under the temperature and other conditions prevailing in the clam body.

The relatively wider distribution of the coliform group in nature would suggest that this group is more fit than the more fastidious pathogens for life in the clam body. At the present time the senior author has experiments under way that are designed to provide more specific information on this subject.

If our hypothesis and our reasoning based on it held true, then, a coliform count of 2,400 per 100 ml. in self-cleansed clams would indicate a lower frequency of an accompanying intestinal pathogens than the same count in clams taken directly from the flats.

7. The extent of self-cleansing regularly achieved in 72 hours during the experiments reported here and in the 1952 report (1) reduced the clams' coliform content from values as high as 115,000 to well below the 2400 per 100 ml. level. The only exception was in Experiment 1, in early April 1952, when either the clams' physiological state or upward fluctuations in the coliform content of the water delayed self-cleansing to some extent.

8. Preliminary washing of the polluted shell stock hastens self-cleansing.

9. "Dug-age" up to 72 hours does not impair the clams' self-cleansing ability, and in the case of early spring clams may even hasten self-cleansing processes.

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IV. EXPERIMENTS IN THE SELF-CLEANSING OF CLAMS, CONDUCTED IN THE MOBILE LABORATORY OF THE LABORATORY OF HYGIENE, ST. ANDREWS, N.B., MAY 6 TO JUNE 27, 1952

by

A. D. Tennant and I. E. Erdman

The Mobile Unit of the Laboratory of Hygiene conducted a series of experiments in the self-cleansing of soft shell clams during the period May 5 to June 27 in a continuation of the studies initiated by a mobile unit of the Fish Inspection Laboratory in 1951 and 1952. An attempt was made to evaluate the cleansing process on a semi-commercial basis. The Bar Road, Charlotte County, N.B., was again selected as the cleansing station, and shell stock was again obtained from St. Andrews Harbour (Mullan et al 1952).

1. Experiments 1 and 2 (May 5 to 16)

To determine the effect of the period and conditions of air storage of sewage-contaminated clams on their subsequent self-cleansing.

Procedure

Pending completion of a large float, these two tests were carried out using the small experimental car constructed in 1951. On May 5 clams were obtained in the regular fashion. Four full hods were used in the first experiment and three in the second. Their treatment is described in the table below.

Experiment No.	Hod No.	Storage Temperature	Storage Site	Storage Time (Hrs.)
1	1	1 - 5° C.	Cold Room, A.B.S.	24
1	2	22° C.	Clam Lab., A.B.S.	24
1	3	-	No storage	0
1	4	1 - 22° C.	Outside Laboratory (Open Air and Sunlight)	24
2	1	1 - 5° C.	Cold Room, A.B.S.	48
2	2	6 - 14.5° C.	Outside Laboratory (Open Air, in shade)	48
2	3	-	No storage	0

Water samples for bacteriological analyses and salinity determinations were taken from four sites in the immediate vicinity of the cleansing car at 12-hour intervals during the experiments. At the same times, surface water temperature readings were made at the site nearest the car. Clam samples were taken for analyses from each hod immediately after digging, after the storage period, and at 12-hour intervals during the cleansing period. In the case of hod 4 in Experiment 1 only one sample of clams was taken after cleansing began. Bacteriological results obtained during these experiments appear in Tables 12 and 13 and in Figures 12* and 13.

In the figures the numbers within the circles on the graphs showing the M.P.N. changes for clams, indicate the number of hours self-cleansing has been under way for each particular lot of clams.

Conclusions

The results of experiments 1 and 2 considered together support the following conclusions:

1. During air storage of 2 samples for 24 hours and 2 for 48 hours the changes in the coliform M.P.N.'s of the clams followed no definite pattern: in 2 lots they were sharply reduced and in 2 lots there was an increase.

2. During the first experiment the water at the cleansing station remained relatively clean.

3. The initial M.P.N.'s of the stocks used in experiment 1 were low and none of the changes during cleansing were great. Nevertheless they were all of the sort to be anticipated from last year's results. Within 48 hours after cleansing began, all samples showed coliform M.P.N.'s of less than 1000 and they remained low.

4. The clams stored at both high and low temperatures cleansed themselves in the same fashion as those that were treated immediately after digging. This fact would be most important in an industrial development because it would be difficult to endure initiation of cleansing treatment on the day of digging.

5. During the second experiment the water became seriously contaminated and the changes in the clam M.P.N.'s were so confused as to be almost meaningless. Obviously the clams did not cleanse themselves satisfactorily and it must be concluded that the Bar Road is not a satisfactory site for a cleansing station.

*For convenience in reference the numbers of figures have been made to conform, as far as possible, with the numbers of the tables listing the data they illustrate. In following this convention some figure numbers have been skipped over, e.g., there are no figures numbered 8 - 11 (inclusive).

Figure 12. Effect of Storage on Cleansing (Experiment 1). Hours of cleansing circled.

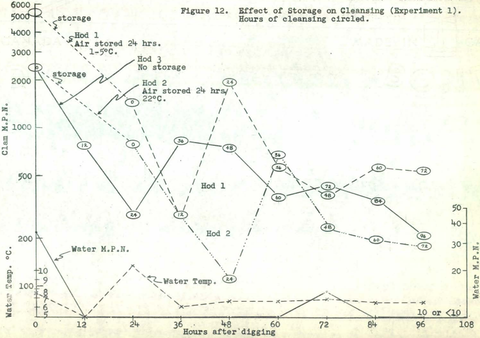


Fig. 13. Effect of storage on cleansing (Experiment 2). Hours of cleansing circled.

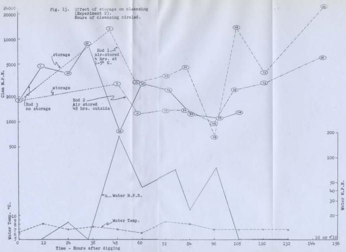


Table 12. Storage experiment 1.

Treatment	Water Coliform M.P.N. per 100 ml.	Clams: Coliform M.P.N. per 100 ml. Hod 1	Hod 2	Hod 3	*Hod 4
Nil (Fresh dug)	6.8 22 4.5 110 Av.35.8	5,400	2,400	no storage 2,400	92,000
24 hours storage	-	1,300 790 Av.1,045	790 790 Av.790	-	92,000 92,000 Av.92,000
12 hours cleansing	2 2 <1.8 2 Av.<2	230 330 Av. 280	330 230 Av.280	790 790 Av.790	24,000 54,000 Av.39,000
24 hours cleansing	7.8 4.5 2 <1.8 Av. 3.8	1,400 2,400 Av.1,900	110 110 Av.110	340 220 Av.280	-
36 hours cleansing	<1.8 2 <1.8 2 Av.<2	790 330 Av. 560	790 490 Av.640	1,300 330 Av.815	-
48 hours cleansing	4.5 4.5 2 2 Av. 3.2	230 490 Av. 360	230 230 Av.230	700 790 Av.745	
60 hours cleansing	2 7.8 7.8 2 Av. 4.9	790 330 Av. 560	170 220 Av.195	490 230 Av.360	
72 hours cleansing	7.8 22 23 6.8 Av.14.9	230 790 Av. 510	130 230 Av.180	490 330 Av.410	
84 hours cleansing	7.8 2 4.5 4.5 Av. 4.7	-	-	170 490 Av.330	
96 hours cleansing	6.8 <1.8 <1.8 4.5 Av. 3.3	-	-	170 230 Av.200	

* Clams removed from Hod #4 by persons unknown after 12-hour sampling.

Table 13. Storage experiment 2.

