AN INVESTIGATION OF THE BACTERIAL FLORA OF THE SOFT SHELL CLAM (MYA ARENARIA) IN NEW BRUNSWICK AND NOVA SCOTIA

By

Alan Dawson Tennant

A Thesis

Submitted to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the Degree of Master of Science.

May, 1949.

ACKNOWLEDGMENTS

Experiments were conducted during the 1947 and 1948 soft shell clam investigations in the Mobile Laboratory of the Department of National Health and Welfare. The author is indebted to Mr. J. Gibbard, Chief of the Laboratory of Hygiene, and to Dr. E.T. Bynoe, Bacteriologist, Laboratory of Hygiene, for the selection of the problem and making his participation in it possible.

The author wishes to thank Dr. E.T. Bynoe, under whose direction this work was undertaken, for his careful supervision, advice, and generous assistance throughout the entire investigation.

He is also grateful to Dr. J.C. Medcof of the Fisheries Research Board of Canada for his many helpful suggestions.

TABLE OF CONTENTS

I INTRODUCTION pg. 1

II REVIEW OF LITERATURE 8

III OBJECT OF INVESTIGATIONS 24

IV EXPERIMENTAL 25

(a) Methods Used for the Bacteriological Examination of Shellfish Growing Waters. 25

(b) Methods Used for the Bacteriological Examination of Mud and Silt. 26

(c) Methods Used for the Bacteriological Examination of Clams. 27

(d) Classification of Coliform Bacteria. 30

(e) Tests for the Enumeration of Fecal Streptococci from Water and Clam Samples. 31

(f) Survey of Charlotte County, N. B. Clam Producing Areas, 1947 and 1948 35

(g) Survey of Clam Producing Areas in Digby and Annapolis Counties, N. S., 1947. 55

(h) Survey of Clam Producing Areas in Halifax County, N. S., 1947. 60

(i) The Bacterial Flora of Clams from "Clean" and Polluted Areas. 67

(j) Classification of Coli-Aerogenes Group Bacteria Isolated from Clam and Water Samples, 1947 and 1948. 70

(k) Relationship Between Coliform and Fecal Streptococci Most Probable Numbers from Clam and Water Samples, and Their Relationship to the Sanitary Quality of the Producing Area. 73

TABLE OF CONTENTS

(Continued)

IV EXPERIMENTAL

(1) Investigation of Some Aspects of the Commercial Packing of Clam Meats. 74
1. The Bactericidal Effect of Dipping Shellstock Clams in Boiling Water for Short Periods of Time. 75
2. Bacteriological Examination of Clam Meats and Clam Liquors. 81
3. Clam Flushing Experiments. 82

V DISCUSSION 84

VI SUMMARY 91

VII REFERENCES 94

MOBILE LABORATORY

Where Experiments were Conducted During the Present Investigations.

INTRODUCTION

While a considerable number of scientists have investigated pollution problems in relation to sea water, oysters, mussels, and other shellfish, a review of the literature reveals a decided paucity of information regarding the bacterial flora of the soft shell clam, Mya arenaria. The importance of the soft shell clam fishing industry in the Atlantic Maritime Provinces of Canada is considerable, and a comprehensive assessment of the bacterial flora of Mya arenaria on the Canadian Atlantic seaboard was therefore deemed essential before possible public health hazards connected with the industry could be properly evaluated, and control measures and significant bacteriological standards for the product could be established.

The Soft Shell Clam, Mya arenaria.

Soft shell clams (Mya arenaria), which are also known as "soft clams", "long-necked clams", "long clams", and "sand clams", are found along the Atlantic coast from South Carolina to the Arctic Ocean. They are very abundant along the coast of Nova Scotia, New Brunswick, and New England, but are rare south of Cape Hatteras. The soft clam was introduced in the middle of the last century to the Pacific coast, where it is now found in large numbers in some areas.

Mya arenaria lives on tidal flats and beaches of inlets, bays, harbours, and river estuaries, and is not to be found on open sea beaches. Weymouth (1930) states in explanation that,

as it increases in size, Mya loses the ability to move quickly, and hence the ability to care for itself when exposed; storms are thus fatal to the larger specimens. Moreover, it is incapable of renewing communication with the surface of the ground when its siphons have been choked by shifting sand; it needs protection, and a firm sub-stratum in which a hole is semi-permanent. Thus the best soil for clams is a mixture of fine sand and mud, although these animals will live in every type of bottom soil ranging from gravel to mud, except very soft mud or shifting sand. At the same time the water from which it strains its food must not be stagnant but moving, and contain a supply of microorganisms sufficient for the needs of the animal Provided the above conditions are fulfilled, Mya will thrive in water that is brackish, even though the temperature falls below $0^{\circ}C$.

It is obvious that these ideal conditions will be found in the tidal mud of a river estuary, and it is here that Mya occurs as one of the characteristic members of the brackish water fauna.

During adult life, the clam lies entirely buried, anterior end downward, in the mud, with only the tip of the siphons level with the surface. Alder and Hancock (1851) have described this as it occurs at the mouth of the River Tyne. "Mya buries itself to a depth of six to eight inches in a stiffish clay mixed with shingle; in shallow pools left by the tide the siphonal tubes may be seen just level with the surface of the

muddy bottom in full action. The mud lies closely packed against the walls of the tubes, so that nothing is to be seen but the internal surface of the expanded lips of the siphonal orifices fringed with numerous tentacles. When it happens that the surface of the water is only a little above the orifices, a strong current can be distinctly seen to boil up from the anal siphon, and another, with a constant steady flow, to set into the branchial one."

The growth of clams depends upon the temperature of the water, the character of the bottom soil, and the abundance of food available. Circulation of water over the clam bed is necessary to provide food organisms and dissolved oxygen. Products of decomposition are carried away by tidal currents; this prevents the fouling of thickly populated beds. Clams are usually very tolerant of changes in the salt content of sea water, and will grow in water ranging from eight to twenty-eight parts of salt per thousand.

Higher temperatures promote more rapid growth along the coasts of New Jersey and southern New England than north of Boston, but even in the latter zone the animals may reach the marketable size of more than two and one-half inches in one and one-half to two years. In some warm localities clams grow during the entire year, but usually the most rapid increase in size is noted in July, August, and September. The normal life-span of a clam is estimated to be at least ten to twelve years.

In adult clams the sexes are separate and occur in approximately equal numbers. Most of the young clams mature for their first spawning when they are about one year old, but some individuals, usually males living in favourable surroundings, may contain spawn at the age of four months. Soft shell clams begin spawning in the spring and early summer after the water temperature reaches 55 to 60° F. This occurs late in July in the Eastern Maritime provinces of Canada. Spawning may continue for about three months; the soft shell clam is very prolific, discharging millions of eggs each summer. Insemination of the egg takes place in the water outside the clam's body. Within ten to twelve hours after insemination the egg develops into a free-swimming larva, and in ten to fourteen days, depending on the temperature of the surrounding water, the larva changes into a young clam, which settles to the bottom and attaches itself to various objects, such as broken shells, rocks, and sea grass, with a byssus. It loses this organ of attachment when about three-quarters of an inch in length, and is capable of burrowing into the bottom soil, which becomes its new habitat.

In Mya arenaria the two shell valves are practically equal in size. When the valves are adducted to the utmost extent there is still a large gape at the posterior end in which lie the retracted siphons. When fully extended the siphons may reach a length of 50 cms., according to Vlès (1908). The mantle edges are fused except for a short extent on the antero-ventral

surface, where the pedal opening is found. The small, wedge-shaped foot, which can be protruded through the opening, is situated, as in all burrowing forms, on the anterior edge of the visceral mass. (Yonge, 1923).

The stomach contents consist of very finely divided particles of organic debris, sand, and microorganisms, e.g., diatoms, singly and in chains; Foraminifera; minute, probably larval, bivalves; ostracods, and other microscopic Crustacea, with parts of larger specimens; spores and eggs of various kinds; sponge spicules, and spines of all sizes. The great mass of material consists of small sand grains. The largest particles present are thin, filamentous strips of alga up to one mm. in length; the largest solid particles are not more than one-fifth mm. in diameter. This fine division is the result of the very excellent sorting mechanism present on the palps.

Pollution of Soft Shell Clams.

Soft shell clams, in common with other molluscan shellfish, are liable to pollution with sewage. If such polluted shellfish be eaten, in an uncooked or partially cooked condition, by human beings, serious or even fatal illness may - and, from time to time, does ensue. It is therefore obvious that the consumption of such shellfish is highly undesirable, not only in the interests of the individual consumer, who may contract disease from the shellfish, but also in that of the general public,

amongst whom the disease may indirectly be spread, possibly on a large scale.

A mass of epidemiological evidence has been accumulated, especially during the last fifty-seven years (1893-1949), which places the causal relationship between polluted shellfish and the incidence of certain outbreaks of disease beyond reasonable doubt. The disease predominantly associated with the consumption of polluted shellfish is typhoid or enteric fever, (including the group of allied diseases, e.g. paratyphoid fever, etc.). It must be borne in mind, however, that polluted shellfish are potentially liable to convey any infection which may exist in sewage, and which can produce disease in man if introduced by mouth. The only essential factor necessary for the conversion of this potential power into an actual danger is the capacity of the organism, responsible for a given infection, of living for a comparatively short time, under the conditions to which it is subjected during its travels from the original source of infection to the consumer of the shellfish. As a rule, this means that the infective agent must be able to survive, firstly, immersion in sewage, secondly, the effects of sea or brackish water, and thirdly, the conditions to which it is exposed while within the shell of the mollusc. It may so happen, however, that the organism may not have to face all or any of these conditions. Infective material need not necessarily get into the water via a sewer; it may be exposed only to fresh or almost

fresh water, e.g., in the case of shellfish taken from river estuaries, at low tide, or, in the case of those "washed" after collection, in polluted streams; and finally, it may be directly conveyed to the consumer in the mud which may be adhering to the outside of the shells.

Probably the most important group of infective bacteria, other than the typhoid bacillus, transmissible by shellfish, comprises those which are responsible for various gastro-intestinal disturbances, ranging from the true dysenteries, to more or less ill-defined conditions characterised by sickness and diarrhoea of varying severity; this group also includes the bacteria responsible for various forms of food poisoning. Among other diseases in this category may be mentioned asiatic cholera dissemination of which by shellfish, was demonstrated in 1893 in England.