Treatment	Water Coliform M.P.N. per 100 ml.	Clams: Coliform M.P.N. per 100 ml. Hod 1	Hod 2	Hod 3
Nil	<1.8	2,400		no storage
(Fresh dug)	<1.8	1,700		
	<1.8	1,400		
	11			
	Av. 3.7	Av. 1,830	Av. 1,830	Av. 1,830
	13			5,400
11 hours	2	-	-	3,500
cleansing (Hod 3)	<1.8			
	4.5			
	Av. 5.1			Av. 4,550
	6.8			5,400
24 hours	4.5			2,400
cleansing (Hod 3)	49	-	-	
	4.5			
	Av. 16.2			Av. 3,900
	4.5			1,400
33 hours	2	-	-	16,000
cleansing (Hod 3)	4.5			
	2			
	Av. 3.2			Av. 8,700
End of storage (Hods 1 and 2)	79	16,000	3,500	1,100
	23	9,200	2,200	490
50 hours cleansing (Hod 3)	79			
	540			
	Av. 180.2	Av. 12,600	Av. 2,850	Av. 795
	27	2,400	790	3,500
10 hours cleansing (Hods 1 and 2)	49	3,500	1,700	2,400
	49			
60 hours cleansing (Hod 3)	49			
	Av. 43.5	Av. 2,950	Av. 1,245	Av. 2,950

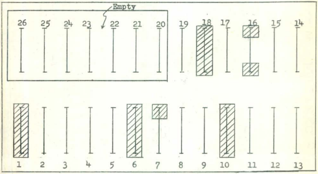
2. Experiment 3 (May 19 to 21)

To study the efficiency of self-cleansing of clams in different parts of the 26-hod car and its loading and handling characteristics.

Procedure

This was the first study with the large (26-hod) car. It was moored in the lower part of the intertidal zone. Nineteen hods of clams were considered sufficient for this test. They were dug and loaded during the same low-tide period as shown in the text figure below. In this figure the hods or parts of hods sampled are blocked in. Clam samples were removed for analysis from six of the hods considered to be representative of all parts of the car at 12-hour intervals and water samples as in experiments 1 and 2 were taken but more frequently from four stations adjacent to the car during the test period. Following the 48-hour sampling, the entire load of clams was stolen at night by persons unknown.

Bacteriological data obtained from the analyses appear in Table 14 and some are shown in Figure 14.



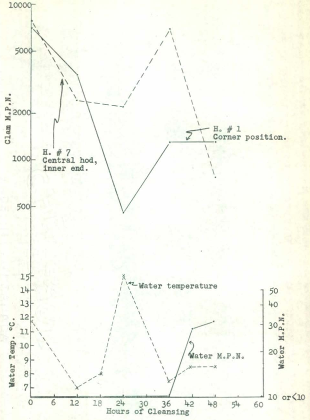


Fig. 14 Cleansing in different parts of the car
(Experiment 3).

Table 14. Experiment 3. Cleansing in different parts of the float

Hours of Cleansing	Water coliform M.P.N. per 100 ml.	Clams: coliform M.P.N. per 100 ml.							
		Hod 1	Hod 6	Hod 7 inside end	Hod 10 inside end	Hod 10 outside end	Hod 16 inside end	Hod 16 outside end	Hod 18
0	13 11 4.5 7.8 Av. 9.1	7,000	11,000	7,900	17,000	17,000	2,400	2,400	9,500
12	13 2 13 4.5 Av. 8.1	3,500	2,200	2,400	14,000	9,200	3,500	11,000	2,400
18	4 2 2 4.5 Av. 3.1	-	-	-	-	-	-	-	-
24	<1.8 2 2 2 Av. <2	460	2,400	2,200	9,200	2,400	3,500	1,300	5,400
36	6.1 7.8 7.8 7.8 Av. 7.4	1,300	7,000	7,000	22,000	3,500	790	3,300	4,600
42	4.5 6.1 79 6.8 Av. 28.2	-	-	-	-	-	-	-	-
46	17 14 23 70 Av. 31	1,300	1,300	790	2,400	2,400	4,900	3,300	2,200

Conclusions

1. The records cover only the initial phases of the cleansing process and only limited deductions can be drawn.

2. Up to 36 hours the water was relatively clean but thereafter the M.P.N.'s rose suddenly suggesting that the Bar Road is not a satisfactory site for a cleansing station.

3. When they were dug the clams were contaminated more heavily than those used in the first two experiments. Besides this they showed a good deal of sample-to-sample variation in their bacterial load.

4. Self-cleansing apparently went on more or less normally for the first 24 hours but thereafter the process was upset probably for the reason outlined in (2) above.

5. The M.P.N.'s for clams from various positions in the car indicate that cleansing and re-contamination went on at about the same rate everywhere. Apparently water circulation is so good that all clams have about the same chance to discharge their pollutional loads when water conditions permit this.

6. The rush involved in digging, transporting and culling clams in time to load them onto the cleansing car during the low-tide period in which they are dug is too great to be practicable even for experimental tests and would be certainly out of the question in an industrial operation.

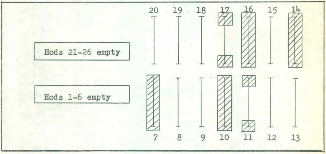
3. Experiment 4 (May 26-29).

To determine the efficiency of self-cleansing of clams in different parts of the 26-hod car and to compare the rate of cleansing at different levels in the intertidal zone.

Procedure

Following the theft of clams during the course of Experiment 3, an officer of the local detachment of the Royal Canadian Mounted Police agreed to make nightly visits to the Bar Road to discourage further pilfering. Besides this it was possible to lock the hods in position.

A second series of fourteen hods of St. Andrews Harbour clams was dug on May 26 at the morning tide and placed in a shed at the Biological Station (temperature approx. 13° C.) until the evening low tide interval. Twelve of these were then loaded into the large car as shown in the text diagram below. At the same time, the other two hods were loaded into the small experimental car, which was moored higher on the tidal flat so that longer air exposure occurred between the high-tide cleansing periods. Clam samples were removed for bacteriological analysis from six representative hods in the large car (see text figure) and the two in the small car at 6-hour and/or 12-hour intervals. Water samples from four stations adjacent to the float were taken for analysis at the same intervals throughout the cleansing period. Bacteriological data obtained during the experiment appear in Table 15 and in Figure 15.



Conclusions

1. The water at the cleansing station was relatively clean when the experiment began but was contaminated seriously during the first 12 hours. Thereafter it cleared rapidly and remained clean. The Bar Road is not therefore a suitable site for a cleansing station.

2. As might be expected the clams failed to cleanse themselves in the early phases of the test (Their M.P.N.'s rose in most cases) but as soon as the water cleared, they cleansed themselves rapidly. Within 48 hours after the water cleared (i.e. at 72 hours cleansing) the M.P.N.'s of all the lots (with one exception) had dropped to less than 2000 and the average for all lots was 1000.

3. The cleansing pattern after the water cleared seemed to show the three phases described by Mullan et al (1952) but the variable contamination of the water and the considerable lot-to-lot variation obscured the picture.

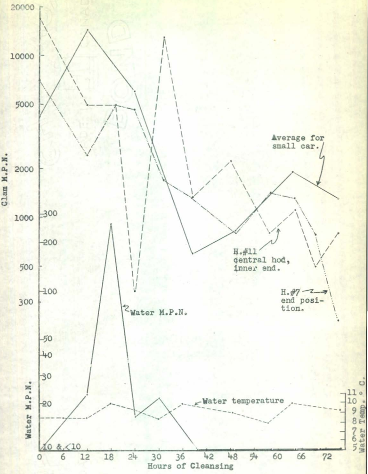


Fig. 15. Cleansing in different parts of the car (Experiment 4).

4. There were no remarkable differences in the rate or extent of cleansing among the lots tested that could be attributed to position in a car or to position in the hod.

5. The extra air exposure suffered by the clams in the small car apparently had no important effect on their cleansing.

Table 15. Results of bacteriological analysis - Experiment 4.

				Clams: Coliform M.P.N. per 100 ml.													
	Hours of clam	Water M.P.N.'s from four		Large car								Small car		Average of all	Average of samples	Average of samples
Date and Time	cleansing	stations	Av.	Hod 7	Hod 10	Hod 11 inside	Hod 11 outside	Hod 14	Hod 16	Hod 17 inside	Hod 17 outside	Hod 1	Hod 2	samples	large car	small car
May 26, 7:00 p.m.	0	4.5 4.5 2 7.8	4.7	7,000	4,600	17,000		7,900	4,900	11,000		3,500	4,900	7,600	8,733	4,200
May 27, 7:30 a.m.	12:30	49 17 7.8 21	23.7	2,400	2,400	4,900	4,600	4,900	2,400	54,000	1,300	13,000	7,900	9,780	9,613	10,450
May 27, 2:00 p.m.	19:00	540 350 280 79	312.3	4,900	28,000	4,900	7,900	13,000	7,900	2,400	13,000	-	-	10,250	10,250	-
May 27, 7:30 p.m.	24:30	13 33 17 2	16.3	4,600	1,700	350	4,900	54,000	7,000	4,600	7,900	4,900	7,000	9,695	10,631	5,950
May 28, 2:15 a.m.	31:15	13 23 33 17	21.5	1,700	3,500	13,000	4,600	1,300	2,800	1,300	700	-	-	3,613	3,613	-
May 28, 9:15 a.m.	38:15	2 4.5 2 11	4.8	1,300	3,500	1,300	3,500	3,500	7,900	4,900	790	490	700	2,788	3,335	595
May 28, 8:30 p.m.	49:30	13 6.8 2 4.5	6.6	790	1,300	2,200	2,400	3,500	2,400	700	3,500	1,300	350	1,844	2,099	825
May 29, 4:00 a.m	58:15	2 4.5 13 4.5	6.0	1,400	1,300	790	1,700	1,700	1,300	490	1,300	-	-	1,248	1,248	-
May 29,10:00 a.m.	64:15	2 4.5 21 2	7.4	1,300	490	1,100	4,900	1,100	2,200	790	790	2,400	1,400	1,647	1,584	1,900
May 29, 3:00 p.m.	69:15	<1.8 2 7.8 2	3.2	790	490	490	1,700	790	490	790	790	-	-	791	791	-
May 29, 9:00 p.m.	75:15	5.6 <1.8 7.8 4.5	4.9	230	330	790	2,400	1,300	790	790	790	1,300	1,300	1,002	927.5	1,300

Experiment 5. (June 3-11).

To compare the rates of self-cleansing in different parts of the car and the effect of sampling disturbance on the rate, using stocks of clams with uniform bacterial loads.

Procedure

Clams were dug from St. Andrews Harbour during the afternoon low tide of June 3 and stored in the cool Biological Station tank room until the following morning. They were then exposed to sunlight on the Biological Station wharf for one and one half hours during which time they were washed with sea water.

A special effort was made to mix the stock thoroughly to eliminate lot-to-lot variations in bacterial loads that tend to obscure differences in the self-cleansing rates of clams when subjected to different treatments (See conclusion 3 from Experiment 4). When they were brought from the tank room, the hodfuls were dumped in a long pile on the wharf then the 18 hods were re-filled by picking up clams in small lots at random from the full length of the pile.

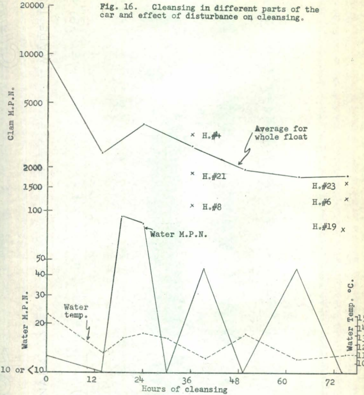
It was decided to load the float to capacity (26 hods) to avoid "border effects" that blank spaces might induce in the cleansing pattern. Hence an additional 8 hods of clams were dug at low tide on the afternoon of June 4. These (#9-12 and 15-18) were washed and placed in the float simultaneously with the other 18 hods but separate from them (See diagram below) and were never sampled.

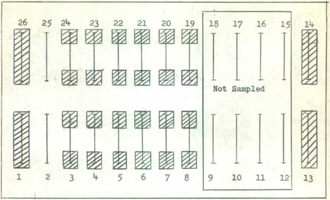
Since the three hods which were to be regularly sampled at both their inner and outer ends would be depleted twice as fast as those from which single samples were to be taken (#1, 13, 14 and 26) the "inside and outside" samples were taken alternately from hods in corresponding positions on opposite sides of the float, i.e. sampling of #3, 22 and 7 alternated with that of #24, 5 and 20 respectively.

It was thought that the sampling disturbance might affect cleansing rates and to see if this were true, three hods (#4, 8 and 21) were left undisturbed until 36 hours and 3 other (#6, 19 and 23) until 72 hours of cleansing before sampling.

Bacteriological data obtained from this study and from water samples taken periodically from four stations adjacent to the float are recorded in Table 16 and some of these are plotted in Figure 16.

Fig. 16. Cleansing in different parts of the car and effect of disturbance on cleansing.





Conclusions

1. The water at the cleansing station was relatively clean when the experiment began but within 24 hours it showed serious contamination which continued intermittently throughout the test at a somewhat lower level until the end of sampling of the stocks (72 hours cleansing). Thereafter (June 8-11) the water scores rose again and much worse conditions were encountered than in the first 72 hours.

2. As might be expected the clams showed a relatively slight drop in their M.P.N.'s during their exposure but it is considered to have been sufficient to show up gross differences in cleansing characteristics had these obtained in different parts of the float.

3. There were no great differences in the behaviour of clams in various parts of the car, so with the reservations mentioned in (2) above, it would seem that cleansing conditions were approximately the same for clams throughout the float irrespective of the position of the hod they were in or their position in that hod.

4. The disturbance of hods of clams which attends the removal of samples apparently has no remarkable effect on their cleansing behaviour. If anything it tends to reduce the cleansing rate.

If this observed effect is typical then the cleansing during commercial treatments which would involve no disturbance for sampling, may be slightly better than, rather than poorer than, the experimental data collected heretofore would lead us to expect.

Table 16. Bacteriological results - Experiment 5.

Clams: Coliform M.P.N. per 100 ml.

Date and Time	Hours of clam cleansing	Water M.P.N. Stations 1-4	Av.	Hod 1	Hod 13	Hod 14	Hod 26	Hod 3		Hod 7		Hod 22		Hod 5		Hod 20		Hod 24		Hod 4		Hod 8	Hod 21		Hod 6		Hod 19	Hod 23		Average of all samples	Average of inside samples	Average of outside samples	Average of end hods	Average of central hods
								In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out		In	Out	In	Out		In	Out					
June 3, 1-4p.m.	Dug	-	-			Composite samples taken by removing some from each hod. 2,300		7,900		3,300			4,900			7,900														5,260			5,260	5,260
June 4, 2:45p.m.	0	2 6.8 8.1 33	12.8	4,900	7,900	4,900	7,900	3,300		13,000		11,000		3,300		3,300		35,000		-		-	-							9,750			5,900	11,843
June 5, 4:30a.m.	13:45	5.5 6.8 7.8 14	8.5	2,400	3,500	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	-		-		-		-		-	-							2,510	2,400	2,400	2,675	2,400
June 5, 9:30a.m.	18:45	79 110 110 79	94.5	-		-	-	-	-	-	-	-	-	-		-		-		-		-	-		-		-	-		-	-	-	-	-
June 5, 3:00p.m.	24:15	15 240 46 33	83.5	3,500	160,000	54,000	3,500	-	-	-	-	-	-	3,500	3,500	2,400	1,300	3,500	3,500	-		-	-							25,870 3,788*	3,133	2,766	20,333	2,950
June 5, 9:00p.m.	30:15	4.5 11 11 13	9.9	-	-	-	-	-	-	-	-	-	-	-		-		-		-		-	-		-		-	-		-	-	-	-	-
June 6, 5:30a.m.	38:45	49 49 31 49	44.5	2,200	2,400	3,500	3,500	1,300	9,200	2,400	3,500	1,300	16,000	-		-		-		2,400	3,500	1,100	1,100	2,400						3,720 2,292** 2,786+	1,700	6,920 3,133** 4,650+	2,900	4,018
June 6, 4:30p.m.	49:45	4 4.5 7.8 11	6.8	1,300	2,400	1,700	2,400	-	-	-	-	-	-	790	3,500	1,300	2,400	2,400	1,300	-		-	-							1,949	1,477	2,400	1,950	1,950
June 7, 6:30a.m.	63.45	23 33 70 49	44.8	1,300	2,400	1,300	790	2,400	1,700	3,500	1,300	2,400	1,300	-		-		-		-		-	-							1,739	1,497	1,434	1,450	2,100
June 7, 4:30p.m.	73:45	4.5 2 4.5 11	5.5	1,300	1,100	1,700	1,300	-	-	-	-	-	-	3,500	5,400	2,400	1,300	1,300	1,300	-		-	-		790	1,700	790	1,300	1,700	1,787	1,858	2,280	1,350	1,953
June 8, 5:30p.m.		6.8 6.8 4.5 7.8	6.5																															
June 9, 5:00p.m.		49 79 49 110	71.8																															
June 10, 8:00p.m.		70 79 46 79	68.5																															
June 11, 8:30a.m.		79 240 240 79	154.5																															
June 11, 2:30p.m.		170 220 220 1,600	552.5																															