Entirely apart from the dangers of contracting serious disease from the consumption of polluted shellfish, it is not particularly pleasant, from a purely aesthetic point of view, to contemplate the fact that, when eating shellfish from a sewage-polluted area, we may be sampling the filth of all sorts which goes to make up the sewage of towns or villages. Shellfish may be, and frequently are, a dangerous food. The importance of limiting the fishing of clams and other shellfish to areas shown, by bacteriological and sanitary engineering surveys, to be free from sewage contamination cannot be over-emphasized.

REVIEW of LITERATURE

Viability of Adventitious Pathogens in Sea Water.

It is the concensus of opinion that there are no autochthonous marine bacteria capable of infecting man, but the literature is replete with contradictory accounts of the viability of adventitious pathogens in sea water. Some workers claim that sea water is highly lethal for bacteria from land-dwelling animals, while others have presented data which indicate that such bacteria can live almost indefinitely in the sea. Several factors which influence the survival of bacteria in sea water must be taken into consideration in order to effect a reconciliation of these divergent views. The biological properties of sea water may vary widely; the synthetic, diluted, or autoclaved sea water used in many of the experiments may not have simulated natural sea water.

De Giaxia (1889) observed that enteric bacteria perish quickly in the sea. He found more than 100,000 bacteria per ml. of sea water 50 meters from a sewage outfall in the Gulf of Naples, 26,000 at a distance of 350 meters, and fewer than 100 per ml. 3,000 meters from the sewage outfall. Controlled experiments showed that Bacillus anthracis and Vibrio comma were unable to compete with saprophytes in polluted sea water; Salmonella typhosa and pathogenic species of Staphylococcus were even less resistant. The period of survival was a function of the organic content and bacterial population of the water. In grossly polluted water these organisms survived for less than 24 hours.

In a summary of the experiments of various authors, Frankland and Frankland (1894) concluded that, in general, human pathogens do not survive as long in sea water as in fresh water. Soper (1909), however, found that the virulence of Salmonella typhosa was not reduced by sea water in two or three weeks.

Beard and Meadowcroft (1935) designed experiments to simulate natural conditions in polluted sea water, and noted a rapid diminution in numbers of both Salmonella typhosa and Escherichia coli, although some of each survived for more than a month. The test bacteria were suspended in freshly collected, unfiltered water from San Francisco Bay in semi-permeable membrane cells which were immersed in the bay. The death rate of the bacteria was invariably higher in unfiltered water which was sterilized by passage through an L-3 Chamberland candle.

ZoBell (1936) noted that, although 99.9 per cent of the sewage organisms were killed after two days suspension in sea water, a few survived for nearly a month.

Carpenter, et al (1938) found that 80 per cent of the organisms in sewage were killed by natural sea water within thirty minutes.

Krassilnikov (1938) found that water from the Black Sea was germicidal for terrestrial bacteria until boiled. Passage of the water through a Seitz filter rendered it less bactericidal for adventitious organisms. Krassilnikov stressed the importance of the organic content of water as a factor affecting the

survival of bacteria.

ZoBell (1946) considers that the sanitary significance of Escherichia coli, Aerobacter aerogenes, and other coliform bacteria in sea water depends upon two important considerations. First, do the intestinal tracts of marine animals normally harbour coliform organisms, and secondly, how long do coliform bacteria of fecal origin survive in the sea?

Browne (1917) reported the absence of E. coli from Buzzards Bay and Vineyard Sound near Woods Hole, Mass. E. coli was found in only 39.8 per cent of 93 scup which he examined; the presence of these bacteria in the fishes' intestines seemed to be a function of the amount and type of food present.

Gibbons (1934) noted that the bacteria in fewer than half of the offshore fish which he examined produced gas in lactose broth, and concluded that E. coli and A. aerogenes are present only in fish from contaminated waters.

Griffiths and Fuller (1936) detected only a few E. coli in commercial fish, and concluded that E. coli is not a normal intestinal inhabitant of marine fishes.

ZoBell (1941) failed to find coliform bacteria in any of 961 samples of sea water collected at stations remote from any possibility of terrigenous contamination. Large numbers were found in polluted bays and estuaries. Positive presumptive tests were obtained from the intestinal contents of 203 of the 387 marine fishes examined; the coliform bacteria isolated from the fishes were classified as follows using the "I M V I C" tests:

Escherichia coli	6 per cent
Aerobacter species	73 per cent
Citrobacter species	21 per cent

E. coli was found only in feedy fishes taken relatively near shore. ZoBell concluded that E. coli or other bacteria which ferment lactose with the production of gas do not constitute part of the normal intestinal flora of marine fishes, although such bacteria may survive for considerable periods of time if ingested.

ZoBell (1946) reports that the intestinal contents of sea birds are generally free from coliform bacteria, except in certain cases where the fowls have been feeding in polluted waters. Very little is known about the intestinal flora of marine mammals. E. coli does not appear to be a normal inhabitant of the intestines of seals in captivity.

Land drainage and raw and partially treated sewage contributes huge numbers of coliform bacteria to the sea. Warren and Rawn (1938) estimate from their data that enough coliform bacteria are discharged by sewage effluents along the west coast of the United States each day to give over a hundred for every liter of water in the North Pacific Ocean, if evenly distributed. Comparable sanitary conditions were found on the east coast of the United States by Weston (1938). Such organisms, however, are found only in harbours, bays, and tide water, which are often heavily polluted.

Data obtained during extensive surveys around the Hyperion

outfall, summarized by Knowlton (1929), show the rapidity with which coliform bacteria succumb in the sea. The Hyperion outfall carries raw sewage from nearly two million inhabitants of Los Angeles a mile into the ocean. Millions of coliform bacteria per ml. are found near the sewer outlet, but the number decreases with distance from the outlet much more rapidly than can be explained by dilution. Even during onshore winds and currents, the count is often less than ten coliform bacteria per ml. of surf; as many or more E. coli could easily be introduced by bathers on the beach. Coliform bacteria were never traced more than a mile or two from sewer outfalls in the open ocean. A commission appointed by the California State Bureau of Sanitary Engineering (1943) considered the effect of air currents, water movements, composition of sewage, and other factors which influence the distribution of E. coli; they concluded that only in solids and greases were coliform bacteria able to survive for long periods of time in the sea.

Dienert and Guillerd (1940) conducted similar experiments in Europe; they concluded that, while sea water is neither antiseptic nor inimical to E. coli, sewage discharged into the sea is rapidly purified by predatory organisms, sedimentation, and dilution. Lloyd (1930) found that Clyde sea water was virtually free from coliform bacteria of fecal origin.

Multiplication of coliform bacteria in sea water or living shellfish has not been definitely proven, but several observers have recorded great and rapid increase of coliform organisms in

ordinary tap water, and fresh water from other sources. Savage and Wood (1918) and Platt (1935) recorded multiplication in tap water sterilized by heat; Harold (1934) noted rapid multiplication and survival for some months of an aerogenes strain in Thames River water sterilized by boiling, while a very comprehensive research by Bigger (1937), in connection with the multiplication and survival of E. coli, indicated that this organism increased enormously in Dublin tap water, the numbers waxing and waning from day to day over periods of weeks or months. He found that autoclaving, filtration, or exposure to adsorbents or to a vacuum, greatly enhanced the value of the water as a culture medium. A striking parallelism, in many respects, is to be noted between Bigger's results and those obtained by Dodgson (1928) at Conway, England.

The Coliform Index as a Measure of the Pollution of Shellfish and Shellfish Growing Waters.

Considerable difference of opinion has arisen over the validity of the Standard Methods test for the presence of members of the coli-aerogenes group as an index of true fecal pollution. Many of those who have studied shellfish pollution problems in more southern waters (20) (35) (59) (60) have long been convinced that high scores based on coli-aerogenes bacteria may have little value as indicators of fecal pollution, especially where water temperatures rise rapidly to a high level during the summer months. Hunter (1939) states that, while "the coliform bacteria have long been considered the only bacterial group which can be used with

even a reasonable degree of accuracy as a measure of pollution", it must be remembered that the presence of coliform bacteria in water and in or on other foods depends on many environmental factors, each of which must be determined for each item of food in question. Hunter considers that only when a correlation is found to exist between unsanitary methods of food production and the presence of coliform organisms in the finished product can the presence of confirmed fecal strains of coliform organisms be interpreted as having sanitary significance; if environmental factors tend to permit or promote multiplication of the normal flora of organisms, together with the coliform contaminants, an interpretation of the sanitary significance of the coliform group becomes involved.

Eliot (1926) reports that B. cloacae was often found in oysters when E. coli and A. aerogenes were absent, and considers that its presence in market shellfish can be disregarded. The investigations of Perry (1939) indicate that many coliform bacteria, particularly of the B. cloacae types, are present in shucked market oysters or shell oysters when the temperature exceeds 15.6°C. (60°F.); Perry concludes that these bacteria are without significance as indicating pollution.

On the other hand, studies by other investigators (3) (22) (56) (57) indicate that any content of coliform bacteria, however atypical, in a water or food sample must be taken as an indication of fecal contamination. Investigations by Parr (1938) indicate that E. coli is the most characteristic and constant coliform

bacterium of the human intestine and of fresh human feces, but is shortly replaced by A.aerogenes and intermediates, particularly in cold stored material. Fecal specimens did occur, however, in which no viable A.aerogenes or intermediates are found, and in such no succession of forms takes place; less frequently A.aerogenes may also be found in large numbers, and at times it may become the predominant, and rarely, the only type. The work of Bardsley (1934) confirms these observations; Bardsley found that most virgin soils were free from coliform bacilli, but that these organisms were contained in enormous numbers in feces. The dominant type in feces was E.coli, but by preliminary incubation in Koser's citrate medium it was found possible to demonstrate the presence of small numbers of A.aerogenes and intermediate types, which, by the ordinary methods of isolation, were usually overgrown by E.coli. Bardsley concluded that the occurrence of A.aerogenes and the intermediates in food and water may be due to fecal pollution.

In a study of the coliform group in market oysters, Eliot (1926) found that the E.coli score of shell oysters may increase enormously during storage. E.coli and A.aerogenes were invariably found together when either of them was present in market oysters; Eliot considered that the inclusion of both in scoring oysters and other shellfish is therefore probably justified.