* Without Hod #13
\+ Without outside sample of hod #22
** Without outside samples of hod #'s 3 and 22

5. Experiment 6.

Correlation of hydrographic changes and coliform M.P.N.'s of water samples in the Bar Road area.

During the routine water sampling program at the Bar Road cleansing station, considerable day-to-day fluctuations in the coliform M.P.N.'s were noted. These masked some of the features of cleansing that experiments conducted this year were designed to show. No such serious changes occurred in the experiments conducted in 1951 so far as the records reveal (Mullan et al 1952) and this prompted an enquiry into their nature. To this end, some of the meteorological data (Table 17) assembled by the Atlantic Biological Station and hydrographic data extracted from the Department of Mines and Technical Survey's, "Tide Tables for the Atlantic Coast of Canada, 1952", were plotted in Figure 17 along with records listed earlier in this report for the Bar Road.

A study of Figure 17 suggested that the degree of contamination of the water is more closely related to cyclic changes in tidal amplitude than to wind velocity or direction (churning of the water) or to precipitation (runoff from the land). To check this conclusion three series of high- and low-tide water samples (150 samples in all) were taken in the area between Indian Point and the northern end of Minister's Island. Coliform M.P.N.'s for these are reported in Figures 18-20, applying to June 11,18 and 21, 1952, respectively.

The June 11 samples were taken during a period of spring tides. The day was calm, cloudy and showery and there had been showers during the previous night. The 19 low-tide samples were taken from 7:45 to 9:15 a.m.; the 28 high-tide samples, 1:45 to 3:15 p.m. with the tide high-slack or high-falling.

The June 18 samples were taken during neap tides. There had been light showers during the previous 24 hours. The 30 high-tide samples were taken from 8:00 to 10:00 a.m. when it was calm and foggy; the 18 low-tide taken from 3:00 to 4:00 p.m. when it was calm and sunny. The June 21 samples were also made at neap tides. The weather was fine all day with light north winds. There had been no rain in the previous 48 hours. The 34 high-tide samples were taken from 10:00 to 11:30 a.m.; the 21 low-tide from 3:00 to 4:00 p.m.

A study of Figures 18-20 confirms the conclusion drawn from the study of Figure 17. Both high- and low-tide samples show that pollution is heavier and wider-spread during spring than during neap tides. On any one day there was little difference between the high- and low-tide readings. St. Andrews Harbour seems to be the principal source of contamination but there are peculiar features of the spread for which no ready explanation can be offered, e.g., the very high M.P.N.'s observed just south of Minister's Island on June 11.

Fig. 17. Relationship of M.P.N.'s of coliform bacteria in Bar Road water samples to wind force, tidal amplitude, rainfall and salinity and temperature of the seawater - May and June 1952.

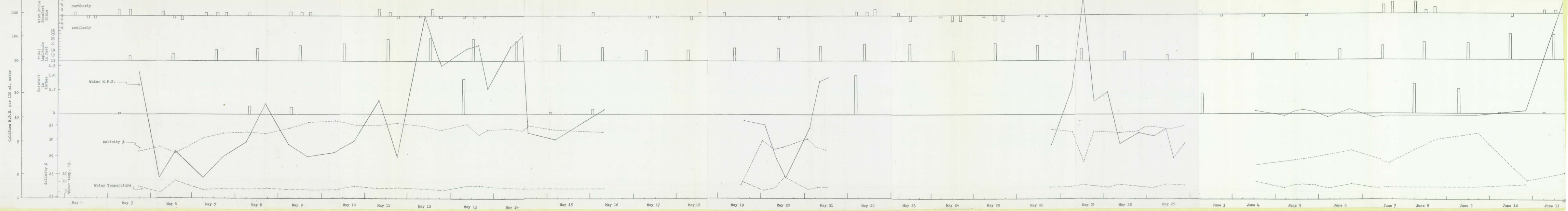


Fig. 18. Spring-Tide water sampling of Bar Road area, June 11, 1952. Coliform M.P.N.'s for 28 high-tide (underlined) and 19 low-tide samples are indicated by the numbers opposite "x's" which show positions of sampling stations.

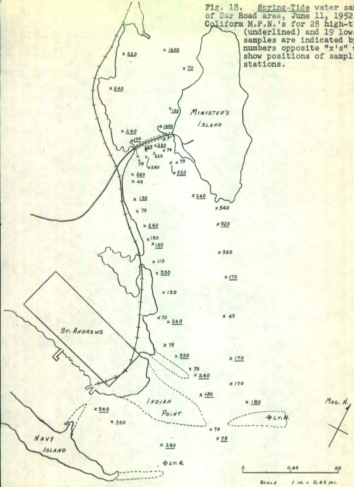
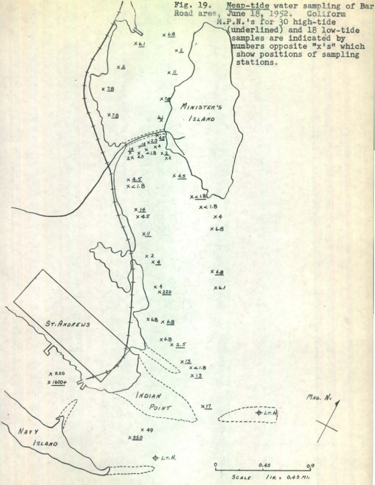


Fig. 19. Neap-tide water sampling of Bar Road area, June 18, 1952. Coliform M.P.N.'s for 30 high-tide (underlined) and 18 low-tide samples are indicated by numbers opposite "x's" which show positions of sampling stations.



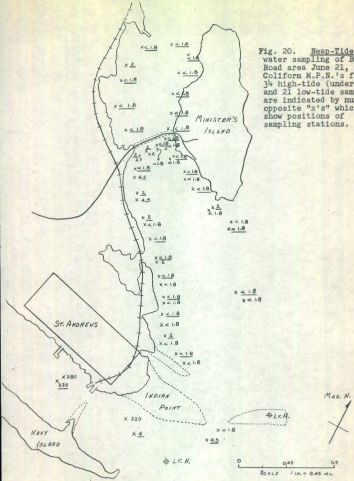


Fig. 20. Neap-Tide water sampling of Bar Road area June 21, 1952. Coliform M.P.N.'s for 34 high-tide (underlined) and 21 low-tide samples are indicated by numbers opposite "x's" which show positions of sampling stations.

Table 17. Meteorological observations from Atlantic Biological Station Records - April 1 to June 30, 1952.