Gard and Nilsson (1942) consider that, in studies of the classification of coliform organisms, too little attention has been paid to the variations in the fermentative qualities of the

individual strains. Through studies on strains of coliform organisms derived from feces, urine, and sewage, it was found that the power of lactose fermentation and the ability to grow in Koser's citrate medium often wears off rather quickly when bacteria are growing outside the intestine, and that other biochemical activities may also decrease. These authors conclude that any content of coliform bacteria, however atypical, in a water or food sample must be taken as a sign of fecal contamination.

It may be concluded that the general absence of coliform bacteria in the sea except in areas known to be polluted with sewage confirms the validity of the test for coliform bacteria as an indicator of sewage pollution. It is possible that in some areas high water temperatures may promote multiplication of coliform organisms in sea water and shellfish; in such instances it is probable that coliform numbers will not accurately reflect the extent of sewage contamination in the area. Under normal conditions, however, it is believed that the Standard Methods test for the presence of members of the coli-aerogenes group serves as a reliable index of sewage contamination of sea water, and of clams and other shellfish.

Enteric Pathogens in Water, Sewage, and Shellfish.

During the period of establishment of the coliform group as an indicator of unsafe waters and shellfish, considerable effort was devoted to the isolation of enteric pathogens, particularly Salmonella typhosa, and to the relative rates of decrease of

coliforms and enteric pathogens under various conditions. The introduction, in 1927, of Wilson and Blair's bismuth sulphite agar resulted in much more work being done on the isolation of certain enteric pathogens during the next ten or twelve years by a number of investigators. These workers were successful in isolating Salmonella typhosa from sewage and polluted waters, using Wilson and Blair's media, or various modifications.

The work of the London Metropolitan Board Laboratories (1927-1938) is perhaps the most complete and carefully controlled; each annual report from 1927 to 1938 carries some reference to isolations of enteric pathogens. Wilson (1928), and Wilson and Blair (1931) have reported numerous isolations of S. typhosa from polluted waters, sewage, and shellfish. Green and Beard (1938) have reported the isolation of S.typhosa from Palo Alto sewage in nine of fifty-one one ml. samples; Ruchhoft (1934) has reported isolations in two 0.1 ml. samples of Chicago activated sludge, while Heukelekian and Schuloff (1935) reported failure to isolate S.typhosa from the sewages of fifteen municipalities in 0.1 ml. amounts. Hajna (1935) isolated S.typhosa from six of twenty-two samples of crude sewage from Baltimore and vicinity. Mom and Schaeffer (1940) reported an extensive series of isolations from sewage, sludge, and river water at Bandoeng, Dutch East Indies, where the morbidity rates for typhoid are around thirty cases per thousand per year. Mom and Schaeffer, and Wilson (1933) stress the relationship between the typhoid morbidity rate and the concentration of S.typhosa found in the sewage of the community.

Dodgson (1938) cites several epidemiological instances of the prolonged survival of virulent typhoid bacilli in mussels and oysters. His experimental evidence indicates that S.typhosa survived in oysters, mussels, and other shellfish in sea water for more than three weeks. Hunter and Harrison (1928) related several instances of the demonstration of typhoid bacilli in shellfish. They record evidence for the survival of the typhoid bacilli in oysters for from nine to forty-two days, and of E. coli for from seven to seventeen days. Tonney and White (1925) found that the longevity of S.typhosa in the shell liquor of both shucked and shell oysters in storage varies with the temperature at which they are kept. In general, the temperature best suited for the preservation of the shellfish tends to prolong the life of the typhoid bacilli in the oyster. Under the conditions of the trade, viz., at icing temperature (45°F.), S.typhosa survived for twenty-two days in shucked oysters, and for sixty days in shell oysters.

Shell oysters artificially contaminated with typhoid bacilli by floating for one hour in sea water to which typhoid bacilli had been added, and then placed at ice-box temperature were found to contain living typhoid bacilli for as long as twenty-four days by Jordan (1925). There was no evidence of multiplication, and after the first few days a diminution in numbers was plainly apparent. Of the three strains used, one was found to survive to the twenty-first day, one to the twenty-second, and one to the twenty-fourth day.

Since fresh clams have been incriminated in typhoid fever

outbreaks, it is obvious that they are subject to the same type of contamination as other shellfish. An account of eight cases of typhoid fever, caused by eating raw clams dug from the Thames River not far from a sewer outlet, appears in the Annual Report of the Connecticut State Board of Health for 1927 (11). Clams dug from a posted area along the shore of a New Haven harbour caused the first recorded death from typhoid fever in Connecticut for 1948 (1).

The following cases of typhoid fever from 1933 to the end of 1948 were investigated by the New York City Department of Health (47) and finally judged as having had their sources of infection in shellfish originating from condemned areas within the City of New York. In most cases, Jamaica Bay was involved, but some cases were also reported from Eastchester, Pelham, Raritan, and Princess Bays.

Year	Typhoid Cases	Type of Shellfish		
		Clams	Mussels	Oysters
1933	81	Not available		
1934	9	7	1	1
1935	35	35	-	-
1936	2	2	-	-
1937	9	9	-	-
1938	19	17	2	-
1939	6	6	-	-
1940	19	19	-	-
1941	2	2	-	-
1942	2	2	-	-
1943	4	2	2	-
1944	0	-	-	-
1945	3	3	-	-
1946	0	-	-	-
1947	1	1	-	-
1948	1	1	-	-

These figures do not include typhoid fever cases from shellfish which were obtained from peddlers or other persons whose sources of supply could not be traced, or investigations that could

not be thoroughly completed. An unestimated number of food poisonings and other gastro-intestinal illnesses traceable to shellfish from uncertified areas is known to have occurred during this period, but statistics could not be assembled as many such cases were not reported to the Public Health authoities.

The studies made by Kehr and Butterfield (1943) of the available data in the literature emphasize the basic value of the coliform test as an indicator of the possible presence of pathogens and indicate that a very real danger may exist when coliforms are present in even moderately high concentrations. The factor of safety provided by the ratio of a million or so coliforms present for each S.typhosa would, it is believed, take care of the usual fluctuations in the ratio of S.typhosa to the coliforms, provided the density of coliforms in ingested media be kept low or eliminated entirely by methods which reduce the general bacterial population.

Fecal Streptococci as an Index of Pollution.

For a number of years the fishing industry has been dissatisfied with the bacteriological methods for detecting domestic pollution; the present methods frequently indicate that an area is unfit for shellfish production or that a fishery product is not suitable for human consumption even though there is no other evidence to indicate that this may be so. As a result many investigators have been engaged in a search for a more reliable criterion of pollution than the coliform bacteria.

Some authors consider that the fecal streptococci may prove to be the solution to the problem of a more certain index of fecal pollution. This group of organisms, commonly known as the enterococci, was first reported by Lawes and Andrews (1894). Six years later, Houston (1900) began to lay stress upon the fact that these organisms were readily found in polluted waters and seemingly absent in non-polluted samples. The work of these and other British workers led Suckling in 1943 to state:

"Streptococci are used as an indicator of pollution on the same grounds as Bacterium coli, namely:

1. They are present in faeces and sewage and are found in known polluted waters.

2. They are not found in pure waters, virgin soil, and sites out of contact with human and animal life.

3. They do not multiply outside the animal body (except in such media as milk.)"

In the United States, Winslow and Hunnewell made the first report on these organisms in 1902. Since then, several methods of isolation have been suggested, and in 1939, Darby and Mallman suggested the use of a selective medium which would permit these organisms to grow without competition from certain other bacteria. Four years later, Hajna and Perry (1943) proposed their "SF medium" which they reported to be highly selective for enterococci if the incubation temperature was 45°C.

In 1945, White and Sherman indicated that a medium containing penicillin was highly selective for this group of

organisms. The possibilities of this medium for routine use in sanitation bacteriology led Winter and Sandholzer (1946) to investigate it in detail; they found that fecal streptococci were present in all of the samples of human, domestic-animal and wild animal feces that were tested, and that soils of virgin wooded areas do not show the presence of enterococci, whereas, the soil of pasture land does. In fecal samples, the fecal streptococci were outnumbered approximately a hundred times by the coliform organisms. It is of interest, however, that samples of polluted waters taken right at the source of pollution show the average enterococci count to be twenty times that of the coliforms. Then, as the distance from the point of pollution increased, the numbers of enterococci decreased more rapidly than the numbers of coliforms, so that beyond a hundred yards or more, the coliforms are greater in number. These workers concluded that the enterococci may serve as a more specific and reliable index of fecal pollution than the coliform bacteria, even though the former may be present in fewer numbers than the latter in polluted areas.

Data obtained from food-producing establishments by Ostrolenk, Kramer, and Cleverdon (1947) show excellent correlation to exist between sanitation and recovery of both E.coli and enterococci. Enterococci in artificially contaminated soils and in normal feces appear to survive longer than E.coli under identical conditions.

Investigations by Ostrolenk and Hunter (1946) demonstrated

that fecal streptococci are common in the excreta of ten animal species, and that they occur in significant numbers. These authors consider that, although the fecal streptococci are generally outnumbered by E.coli, their resistance to chemical agents and possibly to other environmental factors makes them of sanitary significance as indices of fecal contamination and pollution.

Brown and Gibbons (1949) report that the examination of 162 egg powders from various sources revealed that one hundred per cent contained enterococci in relatively large numbers, while 26.5 per cent were negative for E.coli, and 25.9 per cent contained less than one per gram. These authors conclude that, in egg powder, enterococci seem to furnish a better index of the number of organisms of fecal origin in the liquid egg than E.coli or the coliforms.

Several outbreaks of food poisoning of a mild type in which enterococci were the predominant organisms isolated from the suspected foods are described in a paper by Buchbinder, et al (1948). The literature on food poisoning is also discussed, and it is pointed out that, in several instances in which the inculpated organisms were closely identified, they were found to be enterococci.

The relatively small numbers of outbreaks reported as caused by Streptococcus faecalis is in sharp contrast to the widespread occurrence of this organism in nature. Some success in producing experimental Streptococcus faecalis food poisoning

in man is presented in a paper by Osler, et al (1948). Symptoms of acute gastric or intestinal disturbance, or of both, were produced in six, or possibly seven, of twenty-six human volunteers who ate foods in which strains of Streptococcus faecalis had grown for five hours. These workers concluded that the experimental production of acute mild intestinal or gastric disturbance or both in man tends to confirm the etiological role of Streptococcus faecalis in naturally occurring outbreaks of food poisoning. Dangler and Steffen (1948) consider that the enterococci may be as important as the staphylococci as causal organisms in outbreaks of food poisoning.