		Precipitation during 24 preceding hours - read at 8:00a.m.	Wind Direction and Force (Beaufort Scale) 7:30a.m. D.	F.	1:00p.m. D.	F.	5:30p.m. D.	F.
April	1	-	NW	2	Calm		SE	1
	2	-	NE	1	NE	1	NE	4
	3	0.68	NE	1	NE	1	Calm	
	4	-	Calm		Calm		NW	1
	5	-	Calm		Calm		Calm	
	6	0.42	E	4	SE	2	S	4
	7	-	S	2	Calm		Calm	
	8	-	NE	2	NE	4	NE	4
	9	-	NE	2	E	1	SE	1
	10	-	Calm		SE	1	SE	3
	11	-	S	1	SW	1	W	3
	12	-	NW	4	NW	5	NW	5
	13	-	NW	3	NW	3	NW	1
	14	-	Calm		Calm		Calm	
	15	1.21	NE	4	NE	4	NE	7
	16	-	Calm		N	2	N	3
	17	-	NE	1	SE	2	SE	4
	18	-	Calm		E	1	E	2
	19	-	Calm		SW	2	SW	4
	20	-	NW	3	NW	4	NW	6
	21	0.09	N	4	N	3	N	5
	22	-	NE	1	Calm		Calm	
	23	0.02	Calm		NW	6	NW	6
	24	-	NW	4	NW	5	NW	4
	25	-	Calm		W	1	SW	4
	26	-	Calm		S	1	S	2
	27	-	Calm		E	1	E	1
	28	-	Calm		Calm		NE	2
	29	0.34	Calm		Calm		Calm	
	30	0.08	NE	4	NE	4	NE	6
May	1	-	NE	4	NE	3	NE	4
	2	-	W	2	W	1	NW	1
	3	-	W	1	SW	1	S	1
	4	-	NE	1	S	1	E	1
	5	0.02	NNE	2	NE	2	Calm	
	6	-	NE	1	S	1	S	2
	7	-	NW	1	NE	2	NE	1
	8	0.13	NE	1	Calm		Calm	
	9	0.11	N	1	N	1	N	1
	10	-	Calm		Calm		Calm	
	11	-	NE	2	NE	1	S	1
	12	-	E	1	NE	2	SE	1
	13	0.87	SE	1	SE	1	SE	1
	14	-	Calm		Calm		Calm	
	15	0.03	Calm		Calm		Calm	
	16	0.08	NE	1	Calm		Calm	
	17	-	Calm		S	1	E	1
	18	-	Calm		S	2	NW	1
	19	-	NE	1	Calm		Calm	
	20	-	Calm		S	2	S	1
	21	-	Calm		Calm		Calm	
	22	1.03	NE	1	NE	1	NE	2

Table 17 continued.

Date		a.m. (8:00) Precipitation	7:30 D.	7:30 F.	1:00 D.	1:00 F.	5:30 D.	5:30 F.
May	23	-	NE	1	E	3		
	24	-	E	1	S	3	S	3
	25	-	S	1	S	3	S	3
	26		Calm		SW	1	SW	1
	27							
	28							
	29							
	30							
	31							
June	1		Calm		Calm		SE	1
	2		SE	1	Calm		NW	1
	3	0.42	NE	1	Calm		SE	1
	4		Calm		Calm		SE	1
	5		Calm		Calm		SE	1
	6		Calm		Calm		Calm	
	7		Calm		NW	3	NW	4
	8	0.67	NW	4	NW	1	NW	2
	9	0.52	Calm		Calm		Calm	
	10	0.01	Calm		SW	2	Calm	
	11	0.02	E	1	E	1	NE	1
	12	1.00	NE	1	NE	1	Calm	
	13	0.05	NE	1	Calm		Calm	
	14		Calm		S	1	S	2
	15		SW	4	SW	1	SW	2
	16	0.06	NE	5	NE	4	NE	4
	17		Calm		Calm		SE	1
	18	0.08	Calm		Calm		NW	2
	19		Calm		SE	1	SE	2
	20	0.13	NW	3	NW	4	NW	4
	21		Calm		NW	1	NW	1
	22		Calm		S	3	S	4
	23		NE	1	SE	1	SE	1
	24		S	1	SE	2	SE	4
	25	0.03	Calm		Calm		Calm	
	26		Calm		Calm		Calm	
	27	0.50	NE	2	Calm		Calm	
	28		NE	2	NE	2	NE	2
	29		Calm		Calm		Calm	
	30		NE	2	Calm		SE	1

Apparently the heavy flushing of the harbour basin at the times of the high-amplitude spring tides spreads pollution over wider areas than usual. Pollution may accumulate by sedimentation (Ketchum et al, 1952). If this be the case, then it is conceivable that it could be "stored up" in basins during neap tides for spreading at springs. Such an effect might account for the great changes recorded.

To the best of the writer's knowledge this relationship between tidal cycles and pollution levels has not been previously demonstrated or even suspected. It has implications beyond the scope of the present investigation. The principle has significance in the execution and interpretation of bacteriological examinations of all shellfish-producing waters.

Conditions encountered by Mullan et al in 1951, so far as they are known, are described in Table 18. The relationship which appeared so clearly in 1952 and which has just been discussed does not appear in the 1951 data. This is understandable because the facilities available for work that year limited the possible number of observations. Furthermore those that were made fell in September and October when pollution levels are generally lower in this area than in summer (Tennant 1947 and 1948).

This periodic pollution is so great as to eliminate the Bar Road as a site for a cleansing station as was concluded from other 1952 observations reported here.

Table 18. Relationship between Bar Road water M.P.N.'s in September and October 1951 (See Mullan et al, 1952, Table 12) and tidal amplitudes during that period.

Date	Tidal amplitude	Water M.P.N.	Date	Tidal amplitude	Water M.P.N.
Sept. 10	17.0	0	Oct. 1	23.0	
11	17.5	30	2	23.9	
12	21.0	79	3	24.2	
13	22.9	6.8	4	24.0	
14	24.4	140	5	23.0	
15	25.1		6	21.5	
16	25.0		7	19.9	
17	24.6		8	18.9	
18	23.6		9	17.5	
19	22.0	11	10	19.4	
20	20.1	14	11	20.8	
21	18.1	49	12	22.2	
22	16.4		13	23.5	
23	14.5		14	24.2	
24	14.1		15	24.5	
25	14.7	4.5	16	23.9	
26	15.9		17	21.7	
27	17.5		18	21.1	
28	19.3	30	19	19.5	
29	20.8	2	20	17.8	
30	21.9	7.8	21	16.1	
			22	15.2	7.8
			23	13.7	
			24	15.1	2.0
			25	16.1	
			26	17.5	
			27	19.2	0
			28	21.1	
			29	23.0	9.2
			30	24.2	0
			31	25.7	

References.

Ketchum, B.H., J. C. Ayres and R. F. Vaccaro. Processes contributing to the decrease of coliform bacteria in a tidal estuary. Ecology 33(2):247-258, 1952.

Mullan, M. W., A. B. Williams and D. R. Colwell. Preliminary experiments in the self-cleansing of clams (Mya arenaria L.). Fish. Res. Bd. Can., M.S. Rep. Biol. Stn's. 440, 1952.

Tennant, A. D. A bacteriological survey of clam-producing areas and shucking plants in Nova Scotia and New Brunswick, June - October, 1947. Dept. Nat. Health and Welfare, Lab. of Hygiene, M.S. Reports, 1947.

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V. BACTERIOLOGICAL SURVEYS OF THE HOLT'S POINT AREA

by

A. D. Tennant and I. E. Erdman

A review of bacteriological data led to the conclusion that the Bar Road could not be considered a suitable site for commercial-scale cleansing and that some area that is more nearly free of intermittent bacterial pollution must be found before commercial-scale clam cleansing could be considered practicable.

Previous studies* of the Passamaquoddy Bay area suggest that Holt's Point might be satisfactory. These early studies were reviewed and more observations made in 1952.

Results of Early Surveys

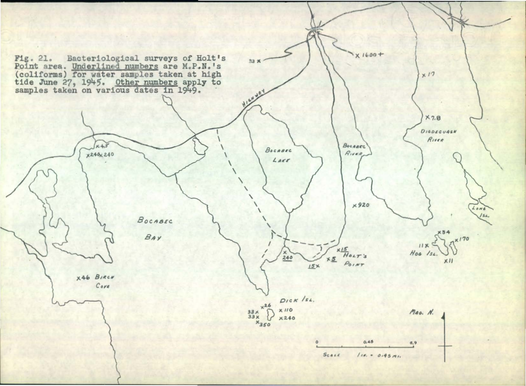
On June 27, 1945, four water samples were taken in the Holt's Point area by the Public Health Engineering Division of the Department of National Health and Welfare. Samples were taken at high tide during a rainstorm. The tide was at an intermediate phase in its cycle (neither neap nor spring) at that time. The results of the bacteriological analyses of these samples are listed below and are plotted (underlined) in figure 21.