OBJECT OF INVESTIGATIONS

While a considerable number of scientists have investigated pollution problems in relation to sea water, oysters, mussels, and other shellfish, a review of the literature reveals a decided paucity of information regarding the bacterial flora of the soft shell clam, Mya arenaria. The importance of the soft shell clam fishing industry in the Atlantic Maritime Provinces of Canada is considerable, and a large proportion of the pack is consigned to the markets in the United States; a comprehensive assessment of the bacterial flora of Mya arenaria on the Canadian Atlantic seaboard was therefore deemed essential before possible public health hazards connected with the industry could be properly evaluated, and control measures and significant bacteriological standards for the product could be established.

This investigation has therefore included : a bacteriological survey of soft shell clams from producing areas in New Brunswick and Nova Scotia; an investigation of the effect of seasonal changes on the fecal bacterial content of soft shell clams; a critique of methods used in the bacteriological analysis of clams; and a bacteriological investigation of methods used in the commercial production of clam meats for export.

EXPERIMENTAL

Bacteriological Examination of Shellfish Growing Waters.

A. Collection and Transportation of Samples.

Samples of sea water from shellfish growing areas were collected at various stages of the tide in sterile bottles, and were fully protected against contamination both during sampling and after collection. They were kept at a temperature at or below 10°C. (50°F.), and were examined as soon as possible after collection. The samples were preferably tested within four hours of the time of collection, and in no case were samples held for longer than eight hours before testing.

B. Field Record.

A record of environmental conditions made at the time of collection of the sample accompanied all water samples collected during the investigations. This record included the date and hour of collection, and the location of the sampling station. Wherever possible the following supplementary data was obtained in order to interpret the bacteriological findings:

1. State of the tide.

2. Temperature of the water.
3. Depth of the water, and depth at which the sample was collected.
4. Notes on any unusual conditions which might effect the sanitary quality of the water.
5. Record of rainfall in the immediate past.

C. Examination for Coliform Bacteria.

Examination for coliform bacteria was made according to the methods outlined in Standard Methods for the Bacteriological Examination of Shellfish and Shellfish Waters (1942).

D. Expression of Results.

The numbers of coliform bacteria were expressed as the most probable numbers (M.P.N.) per 100 ml. of sample. M.P.N.'s were determined from the table of most probable numbers given by Hoskins (1934).

Bacteriological Examination of Mud and Silt.

A. Collection of Samples.

Samples of clams, mud, and silt were taken from the same general location for bacteriological examination during the early phases of the 1947 investigations in Charlotte County, N.B. Samples of mud were obtained at a depth of six to eight inches, while the top quarter-inch of soil surface was sampled as silt. Samples were transferred to sterile sampling bottles by means of sterile spatulas, and taken to the laboratory for immediate examination. In no instance were samples held for longer than four hours before testing.

B. Preparation of Sample for Examination.

Ten grams of the sample were weighed aseptically into a sterile 90 ml. one percent saline dilution blank, and shaken by hand until a homogeneous suspension was obtained.

C. Examination for Coliform Bacteria.

Examination of the prepared dilution of the sample of mud or silt was made in a manner similar to that described for the examination of water samples in Standard Methods for the Bacteriological Examination of Shellfish and Shellfish Waters (1942). Results were expressed as most probable numbers of coliform bacteria per 100 grams of mud or silt (1934).

Bacteriological Examination of Clams.

A. Collection and Transportation of Samples.

Individual containers of clam samples were marked for identification, and the samples were preferably tested within four hours from the time of collection; in no case were samples held for longer than eight hours before testing.

1. Shellstock.

Ten or more clams judged to be representative of the lot under examination were selected for transportation to the laboratory. The quantity procured had to be sufficient to produce not less than 200 ml. of shell liquor and ground meats. The shellfish were placed in a suitable sterilized container and handled aseptically during the period before examination.

2. Shucked Stock.

Samples of not less than 200 ml. of shucked clam meats

were collected with a sterilized spatula. Special glass sample bottles, suitably sterilized, were found to be satisfactory for transporting samples of shucked shellfish.

B. Field Record.

A record of environmental conditions made at the time of collection of the sample accompanied all clam samples taken directly from growing areas. The record included the exact location from which the samples were collected, and the date and hour of collection. Where possible the following supplementary data was obtained:

1. The state of the tide.
2. Temperature of the water.
3. Whether there was heavy, moderate, or very little rain during or immediately preceding the period of collection.
4. Careful notes on any unusual sources of pollution, such as boats, privies, sewers, pasture lands, or animals, which might affect bacteriological results.

When samples were collected from packing plants, records were made of:

1. Date and hour of collection.
2. Name and address of the place from which the samples were collected.
3. Exact location of the growing area from which the shellfish were obtained.
4. Conditions of storage prior to collection of the sample.

5. Sanitary conditions in the packing plant.

C. Procedure.

1. Preparation of sample for examination.

(a) Washing shells: Excessive growth and loose material was scraped off, and the shellfish scrubbed with a stiff brush in running water of known purity until the shells were free of all mud, especially in crevices at the junction of the shells. The hands of the examiner were thoroughly washed with soap and water, and the brush used for scrubbing the shells was sterilized by boiling or autoclaving. The cleaned shellfish were placed on clean paper towels and dried in the air.

(b) Removal of shell content: Clams were held in the left hand, and the point of the sterilized oyster knife inserted between the shells on the dorsal side (at the right of the examiner), just to the right of the hinge. The adductor muscle was cut, and the shells pried wide enough apart to drain the shell liquor into the sterile Waring Blendor jar. The upper shell was then pried loose at the hinge by using the knife as a lever; the removal of shell is the most difficult part of the procedure, and called for careful technique to avoid contact of shell, meats, or liquor with the hands. The entire shell contents were thus aseptically collected in a sterilized Waring Blendor jar graduated at 200 and 400 ml. levels. A sufficient number of clams were opened to obtain 200 ml. of shell liquor and whole meats.

(c) Grinding: An equal amount of sterile 1% salt solution was added to the meats and liquor contained in the Blendor jar.

Grinding for two minutes was found to produce an homogenous mixture. The sample was then tested immediately. The examination of shucked shellfish was made in an analogous manner, that is, 200 ml. of the sample was added to 200 ml. of 1% salt solution, and ground as above.

D. Examination for Coliform Bacteria.

Examination of clams was made according to the methods outlined in Standard Methods for the Bacteriological Examination of Shellfish and Shellfish Waters (1942).

E. Expression of Results.

The number of coliform bacteria was expressed as the most probable number (M.P.N.) per 100 ml. of sample, determined from the table of most probable numbers given by Hoskins (1934).

For the purpose of examination of shellfish and shellfish growing waters undertaken during these investigations, the coliform group was considered as including all bacteria which, upon transfer from a positive presumptive test (gas positive in lactose broth), show fermentation with gas formation in lactose medium containing 0.00133 per cent of brilliant green, and 2.0 per cent of bile (brilliant green lactose bile Broth).

Classification of Coliform Bacteria.

A. Isolation of Coliform Bacteria.

During the 1947 investigations, isolations of coliform bacteria were made from positive presumptive lactose broth tubes; culture material was streaked on Bacto Endo's agar plates, which were then incubated for 24 hours at 37°C. Discrete colonies

as well as in the presence of 6.5% NaCl, and by failing to produce catalase. They will grow in the presence of 0.04% sodium azide and 650 Oxford units of penicillin per liter.

The test developed by Winter and Sandholzer (1946) and used during these investigations for the enumeration of fecal streptococci in water and clam samples consists of two parts:

1. A presumptive test in which the production of acid and growth turbidity in a sodium azide enrichment medium after incubation at 45°C. is interpreted as evidence of the presence of enterococci.

2. The positive presumptive tests are then confirmed by inoculating a slant-broth preparation of a penicillin-sodium azide medium. Pin point colonies on the slant, growth sediment in the broth, the presence of Gram-positive ovoid streptococci in the broth, and a negative catalase test is interpreted as confirmed positive evidence of the presence of enterococci.

Preparation of Media.

1. Presumptive Enrichment Medium (normal strength).

0.5% Dextrose
0.5% Tryptone
0.5% Yeast Extract
0.04% Sodium Azide
0.0032% Brom thymol blue

To save weighing of small amounts, the sodium azide was prepared in a 1% aqueous solution and 40 ml. added to each liter of medium. For the same reason, a 1.6% alcohol solution of brom

were transferred from the plates in the same ratio as colony types appearing thereon, to Tryptone Glucose Extract agar slopes.

In 1948, clam and water samples were plated directly on Bacto Violet Red Bile agar for incubation at 37°C. for 24 hours. Discrete colonies were then transferred from these plates, in the same ratio as colony types appearing thereon, to Tryptone Glucose Extract agar slopes.

B. Differentiation of Coliform Bacteria.

For a satisfactory differentiation of these bacteria into the Escherichia coli, Aerobacter aerogenes, and intermediate types, it has been shown (43) (48) (49) (65) that four tests (indol, methyl red, Voges-Proskauer, and sodium citrate) are required. These four tests were used for such differential determinations, as outlined in Standard Methods for the Examination of Water and Sewage (1946).

Tests for the Enumeration of Fecal Streptococci from Water and Clam Samples.

The enterococci comprise a group of streptococci that have as their normal habitat the intestinal tract of man and other warm blooded animals. They are large, ovoid, Gram-positive streptococci appearing usually in chains of two to seven cells. The group consists of four species, Streptococcus faecalis, Streptococcus liquifaciens, Streptococcus zymogenes, and Streptococcus durans, all of which belong to Lancefield's group D. They are characterized by the fermentation of dextrose with the production of acid, ability to grow at both 10 C. and 45 C.

thymol blue was prepared and 2 ml. of this used in each liter of medium. The medium was then adjusted to a pH of 8.0, tubed in 8 ml. amounts, and autoclaved at 15 pounds pressure for 15 minutes.