Sample No.	Location	Coliform M.P.N. per 100 ml.
1	Outlet from Bocabec (Wheaton) Lake	240
2	Near Holt's Point	15
3	Near Holt's Point	5
4	Near Holt's Point	15

On various dates in 1949, Mr. Tennant of the Laboratory of Hygiene took a number of water samples, mainly from the Islands on either side of Holt's Point. No record of the stage of the tide (high or low) is supplied with these. The results of analyses appear below and are plotted (without underlining) in figure 21.

* See also section of this report by Mr. Dohaney.

Fig. 21. Bacteriological surveys of Holt's Point area. Underlined numbers are M.P.N.'s (coliforms) for water samples taken at high tide June 27, 1945. Other numbers apply to samples taken on various dates in 1949.



Date	Location	Coliform M.P.N. per 100 ml.	
June 17	Dick Island, west side (1)	33	
June 17	Dick Island, west side (2)	33	
June 17	Dick Island, north west tip	26	
June 17	Dick Island, east side	110	Neap tides
June 17	Hog Island, north shore	34	
June 17	Hog Island, east shore	170	
June 17	Hog Island, south shore	11	
June 17	Hog Island, west shore	11	
June 27	Big Bay Cove (Head of Bocabec Bay)	240	
June 27	Digdeguash River (East shore)	17	
July 21	Birch Cove	46	
July 28	Big Bay Cove	240	
July 28	Dick Island, east side	240	Spring tides
July 28	Dick Island, west side	350	
July 28	Bocabec River, near mouth	920	
July 28	Bocabec River, Highway Bridge	1600+	
Oct. 14	Digdeguash River, east side	7.8	
Oct. 15	Bocabec River, Highway Bridge	33	
Oct. 15	Big Bay Cove	4.5	

The results suggest that although there is some contamination of the waters in this region the area immediately about Holt's Point is relatively free.

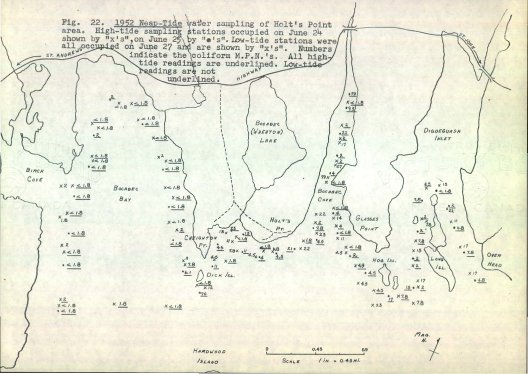
Results of 1952 Surveys

It seemed important to have further information about the Holt's Point area before suggesting that it would be a satisfactory site for clam-cleansing operations. Accordingly three further examinations were made in 1952 - the first by junior author.

The writer's study of water conditions included a wide area and was spread over three days during a period of neap tides. On June 24 between 12:00 noon and 2:00 p.m. 36 high-tide samples were taken. There was a strong southwest wind that day and there had been no rain within the last 48 hours. On June 25 between 12:30 and 2:30 p.m. 42 high-tide samples were taken. The weather then was calm and cloudy and there had been no rain in the preceding 72 hours. On June 27 between 8:00 and 10:30 a.m. 39 low-tide samples were taken. There was a light north wind blowing then and there had been heavy showers in the 48 hours preceding the sampling.

The results of the analyses of these samples are presented in figure 22. Of the 117 samples, 35 were free of coliform contamination (i.e. M.P.N.'s were <1.8); for 50 the M.P.N.'s ranged from 1.8 to 7.8; for 27 they ranged from 9.3 to 23 and for the last 5 they were 27, 49, 70, 79 and 79. The last three were from Hardwood Island, Bocabec River and Bocabec Cove respectively.

Fig. 22. 1952 Neap-Tide water sampling of Holt's Point area. High-tide sampling stations occupied on June 24 shown by "x's", on June 25 by "●'s". Low-tide stations were all occupied on June 27 and are shown by "x's". Numbers indicate the coliform M.P.N.'s. All high-tide readings are underlined. Low-tide readings are not underlined.



The 1952 data suggest that as regards purity of water, the Holt's Point area would qualify as satisfactory for clam cleansing but they all apply to neap-tide conditions. In discussing the results of experiment 6 it was pointed out that a bacteriological survey such as the present should include conditions at different phases of the tidal cycle. In this case it seems desirable that further sampling be done at least during a spring phase before any confident statement is made on the quality of the water.

VI. ASSESSMENT OF HOLT'S POINT AS A SITE FOR A CLAM-CLEANSING STATION

by

M. W. Mullan and A. B. Williams

The 1952 work at the Bar Road showed that a better site was needed for a clam-cleansing station. From what information was available from early surveys, Holt's Point seemed suitable and several observations were made to provide a better basis for judgment.

On July 8, 1952, when tides were in the spring phase a high-tide and low-tide series of 17 water samples each was taken and subjected to bacteriological analysis. On July 10, when the tide was at an intermediate phase between springs and neaps, the same sampling was repeated. On August 3, when the tides were in the same phase as on July 10, four more high-tide samples were taken. The positions of the numbered stations from which these samples were taken are shown in figure 23 and the results of the bacteriological examinations are reported in table 23.

On both September 18 and 19 when the tides were in a spring phase, a high-tide and low-tide series of samples was again taken at Holt's Point and also the Bar Road. There had been no rain during the week preceding September 18 but there was a heavy rain during the night of September 18 to 19. On September 26, when the tide was in a neap phase, the sampling of the two areas was repeated.

The positions of the numbered sampling stations occupied at these times are shown in figures 24 and 25 and the results of the bacteriological examination of the samples appear in tables 24 to 26.

Conclusions

1. A study of these 1952 records shows that even after heavy rain the waters in the vicinity of Holt's Point, during both spring and neap tides, yielded coliform scores in the same low range found to prevail there during previous surveys. This is in sharp contrast with the high scores obtained from samples taken after the same heavy rain, during a spring tide, at the Bar Road. Most other Bar Road samples yielded low coliform counts.

2. The data substantiate Tennant and Erdman's deduction that tidal phases are important in determining the level of pollution at the Bar Road and also show that area to be unsuitable as a site for a clam-cleansing station.

3. Holt's Point water, on the other hand, seems to be suitable.

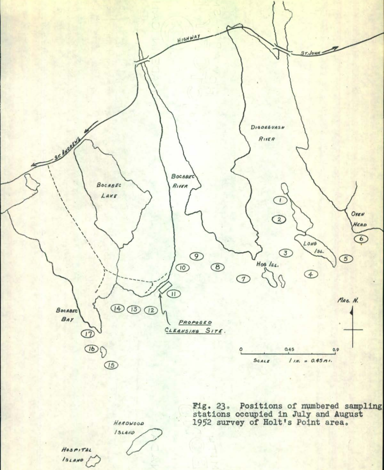


Fig. 23. Positions of numbered sampling stations occupied in July and August 1952 survey of Holt's Point area.

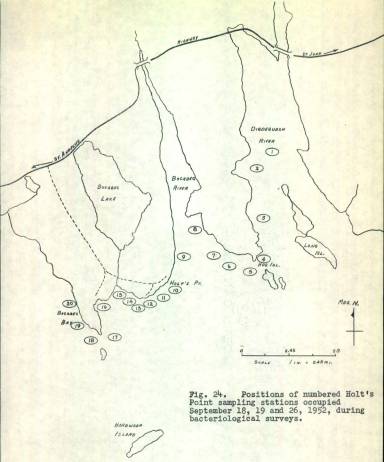


Fig. 24. Positions of numbered Holt's Point sampling stations occupied September 18, 19 and 26, 1952, during bacteriological surveys.