2. Presumptive Enrichment Medium (concentrated).

A concentrated medium, using the same ingredients as the normal strength medium, was prepared by increasing the percentage of each ingredient five fold. The medium was adjusted to a pH of 8.5, tubed in large tubes in 2 ml. amounts, and autoclaved at 15 pounds pressure for 15 minutes.

3. Slant-broth Confirmation Medium.

A. Slants.

0.5% Dextrose
0.5% Tryptone
0.5% Yeast Extract
0.04% Sodium Azide
0.001% Methylene blue
1.5% Agar

The methylene blue was added by using 1 ml. of a 1% aqueous solution. The medium was adjusted to pH 8.0, tubed in amounts for long surfaced slants, autoclaved at 15 pounds pressure for 15 minutes, and slanted.

B. Broth.

The broth medium differs from that of the slants in having no agar, and by having 6.5% NaCl added after the adjustment to pH 8.0. It was autoclaved in flasks at 15 pounds pressure for

15 minutes. After cooling to room temperature, 650 Oxford Units of penicillin per liter were added. A sterile distilled water dilution of penicillin was added by means of a sterile 1 ml. syringe. Enough of this broth was then added aseptically to each slant to cover approximately one half of the surface of the slant.

Procedure for Water and Clam Examination.

Tubes of the presumptive enrichment medium were inoculated in the same dilutions and in the same manner as in the presumptive test for coliform bacteria (71). The inoculated tubes were then incubated in a water bath at 45°C. and observed periodically after eight hours for the production of acid, as shown by the indicator, and for growth, as indicated by turbidity. The production of acid and turbidity was interpreted as a positive presumptive test.

As soon as a positive presumptive reaction appeared, a loopful of the material was transferred to the broth of the slant-broth preparation and the loop zig-zagged on the surface of the slant as the loop was withdrawn. These tubes were then incubated at 37°C. and observed after 12 hours for pin point colonies on the slant surface and for a growth sediment in the broth. After observation, a Gram stain was made. Pin point colonies on the slant, a sediment growth in the broth, and large Gram-positive ovoid streptococci were interpreted as confirmatory evidence of the presence of enterococci. Numbers of fecal streptococci were expressed as the most probable numbers (M.P.N.) per 100 ml. of sample, from the table given by Hoskins (1934).

SURVEY of CHARLOTTE COUNTY, N.B. CLAM PRODUCING AREAS.

As an initial approach to the problem of soft shell clam bacteriology, samples of clams, sea water, mud, and silt were taken from most of the flats in Charlotte County, N.B., for bacteriological analysis, during June to October, 1947. Data obtained from these studies, and from a second series of experiments in June, July, and October, 1948, in some of the same Charlotte County areas, appear below.

Survey by Areas, Charlotte County, N.B., 1947.

Oak Bay : Here extensive flats are divided into East and West Coves by a rocky ridge; clams are also dug on the flats of small, uninhabited Cookson's Island, which lie about one hundred yards from the mainland flats, off the point of the ridge. Small dwellings are situated along a small stream flowing into Oak Bay, and on both coves. Drainage to the bay would include surface runoff from farmlands along the creek. Sewage contamination probably would not be extensive, but danger of pollution certainly exists.

Waweig River : Small clam flats are located just west of the St. John highway, where the Waweig and St. Croix Rivers converge. The surrounding area is sparsely settled; drainage from farmlands to the north-east, carried to the clam flats by the Waweig River, should constitute the only possible source of coliform contamination.

Table I (a)

Coliform and Fecal Streptococci Most Probable Numbers from Clam and Water Samples from Oak Bay, N.B. and Waweig R., N.B.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
June 11	Water	Oak Bay Creek.	4	-
	Water	Oak Bay, West Cove	110	-
June 26	Water	Oak Bay, West Cove	23	-
	Clams		>16,000	-
	Water	Oak Bay, East Cove	23	-
	Clams		>16,000	-
	Water	Waweig R. at bridge	79	-
	Clams		16,000	-
Oct. 13	Water	Oak Bay, West Cove	0	0
	Clams		2,400	1,400
	Water	Oak Bay, East Cove	0	0
	Clams		1,700	1,300
	Water	Cookson's Island	0	0
	Clams		1,300	1,300
	Water	Waweig R., at bridge	7.8	0
	Clams		1,700	490

Johnson's Cove: Small clam-flats are located in a small bay on the St. Croix River, about three miles north-west of St. Andrews, and about three hundred yards from the St. John Highway. As there are no homes in the immediate vicinity, there is very little danger of direct sewage pollution. Run-off from surrounding farmlands, and contamination from the river might affect the clam flats to some extent.

Table I (b) - Johnson's Cove, N.B.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
June 26	Water	Johnson's Cove	49	-
	Clams		5,400	-
Oct. 13	Water	Johnson's Cove	2	0
	Clams		700	330

St. Andrews Harbour: Heavy pollution is contributed by the town sewage system, which dumps raw sewage at four locations in the harbour; these sewer outlets are closely adjacent to the clam flats, which extend the length of the harbour. Besides these outfalls, there are private sewage systems in the summer homes at Niger Point, and at the south end of the Harbour. Another major source of contamination is the town garbage dump located at Pagan Point. The washings from Conley's Lobster plant enter the Harbour beside the C.P.R. Wharf.

Because of the obvious danger of heavy contamination, flats

in the harbour have been closed to the taking of clams. The "Pottery" flats at Niger Point were not closed, although they are adjacent to the Harbour flats, and probably would receive contamination from the Harbour.

Eight stations, from the Pottery flats to the Garbage Dump, were selected and sampled periodically through June and July, and on October 10. Results appear in Table.II.

Table I (c) - St. Andrews Hbr., N.B. Stations 2-8.

	WATER	CLAMS
Total No. of Samples	40	42
Coliform M.P.N. Range	7.8 ->1,600	490 ->16,000
% Samples showing Max. M.P.N.	(20) 50%	(21) 50%
% " M.P.N. 350 or less	(20) 50%	(0) 50%
% " M.P.N. 2,400 or less	-	(9) 21.43%

Table I (d) - Station 1, Pottery Flats, Niger Pt. (Open area) Coliform M.P.N.'s.

Specimen	June 12	June 24	July 1	July 17	July 23	Oct. 10
Water	0	49	13	>1600	40	1.8
Clams	330	1100	>16,000	9200	5400	130

Chamcook Bay: A large bay on the west shore of Passamaquoddy Bay, to the north of St. Andrews, N.B. The area is sparsely settled, well wooded, and somewhat rural in character. The plants of St. Andrews Sardine Packers, Cap'n John Seafoods, and Chamcook Packers, contribute the only direct contamination. Minister's Island is located in the centre of the Bay; pollution from houses and farmlands on the Island should be neglibible. A small stream flows into Chamcook Cove, at the head of the Bay, and may carry surface run-off from adjacent farmlands to the flats.

Clams are found at Bar Road (to Minister's Island), Chamcook Cove, and in McCann's Cove.

Table I (e) - Chamcook Bay, N.B.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
June 26	Water	Bar Road to Minister's Island	46	-
	Clams		1,700	-
July 16	Water	Chamcook Cove	920	-
	Clams		2,400	-
Oct. 13	Water	Chamcook Cove	7.8	2
	Clams		1,100	700
Oct. 13	Water	Bar Road to Minister's Island	0	0
	Clams		0	45
Oct. 15	Water	McCann's Cove	13	2
	Clams		790	330

Bocabec Bay: Bocabec Bay is a large inlet in the north-west section of Passamaquoddy Bay. The surrounding area is very rocky, heavily wooded, and sparsely settled; the few farms and houses are well back from the shoreline, and the clam flats at Birch Cove, and at Big Bay (at the head of Bocabec Bay) should be safe from direct pollution. A small stream flowing into Big Bay may contribute surface run-off from farmlands just north of Big Bay.

Table I (f) - Bacabec Bay, N.B.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
June 27	Water	Big Bay Cove (At Head of Bocabec Bay)	240	-
	Clams		>16,000	-
July 21	Water	Birch Cove	46	-
	Clams		230	-
July 28	Water	Big Bay Cove	240	30
Oct. 15	Water	Big Bay Cove	4.5	2
	Clams		78	45

Bocabec River: The Bocabec River flows into Passamaquoddy Bay east of Bocabec Bay and about two miles west of the Digdequash River Estuary. The river flows through an area which is somewhat rural in character, although rocky and

heavily wooded. Any pollution reaching the small clam flats at the mouth of the river would be from surface run-off carried down by the river; there are no houses near the flats.

Table I (g) - Bocabec River, N.B.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
June 28	Water	Bocabec R., estuary.	240	-
	Clams		>16,000	-
July 28	Water	Bocabec R. at bridge.	>1,600	-
Oct. 15	Water	Bocabec R. at bridge.	33	0

Digdequash River : The Digdequash River enters the north-eastern end of Passamaquoddy Bay through a wide estuary, where extensive clam flats are found. There are a few homes on the high banks of the river, and the country through which the river flows is somewhat rural in character, although rocky and heavily wooded. There would seem to be little danger of direct sewage pollution, but surface run-off from farmlands bordering the river may be a potential source of coliform contamination.

Magaguadavic River : The Magaguadavic River enters Passamaquoddy Bay on the eastern shore, just north of the Mascarene Shore. The area is rocky and wooded, with some farmlands to the north and east. Sewage from the town of

St. George, situated on the river above the clam flats, would probably affect the clam flats to some extent.

L'Etete : Clams are present in the small flats along L'Etete Creek. The area is heavily wooded and sparsely settled, but some sewage may reach the flats from a few homes near the creek.

Back Bay : Small clam flats are located along the shore of Back Bay, just below the town. Sewage from the town and from the sardine cannery may constitute a pollution hazard. Seagulls are numerous in this area.

Table I (h) - Digdequash R., N.B., and Magaguadavic R., N.B.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
June 27	Water	Digdequash River	17	-
	Clams		>16,000	-
	Water	Magaguadavic River	140	-
	Clams		>16,000	-
	Water	L'Etete Creek	70	-
	Clams		9,200	-
	Water	Back Bay, west shore	0	-
	Clams		>16,000	-
Oct. 14	Water	Digdequash River	7.8	4.5
	Clams		330	490

L'Etang River (Craig's Cove) : This is a long, narrow cove with high rock and gravel banks on either side; clams are found along a small creek in the centre. There are about ten acres of clam flats along the half mile of creek. There would be no danger of direct sewage pollution here, but surface run-off from farmlands may affect the flats.

Beaver Harbour : (a) Woodland's Cove is situated in the north-east portion of Beaver Harbour. At the head of the cove is a flat about twenty acres in size; the clam population is very scattered over this area, and is of little commercial importance. A shallow, wide creek flowing into the cove may contribute contamination drained from the pasture lands at the head of the cove.

(b) Beaver Harbour Basin is an almost landlocked basin, with a small outlet to the main harbour to the south at high tide, containing eight acres of clam flats. Sewage contamination may enter the basin from the village of Beaver Harbour situated to the east, and from homes along the road approaching the basin.

(c) Buckman's Creek is a narrow inlet, about one mile long, in the northern portion of Beaver Harbour. There are about ten acres of high, rocky flats in this area; the only possible source of pollution is drainage from farmlands around the creek.

(d) Sturgeon's Cove lies in the southern portion of L'Etang Harbour. Clams are present in good quantities on a gravel-mud bar of about four acres at the mouth of the cove. Some pollution could be expected from the buildings near the cove.

Deadman's Harbour : Extensive flats from a gravel causeway to the low water level. The clam population is scattered over about forty acres of bottom; the clams are slow-growing, and consequently are of little commercial importance. There is little danger of pollution from any source.

Table I (i) - L'Etang R., N.B., and Beaver Hbr., N.B.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
June 28	Water	L'Etang R.(Craig's Cove)	40	-
	Clams		>16,000	-
July 24	Water	Woodland's Cove	>1,600	-
	Clams		9,200	230
	Water	Beaver Hbr. Basin	79	-
	Clams		>16,000	11,000
	Water	Buckman's Creek	540	-
	Clams		16,000	430
	Water	Sturgeon's Cove	220	-
	Clams		9,200	930
	Water	Deadman's Harbour	1.8	-
	Clams		1,800	640

Little Lepreau Basin : (a) The Red Ledge Flat is situated at the west end of Lepreau Basin, near the mouth of Lepreau Harbour. The clam flats border the channel, and are about three acres in size. There is little danger of pollution here.

(b) The Brickyard Flat is situated on the north side of the

channel, and extends from the mouth of Lepreau Harbour to the cannery. Only about fifteen acres contain clams; the upper reaches are barren. Some sewage contamination could be expected from the cannery, and from the dwellings around the basin.

Lepreau Harbour : (a) Old House Flat stretches about one quarter of a mile along the north shore of the Harbour, and is about five hundred feet in width. The flat is steeply sloped, and is dug only during spring tides.

(b) The Jewish Flat lies in the north-east portion of the Harbour, and includes about three acres of producing bottom.

(c) The Rocks Flat is situated in the eastern end of the Harbour, opposite the mouth. It extends from the Mud Flat to the narrows, and includes about twenty-five acres of clam flats.

(d) The Mud Flats are situated on the south side of the Harbour, and extend from the Ledge flats to the Rocks. The flats slope gradually from high to low-water levels; they are good producers, and are dug steadily every season.

The country surrounding Lepreau Harbour is sparsely settled, rocky, and well wooded; there is little danger of direct sewage pollution reaching any flat in the area.

Ward's Creek : The only flats in this area are found along the creek, a channel about one half mile long. The mouth of the inlet is very narrow, and is cut up by high, rocky bars; this limits digging to an extremely small area along the creek, and the flats are therefore of small commercial importance. There would appear to be little danger of sewage contamination; the

surrounding heavily-wooded country supports few habitations.

Crow Harbour : This is a very small inlet, much cut up by high rock ledges. Clams are found along the edge of the channel in scattered areas. Some contamination from a few homes may reach the Harbour.

Table I (j) - Lepreau, N.B.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
July 24	Water	Red Ledge Flat, Little Lepreau Basin	23	-
	Clams		5,400	390
	Water	Brickyard Flat, Little Lepreau Basin	350	-
	Clams		2,400	390
July 16	Water	Old House Flat, Lepreau Harbour	23	-
	Clams		1,700	-
	Water	Jewish Flat, Lepreau Harbour	6	-
	Clams		1,100	-
	Water	The Rocks Flat, Lepreau Harbour	6	-
	Clams		3,500	-
	Water	The Mud Flat, Lepreau Harbour	7.8	-
	Clams		3,500	-
June 30	Water	Ward's Creek	140	-
	Clams		>16,000	-
	Water	Crow Harbour	130	-
	Clams		>16,000	-

Red Head Harbour : The Landslide Flat, located at the head of the Harbour, includes about ten acres of clam flats. A narrow channel running through the Harbour probably carries sewage from numerous cottages over the flats.

Pocologan Harbour : The Slag Flat is the largest and most productive flat in the Harbour; it lies along the north side of the Harbour for an estimated distance of one-half mile, and would include about one hundred acres of producing bottom. There may be some danger of pollution from sewage contributed by a cannery and other buildings in Pocologan village.

Table I (k) - Pocologan Hbr., N.B., and Red Head Hbr., N.B.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
June 30	Water	Landslide Flat, Red Head Harbour	>1,600	-
	Clams		>16,000	-
	Water	Slag Flat, Pocologan Harbour	>1,600	-
	Clams		>16,000	-
July 28	Water	Slag Flat, Pocologan Harbour	540	40
	Clams		200	2,400
	Water	Slag Flat, west, Pocologan Harbour	>1,600	430
	Clams		180	2,400
Oct. 16	Water	Slag Flat, Pocologan Harbour	23	4.5
	Clams		1,100	700
	Water	Landslide Flat, Red Head Harbour	46	13
	Clams		1,700	1,300

Dipper Harbour : Here are located extensive, sheltered flats, mostly sand-mud, with patchy clam populations. There would seem to be some danger of contamination from the village, and a small stream flowing through the flats.

Little Dipper Harbour : There are about fifty acres of flats with scattered clam populations in this harbour. There would seem to be little danger of pollution here.

Chance Harbour : The small sandy flats here are open only at extreme low tide, and very few clams are present. There is some possibility of sewage pollution from Chance Harbour village, and from cottages at the head of the Harbour.

Table I (1) - Dipper Hbr., N.B. and Chance Hbr., N.B.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
July 24	Water	Dipper Harbour	23	-
	Clams		790	1,500
	Water	Little Dipper Harbour	46	-
	Clams		1,300	430
	Water	Chance Harbour	49	-
	Clams		1,700	430

St. Andrews Harbour : (Stations 2 - 8 incl.) Sewage pollution here is very heavy; the area was closed for the taking of clams by the Department of National Health and Welfare Engineering Division. Samples of water, clams, mud, and silt were taken from each of the seven flats for analysis periodically during June and

July, and similarly on October 10, hoping to be able to correlate the coliform M.P.N. values for water, clams, mud, and silt taken at the same time from the same sampling stations, and to see what effect the changing meteorological conditions would have on the coliform flora of the clams. Results from these studies appear in Table II.

Observations, Charlotte County, N.B., 1947.

Coliform M.P.N. values obtained during the survey of clam producing areas in Charlotte County in June and July, 1947, indicated the presence of extremely large numbers of coliform organisms in the meats of clams dug in nearly all of the areas; of ninety samples analysed, only twenty-six (28.9%) showed a coliform M.P.N. of 2,400 or less, forty-six (71.9%) of the remaining sixty four specimens showed a M.P.N. of more than 16,000.

Coliform M.P.N.'s for clams dug during a second sampling of some of the same areas in October, 1947, were consistently lower; of twenty-five samples analysed, twenty (80%) showed a coliform M.P.N. of 2,400 or lower, and none of the other five samples showed a "maximum" M.P.N. Furthermore, these five samples were all from St. Andrews Harbour, a centre of known heavy pollution.

There would appear to be little direct relationship between coliform M.P.N. values of water, clams, mud, and silt. A low M.P.N. in water was not always indicative of a low M.P.N. in clams, and water sampling as a basis for determining the possibility or extent of coliform contamination in clams does

TABLE II

ST. ANDREWS HARBOUR STATION #	SPECIMEN	- DATE - JUNE 12	JUNE 24	JULY 1	JULY 17	JULY 23	1947 OCTOBER 10
#2 Opp. Sewer at Adolphus St.	Water	-	350	>1,600	>1,600	>1,600	350
	Clams	2,400	>16,000	>16,000	>16,000	5,400	5,400
	Mud	-	5,400	790	-	-	-
	Silt	-	16,000	>16,000	-	-	-
#3 Opp. Sewer at King St.	Water	-	>1,600	46	>1,600	>1,600	240
	Clams	5,400	>16,000	>16,000	>16,000	3,500	1,700
	Mud	-	>16,000	9,200	-	-	-
	Silt	-	>16,000	400	-	-	-
#4 Opp. Sewer at Ernest St.	Water	-	>1,600	>1,600	>1,600	>1,600	350
	Clams	2,400	>16,000	>16,000	>16,000	>16,000	5,400
	Mud	-	16,000	>16,000	-	-	-
	Silt	-	4,300	>16,000	-	-	-
#5 Off C.P.R. Wharf.	Water	49	13	220	350	>1,600	70
	Clams	790	>16,000	9,200	>16,000	>16,000	1,700
	Mud	170	210	9,200	-	-	-
	Silt	840	1,700	9,200	-	-	-
#6 South End of Breakwall.	Water	17	49	>1,600	>1,600	>1,600	13
	Clams	790	>16,000	>16,000	>16,000	5,400	700
	Mud	1,100	68	>16,000	-	-	-
	Silt	1,400	230	>16,000	-	-	-
#7 Middle of Breakwall.	Water	130	17	>1,600	>1,600	>1,600	7.8
	Clams	790	>16,000	>16,000	>16,000	>16,000	490
	Mud	210	700	>16,000	-	-	-
	Silt	460	1,700	>16,000	-	-	-
#8 Below Garbage Dump	Water	(130 (72	>1,600	>1,600	>1,600	>1,600	46
	Clams	5,400	>16,000	>16,000	>16,000	>16,000	9,200
	Mud	3,500	230	2,400	-	-	-
	Silt	9,200	700	5,400	-	-	-

COLIFORM M.P.N. VALUES.

TABLE II (a)

RELATIONSHIP BETWEEN COLIFORM MOST PROBABLE NUMBERS FOR WATER, CLAMS, MUD, AND SILT FROM CHARLOTTE COUNTY, N.B. 1947.