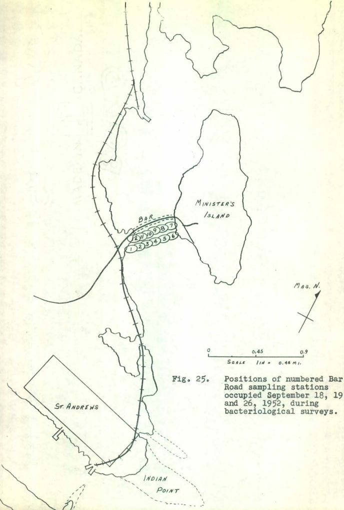


Fig. 25. Positions of numbered Bar Road sampling stations occupied September 18, 19 and 26, 1952, during bacteriological surveys.

Table 23. Results of bacteriological analyses of July and August 1952 Holt's Point water samples.

Sampling station No. (see fig. 23)	Spring Tides, July 8, 1952: Low slack to ½ flood M.P.N.	Spring Tides, July 8, 1952: High slack to ½ ebb M.P.N.	Medium Tides, July 10, 1952: Low slack to ½ flood M.P.N.	Medium Tides, July 10, 1952: High slack to ½ ebb M.P.N.	Medium Tides, Aug. 3, 1952: High slack to ½ ebb M.P.N.
1	<1.8	3.6	3.6	3.6	
2	15.0	<1.8	3.6	9.1	
3	<1.8	<1.8	3.6	9.1	
4	<1.8	43	3.6	<1.8	
5	3.6	<1.8	3.6	<1.8	
6	<1.8	9.1	3.6	3.6	
7	3.6	23	<1.8	3.6	
8	3.6	<1.8	<1.8	3.6	
9	43.0	3.6	15.0	<1.8	
10	9.1	<1.8	43.0	<1.8	<1.8
11	3.6	3.6	<1.8	<1.8	3.6
12	3.6	<1.8	<1.8	<1.8	<1.8
13	<1.8	<1.8	43.0	3.6	<1.8
14	3.6	<1.8	23.0	3.6	
15	3.6	<1.8	<1.8	3.6	
16	<1.8	<1.8	<1.8	<1.8	
17	<1.8	<1.8	<1.8	<1.8	

Table 24. Results of bacteriological examination of water samples taken from Holt's Point and Bar Road areas at <u>spring tides</u> September 18, 1952, after a dry period.

Holt's Point: Sampling station No. (see fig. 24)	M.P.N.	Bar Road: Sampling station No. (see fig. 25)	M.P.N.
High Tide Samples			
1	3.6	1	<3
2	3.6	2	3.6
3	3.6	3	<3
4	3	4	<3
5	43	5	7.2
6	<3	6	3.6
7	<3	7	<3
8	3.6	8	3.6
9	<3	9	<3
10	3.6	10	<3
11	9.1	11	<3
12	3.6	12	<3
13	9.1		
14	15.0		
15	3.6		
16	3.6		
17	<3		
18	<3		
19	<3		
20	<3		
Low Tide Samples			
5	9.1	1	3.6
6	<3	2	<3
7	3.6	3	<3
8	3.6	4	3
9	9.1	5	3.6
10	3.6	6	3.6
11	<3	7	3.6
12	<3	8	9.1
13	3.6	9	<3
14	3.6	10	<3
15	<3	11	7.3
21	<3	12	460

Table 25. Results of bacteriological examination of water samples taken from Holt's Point and Bar Road areas at <u>spring tides</u>, September 19, 1952, after a heavy rain.

Holt's Point: Sampling station No. (see fig. 24)	M.P.N.	Bar Road: Sampling station No. (see fig. 25)	M.P.N.
	High Tide Samples		
1	3.6	1	240
2	3	2	43
3	9.1	3	23
4	9.1	4	43
5	23	5	23
6	9.1	6	43
7	9.1	7	9.1
8	9.1	8	23
9	23	9	150
10	7.2	10	>1500
11	43	11	150
12	39	12	>1500
13	3.6		
14	<3		
15	<3		
16	9.1		
17	<3		
18	3.6		
19	<3		
20	<3		
	Low Tide Samples		
1	23	1	>1500
2	23	2	<3
3	23	3	>1500
4	9.1	4	<3
5	15	5	<3
6	9.1	6	290
7	23	7	>1500
8	23	8	>1500
9	23	9	43
10	23	10	15.0
11	3.6	11	15.0
12	3.6	12	3.6
13	3.6		
14	3.6		
15	3.6		
16	3.6		
17	<3		
18	<3		
19	3.6		
20	3.6		

Table 26. Results of bacteriological examination of water samples taken from Holt's Point and Bar Road areas at neap tides, September 26, 1952, after a dry period.

Holt's Point: Sampling station No. (see fig. 24)	Holt's Point: M.P.N.	Bar Road: Sampling station No. (see fig. 25)	Bar Road: M.P.N.
High Tide Samples			
1	9.1	1	9.1
2	15.0	2	3.6
3	23	3	3
4	3.6	4	<3
5	<3	5	<3
6	<3	6	<3
7	3.6	7	7.3
8	9.1	8	3.6
9	7.2	9	3.6
10	9.1	10	9.1
11	3.6	11	15.0
12	15.0	23	15.0
13	3.6		
14	23		
15	23		
16	3		
17	15.0		
Low Tide Samples			
5	15.0	1	3.6
6	15.0	2	<3
7	43	3	3
8	9.1	4	3
9	23	5	9.1
10	43	6	<3
11	23	7	3.6
12	23	8	3
13	9.1	9	<3
14	43	10	3.6
15	43	11	15.0
16	9.1	12	23
17	23		
18	7.3		
19	9.1		
20	43		

Clam-cleansing trials at Holt's Point

As a check on any conclusions that might be drawn from water sampling as to the suitability of Holt's Point as a site for clam cleansing, two lots of sewage-polluted St. Andrews Harbour clams were exposed at Holt's Point - one from July 31 to August 3, the other from September 22 to 25, 1952.

The first lot consisted of a single hod which was fixed to the beach in the inter-tidal zone by stakes driven into the bottom. Triplicate samples of clams were taken at the time of digging and after 72 hours of cleansing. The second lot comprised 20 hods and was placed in the floating car. Single samples were withdrawn randomly from the stock before exposure and from all parts of the car at various times after cleansing began. The results of the bacteriological examination of these and of water samples taken at the cleansing station at sampling times appear in table 27.

Conclusions

1. The results show that in polluted clams, held off Holt's Point under what could be termed "commercial-cleansing" conditions, the coliform count dropped rapidly to very low levels.

2. The pattern of decreasing counts, in the September test was more nearly logarithmic than the characteristic pattern obtained in trials conducted in 1951 and 1952 at the Bar Road. This may be the result of a more constant frequency of coliform bacteria in Holt's Point water or of the presence, in either one of the areas, of some other biological factor.

3. A cleansing period of 48 hours seems to be all that is required to effect a reduction to a level well below 2400.

4. These data taken with those reported in tables 24 to 26 strongly suggest that Holt's Point would be a satisfactory site for a clam-cleansing station.

5. The floating car seems to provide good conditions throughout for clam-cleansing.

Table 27. Results of bacteriological examination of water samples from Holt's Point and of clams cleansed there July 31 to August 3 and September 22 to 25, 1952.

Time of test	Sample tested	Hours after start of cleansing 0	13	20	48	72
July 31 to August 3	Clams	5400				620
		7000				540
		7900				720
		Av. 6766				Av. 626
	Water					<1.8
						3.6
						<1.8
						<1.8
						Av. <1.8
September 22 to 25	Clams	10,000	1,196	2,210	693	800
	Water		15	23	3	23

VII. SANITARY AND BACTERIOLOGICAL SURVEYS OF HOLT'S POINT, N.B. SHELLFISH AREAS NB 40 AND 41

by

V. C. Dohaney

To aid in the appraisal of Holt's Point as a site for clam cleansing, the writer has reviewed the results of earlier examinations and made further observations in 1952.