Summary of Results for St. Andrews Hbr., Stations 1 - 8.

	WATER	CLAMS	MUD	SILT
Total # of Samples	40	42	18	18
Coliform M.P.N. Range	7.8 ->1,600	490 ->16,000	68 ->16,000	230 ->16,000
% of Samples with Maximum M.P.N.	50	50	16.6	27.7
% of Samples with 2,400 M.P.N. or less	-	21.4	50	44.4
% of Samples with 350 M.P.N. or less	50	0	27.7	5.5

Summary of Results for the Remainder of Charlotte Co., N.B.

	WATER	CLAMS	MUD	SILT
Total # of Samples	53	50	26	26
Coliform M.P.N. Range	0 ->1,600	180 ->16,000	0 ->16,000	170 ->16,000
% of Samples with Maximum M.P.N.	9.4	42	7.7	7.7
% of Samples with 2,400 M.P.N. or less	-	26	88.5	46.2
% of Samples with 350 M.P.N. or less	83	8	53.8	19.2

not seem to be adequate or reliable. In many cases, high count clams were taken from areas showing low M.P.N.'s in the water. In general, the M.P.N. of the clam meats is usually higher than the M.P.N.'s of the silt, mud, or water taken from the same area. Silt usually shows a higher coliform content than mud found just beneath it.

An attempt was made to correlate the coliform M.P.N. values obtained for clams with total count values found by plating on Tryptone-Glucose Extract agar.

M.P.N. values and total counts were obtained for fifty-six specimens of clams from Charlotte County flats.

There was no obvious correlation between coliform M.P.N. values and total counts. An M.P.N. value of more than 16,000 per 100 ml. was accompanied by total count values ranging from 7,000 to more than 120,000 per ml. As a result, specimens collected during the remainder of the summer survey were not plated for total bacterial count.

The results indicate a marked seasonal effect; the M.P.N. values for water, clams, mud, and silt taken periodically from St. Andrews Harbour increased rapidly through June, and reached a maximum in nearly every case in July. A later sampling in October showed a marked reduction in M.P.N. values (See Table I). The rapid rise in coliform numbers experienced during the summer months may be due to several factors:

1. The greatly increased temperatures on the flats.
2. An increase in sewage pollution due to the influx of

TABLE III

COLIFORM M.P.N. per 100 ml.	CORRESPONDING TOTAL COUNT per ml. (IN THOUSANDS)	NUMBER OF SPECIMENS	COLIFORM M.P.N. per 100 ml.	CORRESPONDING TOTAL COUNT per ml. (IN THOUSANDS)	NUMBER OF SPECIMENS
>16,000	MORE THAN 120	9	5,400	15 30 11	3
	50 - 60	5	3,500	9.5 8.8	2
	30 - 40	5	2,400	88 53	2
	20 - 30	3	2,400	31.9 10.4	2
	10 - 20	8	1,300	12	1
	LESS THAN 10	2	1,100	7.6	2
16,000	34.5 30 14.9 11.3	4	790	18.4 12.5 1.4	3
			330	37	1
9,200	35.2 16.4 8.9	3	140	4.3	1

tourists and summer visitors to the area, and a resultant increase in the amount of sewage reaching the flats.

3. Changes in hydrographic conditions.

4. The clams may establish an intestinal coliform flora of their own; they would thus continue to give high M.P.N. values regardless of the presence or absence of further sewage pollution.

Because of the extreme seasonal fluctuations of coliform values obtained for clams, it was difficult to establish the real sanitary significance of a high coliform value from clams dug in a specific area.

Periodic Survey of Twelve Clam-Producing Flats in Charlotte County, N.B., 1948.

From the results of the survey conducted in Charlotte County, N.B. during the summer of 1947, it was apparent that seasonal conditions influence the coliform and fecal streptococci content of Mya arenaria to a marked extent. During the 1948 survey, an attempt was made to demonstrate this seasonal effect by periodic analysis of water and clam samples from twelve key stations in Charlotte County. Results obtained from this survey appear in Tables IV(a), IV(b), and IV(c). Tables V(a) and V(b) show averages for twice-daily water temperature readings made for the months of May to November inclusive for 1947 and 1948 at the Atlantic Biological Station, St. Andrews, N.B. and a graph prepared from this data appears on page 52.

TABLE IV (a)

M.P.N. OF COLIFORM BACTERIA AND FECAL STREPTOCOCCI (PER 100 ml.)

STATION	SPECIMEN	M.P.N. VALUE OBTAINED	JUNE 11	JUNE 14	JUNE 21	JUNE 29	JULY 5	JULY 14	AUG. 19	AUG. 24	OCT. 21
Bocabec Bay (Big Bay)	Stream water	[1] C.	170	>1,600	2,400	170	140	49	>1,600	-	920
		[2] F.S.	0	79	0	0	0	7.8	33	-	7.8
	Sea Water	C.	0	23	0	26	0	6.8	33	-	0
		F.S.	0	0	0	0	0	4.5	2	-	0
	Clams (Low level)	C.	0	230	230	45	45	78	330	-	230
		F.S.	0	0	0	0	0	20	20	-	68
	Clams (Middle level)	C.	0	170	78	460	68	45	130	-	78
		F.S.	0	0	0	0	0	20	78	-	20
	Clams (High level)	C.	0	45	130	45	45	45	230	-	45
		F.S.	0	45	20	0	0	45	130	-	20
Bar Road	Sea Water	C.	7.8	2	4.5	2	240	0	70	4.5	4.5
		F.S.	0	0	0	0	0	0	11	2	0
Chamcook	Clams	C.	230	170	110	20	330	78	460	490	490
		F.S.	39	45	0	0	78	0	130	110	45
Pottery Bridge	Sea Water	C.	11	-	4.5	7.8	7.8	4	27	6.8	4
		F.S.	0	-	2	0	0	2	2	2	0
St. Andrews N.B.	Clams	C.	230	-	1,300	330	330	790	790	490	230
		F.S.	430	-	78	78	78	78	78	130	20
St. Andrews Harb our	Sea Water	C.	>1,600	-	>1,600	9,200	9,200	540	>1,600	-	240
		F.S.	11	-	4.5	920	1,100	350	>1,600	-	79
Station #4	Clams	C.	>16,000	-	>16,000	5,400	9,200	16,000	92,000	-	9,200
		F.S.	2,100	-	790	1,300	5,400	16,000	24,000	-	2,400

Legend: [1]C: = Coliform Bacteria [2]F.S.: = Fecal Streptococci

TABLE IV (b)

STATION	SPECIMEN	M.P.N. VALUE OBTAINED	M.P.N. OF COLIFORM BACTERIA AND FECAL STREPTOCOCCI PER 100 ml. JUNE 11	JUNE 29	JULY 14	AUGUST 19	OCTOBER 21
NIGER REEF	Sea Water	C[1]	170	2	0	49	6.8
		F.S.[2]	0	0	0	4.5	0
(North)	Clams.	C	490	790	330	2,400	330
		F.S.	230	330	140	230	45
ST. ANDREWS HARBOUR	Sea Water	C	13	3,500	4	920	170
		F.S.	0	330	7.8	110	49
STATION #2	Clams	C	230	1,100	16,000	5,400	2,400
		F.S.	230	790	9,200	700	700
ST. ANDREWS HARBOUR	Sea Water	C	4.5	0	3.7	240	23
		F.S.	0	0	0	13	0
STATION #6	Clams	C	700	78	790	1,700	68
		F.S.	430	78	45	330	45
ST. ANDREWS HARBOUR	Sea Water	C	130	130	130	1,600	22
		F.S.	0	23	23	540	4.5
STATION # 8	Clams	C	790	330	310	>16,000	230
		F.S.	230	130	460	>16,000	68
BOCABEC R.	Stream Water	C	33	70	13	240	130
		F.S.	4	2	0	49	0

Legend. [1]C = Coliform [2]F = Fecal Streptococci

TABLE IV (c)

STATION	SPECIMEN	M.P.N. VALUE OBTAINED	M.P.N. OF COLIFORM BACTERIA AND FECAL STREPTOCOCCI PER 100 ml. JUNE 11	JUNE 29	JULY 14	AUGUST 19	OCTOBER 21
DIGDEQUASH RIVER	Stream Water	[1]C.	170	33	46	1,600	2
		[2]F.S.	4	4.5	0	79	0
ESTUARY	Sea Water	C.	0	7.8	0	11	0
		F.S.	0	0	0	0	0
	Clams	C.	230	78	330	790	130
		F.S.	230	0	110	130	68
CHAMCOOK	Sea Water	C.	110	2,400	170	920	110
		F.S.	3	230	13	23	0
COVE	Clams	C.	330	1,700	1,300	1,700	330
		F.S.	430	230	110	230	45
OAK BAY	Sea Water	C.	17	130	79	79	17
		F.S.	0	13	13	4.5	0
(Cookson's Island)	Clams	C.	1,700	1,400	1,300	700	330
		F.S.	430	170	82	110	20
OAK BAY	Sea Water	C.	280	31	22	49	17
		F.S.	43	7.8	9.3	7.8	0
(West Cove)	Clams	C.	5,400	1,300	2,400	1,100	700
		F.S.	390	230	700	330	110

Legend. [1]C = Coliform Bacteria [2]F.S. = Fecal Streptococci

TABLE V (a)