Previous Survey

In July 1951, at times of neap tides a sanitary and bacteriological examination of this area was completed by the author and Mr. E. Nason. On July 3 when the tide was approaching the "high" stage (½ flood), 10 water samples were taken and on July 11, at low tide (¾ ebb), 14 samples were taken. The results of the analyses of these for coliform bacteria are presented in figure 26.

The records show that these organisms were rare in the water at that time.

Two possible sources of contamination were indicated by a sanitary survey. In each case the source was a summer cottage: one, a privy situated on the beach, the other a sewer leading to the beach.

Bacteriological examination of the waters surrounding Holt's Point in September 1952.

On September 8, 1952, 10 water samples for bacteriological examination were collected in the immediate area of Holt's Point when the tide was high and flooding. Another 10 were taken September 9 when the tide was at the half-flood stage. A third series of 10 was gathered September 10 at low tide. These samplings were made when the tide was at a late stage in a "spring" phase of its cycle. The amplitude decreased from 23.9 feet on September 8 to 19.6 feet on September 10.

The results of bacteriological analyses of these samples are presented in figure 27.

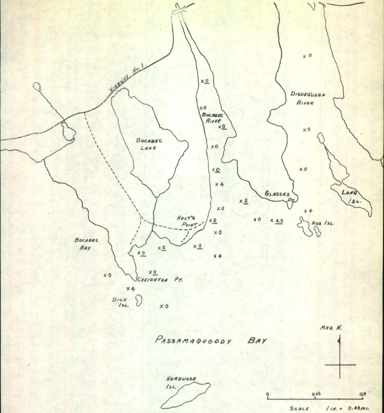


Fig. 26. 1951 Neap-tide bacteriological survey of Holt's Point waters. "x's" show positions of stations occupied. The numbers are the coliform bacteria M.P.N. counts on water samples. Underlined values are for high-tide July 3, those not underlined are for low-tide July 11 samples.

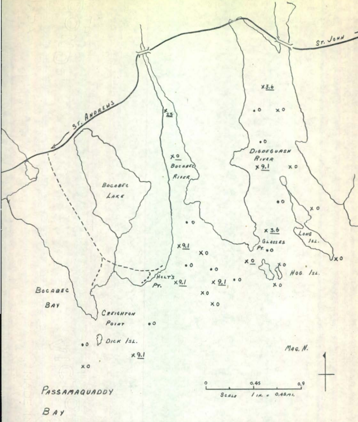


Fig. 27. 1952 bacteriological survey of Holt's Point waters at a late stage in a spring phase of a tidal cycle. "x's" show the positions of the stations occupied on September 8 and 10. The corresponding numbers are the coliform bacteria M.P.N. counts, those underlined applying to the September 8 high-tide samples, those not underlined to the low-tide September 10 samples. The dots show the positions of the stations occupied September 9 when the tide was at the half-flood stage and the corresponding numbers are the M.P.N. counts for coliform bacteria.

The following table is an excerpt from the meteorological and hydrographic records compiled at the Atlantic Biological Station for the immediate area.

Date 1952	Rainfall in previous 24 hours (inches)	Wind Direction and Force (Beaufort Scale) at Various Times 7:30 a.m.	1:00 p.m.	5:00 p.m.	Water temp. °C.
Sept. 7	0	NE 4	NE 2	NE 2	12.7
" 8	0.06	calm	calm	SE 1	12.6
" 9	0	calm	SE 1	S 1	12.9
" 10	0	calm	-	-	12.7

In spite of the rain and heavy winds of September 7 and the heavy flushing of adjacent estuaries that went on during the full spring tides at the time, the data show that the water at Holt's Point contained only small numbers of coliform bacteria on September 8. All the M.P.N.'s were less than 10 except one applying to a station well up Bocabec River.

With calmer weather and some reduction in the tidal flushing (the tides were still in the spring phase, however) the water was virtually free of coliform bacteria on September 9 and 10. These findings accord with those of Erdman for the Bar Road. The level of pollution is highest when the tidal amplitude is greatest, i.e. the worst conditions occur at spring tides.

The water temperatures at the time were close to those recorded by Erdman and Mullan during their summer studies.

A sanitary survey of the area at the time of these samplings indicated that no additional possible sources of contamination have been introduced to the area since July 1951.

These findings support the view that Holt's Point may be a satisfactory site for clam-cleansing.

VIII. TRIAL INDUSTRIAL USE OF SELF-CLEANSED CLAMS

by

J. C. Medcof

Eight and a half hods ($4\frac{1}{4}$ bushels) of clams remained in the floating car after completion of the Laboratory of Hygiene's Bar Road experiment which commenced May 26. On May 31 these were removed (total time in car $4\frac{1}{2}$ days) and taken to Chamcook Seafoods plant and shucked after "hot-dipping".

Approximately 1% of the animals were "sluggish" and discarded. The rest were shucked the same day and yielded 59 pounds of meat -- an average of 14 pounds per bushel of whole clams. This yield is 30 to 40% higher than this plant ordinarily realizes from processing commercial lots. The plant operator observed that the shucking was remarkably easier and quicker than with regular lots and that the meats were so clean as to require less washing than usual before packing. The shuckers preferred opening the cleansed stock to opening regular commercial lots.

All the increase in yield and part of the speed-up in handling is attributed to the absence of broken clams which were removed before cleansing. (Clams with cracked shells are slow to shuck and badly damaged clams have to be discarded.) The rest of the speed-up in processing (shucking and meat-washing) is attributed to the absence of mud and dirt from the shells and the mantle cavities.

The conclusion drawn from this test is that even if the cleansing operationswere costly it might not be a deterrent to commercial use of stocks that might be fished in polluted areas because the higher costs will be offset more or less by the higher yields and reduced costs of processing.

The 59 pounds of meats resulting from this test were distributed among staff members of the Atlantic Biological Station. The meats were fried and used in chowders and clam pies. Everyone agreed that the quality was as good or better than that of regular packs with which they were familiar.

IX. GENERAL SUMMARY

by J. C. Medcof

The reports assembled here greatly improve our understanding of the problems involved in cleansing sewage-contaminated soft-shelled clams.

We have confirmed last year's conclusion that when the water at the cleansing station remains relatively free of coliform bacteria, even heavily polluted clams regularly free themselves of almost all their load of coliform bacteria within 48 hours. This process is hastened by washing the outsides of clams before treatment and is slowed down to some extent by low water temperatures (<10°C.) but it is not affected appreciably by air-storage of clams for periods up to three days preliminary to cleansing or by inter-tidal air-exposure during the cleansing operation. Crowding clams in half-bushel hods and placing hods filled with clams close together in a floating cleansing car has no effect on the rate or extent of cleansing in the actively circulating waters where the tests were conducted. A satisfactory cleansing car has been designed and operated and a semi-commercial size lot of cleansed clams has been processed in an industrial shucking plant and found to compare favourably in handling characteristics with regular lots of clams received through trade channels from approved area.

The three characteristic phases of the cleansing process have been explained in terms of known physiological processes of clams on the basis of a simple hypothesis which seems to satisfy all the observations made so far. The significance of M.P.N.'s of coliform bacteria in cleansed clams as an index to the likely frequency of pathogenic bacteria is fundimentally affected by this hypothesis and it is being examined in the light of laboratory experiments being conducted now at the University of New Brunswick by one of the authors of this combined report.

The Bar Road area has been eliminated as a possible site for a clam-cleansing station because it has been shown to be periodically subject to sewage contamination.

The periodicity of the Bar Road contamination conforms with that of spring phases of tidal cycles. Tidal flushing of adjacent contaminated estuaries (e.g. St. Croix River) brought about by high-amplitude spring tides, seems to carry pollution over wider areas than flushing which occurs during low-amplitude, neap tides. This appears to be the first time such a relationship has been demonstrated. If it obtains generally, as we may reasonably suppose it does, it has significance in planning of and in interpreting results of all bacteriological surveys of marine areas.

An extensive body of data has been assembled showing that Holt's Point waters are practically free of sewage contamination and are likely suitable for clam cleansing. Two cleansing trials conducted there in 1952 corroborate this conclusion and encourage belief that industrial advantage may soon be taken of our findings.