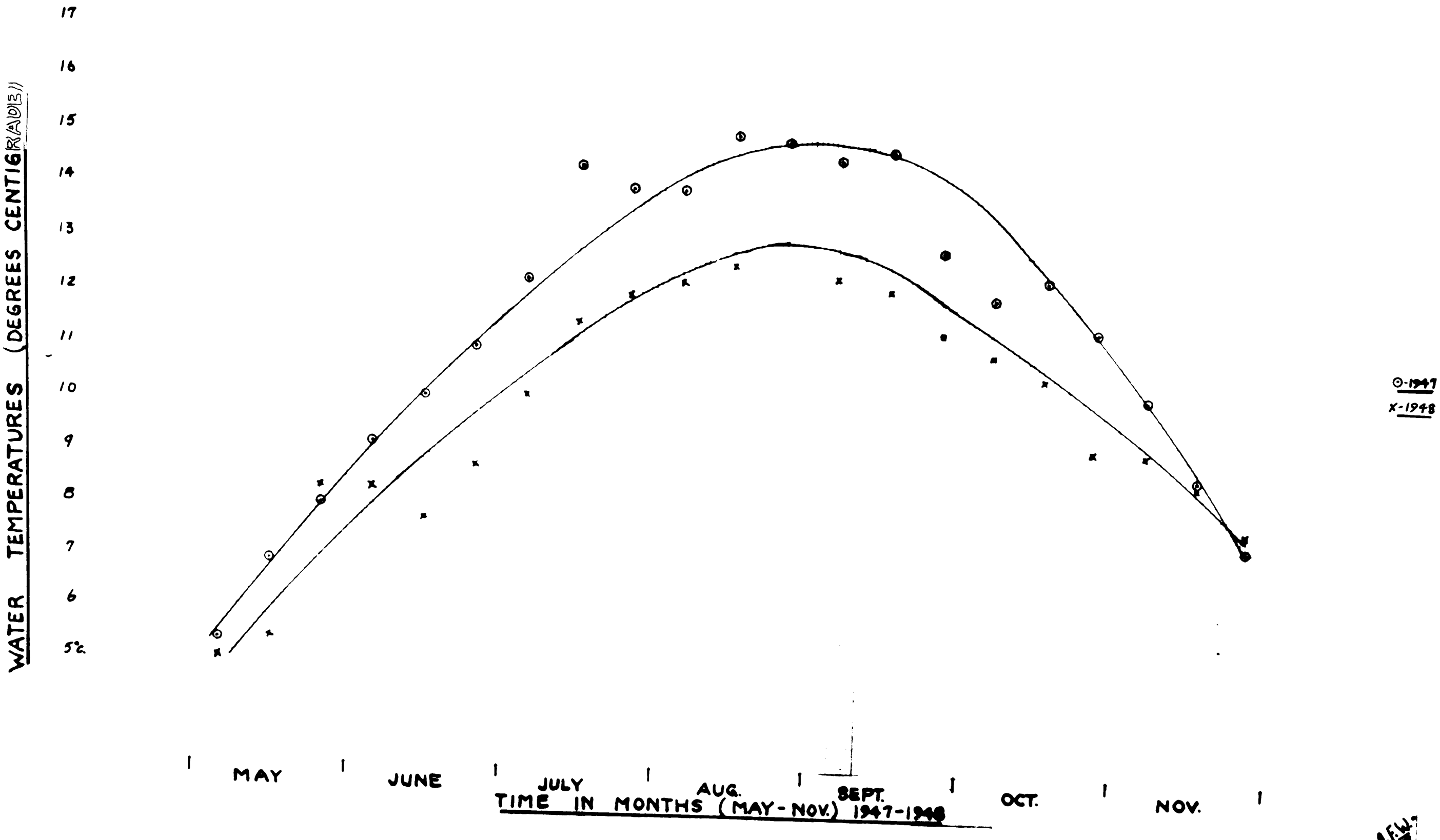
WATER TEMPERATURES AT ATLANTIC BIOLOGICAL STATION - ST.ANDREWS, N.B. - 1947

DATE	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER
1	4.1	8.9	12.9	14.1	14.1	11.7	10.6
2	4.4	8.6	12.8	13.7	14.2	11.7	10.5
3	4.0	8.0	14.2	13.1	14.4	11.7	10.7
4	4.7	8.3	12.2	13.4	14.8	11.8	10.6
5	5.5	8.9	11.9	14.2	14.9	12.1	10.5
6	5.8	10.5	11.1	14.8	14.5	12.3	10.4
7	6.8	9.9	11.4	14.2	14.9	12.8	10.2
8	6.7	9.8	12.5	14.9	15.1	12.6	10.3
9	5.7	8.9	11.8	14.3	14.5	12.3	10.1
10	5.7	10.0	13.4	14.8	15.9	12.1	9.6
11	6.3	10.0	14.2	15.6	15.8		9.5
12	6.1	10.6	14.1	16.2	16.3	12.4	9.7
13	8.3	10.8	14.5	16.1	15.6	12.4	9.2
14	6.7	8.9	14.9	15.3	15.1	12.3	8.7
15	6.5	10.8	15.7	15.4	15.1	12.5	8.7
16	6.6	10.9	15.0	15.2	14.9	12.6	8.5
17		10.8	15.2	14.8	14.7	12.3	8.6
18	7.2	9.6	14.5	14.5	14.4	12.7	8.7
19	7.6	9.0	14.0	14.5	13.7	12.7	8.8
20	6.5	9.9	13.4	14.2	13.7	12.9	8.1
21	7.3	10.1	12.3	14.6	13.6	12.4	7.8
22	7.0	9.9	12.8	15.3	14.1	11.8	8.0
23	7.1	10.5	12.9	16.8	13.5	12.2	8.2
24	7.5	10.3	14.3	14.8	13.3	11.7	7.8
25	7.8	9.7	14.9	14.7	13.4	10.9	7.9
26	8.0	11.8	16.3	15.0	13.0	11.3	7.3
27	8.4	11.3	14.5	15.4	12.5	11.5	7.0
28	8.3	11.3	13.8	15.5	12.4	11.7	7.1
29	8.9	12.6	13.8	14.6	12.6	11.5	6.9
30	8.8	12.9	15.1	14.2	12.3	11.1	6.9
31	8.9		15.2	14.6		10.8	

TABLE V (b)

WATER TEMPERATURES AT ATLANTIC BIOLOGICAL STATION - ST.ANDREWS, N.B. - 1948

DATE	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER
1	3.7	10.0	10.6	13.5	12.55	11.5	9.0
2	4.8	10.0	11.2	12.6	12.45	11.6	8.9
3	4.7	10.3	11.2	13.1	12.45	11.3	8.9
4	5.1	10.1	10.4	12.4	12.40	10.8	9.2
5	4.9	8.4	10.2	12.5	12.35	10.9	9.5
6	5.5	7.7	9.2	11.8	12.60	11.0	9.3
7	5.4	7.2	9.4	12.2	12.60	11.0	9.9
8	5.9	6.7	9.8	12.3	12.40	11.0	9.3
9	5.8	6.7	10.0	11.9	12.55	11.1	9.4
10	5.6	6.6	9.5	11.8	12.45	11.2	9.6
11	5.5	7.0	10.5	12.0	13.40	11.2	9.7
12	5.3	6.5	11.2	11.7	12.90	11.3	9.1
13	5.5	6.7	10.2	11.9	13.20	11.4	9.3
14	5.3	6.9	11.4	12.9	12.95	11.0	8.7
15	4.9	7.0	11.6	13.5	12.35	10.9	8.6
16	4.9	9.1	11.3	13.5	11.80	10.7	8.4
17	4.6	8.8	11.7	12.9	11.65	10.9	8.4
18	4.9	9.0	11.9	13.3	11.50	10.5	8.2
19	6.6	8.1	12.9	12.9	11.80	9.5	8.3
20	7.3	8.7	13.2	12.9	11.48	9.7	8.2
21	7.4	8.2	12.5	13.1	11.53	9.5	8.1
22	6.6	8.4	11.9	13.4	11.73	9.2	7.9
23	6.4	9.0	11.9	12.5	11.15	9.3	8.1
24	7.7	8.4	10.8	13.1	11.05	9.5	8.1
25	7.8	9.0	11.6	13.5	11.15	9.4	8.2
26	8.4	9.7	13.1	14.0	11.65	9.5	7.7
27	8.0	9.5	11.9	13.9	11.90	9.5	8.0
28	8.6	8.2	11.4	13.0	11.70	9.5	7.6
29	10.3	8.5	11.9	14.0	11.50	9.5	7.4
30	10.5	8.7	13.8	13.3	11.60	8.8	7.3
31	10.2		13.3	13.1		9.4	


WATER TEMPERATURES (DEGREES CENTIGRADES)
17
16
15
14
13
12
11
10
9
8
7
6
5°c.
⊙-1947
X-1948
MAY
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
TIME IN MONTHS (MAY-NOV.) 1947-1948

Observations

Results obtained during the 1948 survey differ very markedly from those recorded during the summer of 1947. Very few of the clams produced in these areas during the June-August 1947 period could meet the requirements suggested by the U.S.P.H.S. (viz: an M.P.N. of no more than 2,400 coliforms bacteria per 100 ml.); when repeat tests were conducted in some of these areas in October 1947, a general improvement in quality, as measured by bacteriological tests, was observed. In 1948, however, M.P.N. values remained relatively low throughout the entire survey period, although a slight general rise was observed in the middle of August; unfortunately, it was impossible to continue the survey through the latter part of August, and September. It is considered likely that a further rise in M.P.N. values would have been noted during this period. M.P.N. values obtained during a final survey on October 21 approximated the low M.P.N. values noted in June.

Water temperatures recorded at St. Andrews during May to November, 1948, were consistently lower than those for the same period in 1947. It has been shown that the swarmings of the dinoflagellate, Gonyaulax, strongly suspected as being the ultimate source of shellfish toxicity in the Fundy area, are apparently controlled by water temperature, and that the toxicity develops suddenly, and succeeds a sharp rise in water temperature to or near the season's maximum. It is considered probable that a similar relationship exists between water

temperature and the coliform content of soft-shelled clams. As long as water temperatures remain low, M.P.N. values will be significant, but when the temperature of water over the clam flats reaches a critical "threshold" level, multiplication of fecal bacteria within the clam may occur; the resultant increase in M.P.N. values would not be of sanitary significance, since it could occur in the absence of further sewage pollution.

In 1947, peak toxicities recorded for clams came nearly two months earlier than in 1948; the water temperature maximum was correspondingly higher and earlier. Similarly, coliform M.P.N. values recorded during the 1947 survey reached a maximum early in July and remained at a high level throughout the summer; in 1948, however, coliform M.P.N. values remained relatively low throughout the entire survey, although indications were that a general increase in the coliform content of clams occurred in late August, at approximately the same time as the sudden rise in toxicity.

Hachey and McLellan (1948) in a study of trends and cycles in surface temperatures of the Canadian Atlantic prepared from data compiled at the Atlantic Biological Station, St. Andrews, N.B., indicate the possibility that there is a cycle including approximately four years of sub-normal temperatures and four years of supra-normal temperatures; supra-normal temperatures prevailed in the years 1944 to 1947 inclusive, while 1948 was the first of four years of sub-normal water temperatures.

SURVEY OF CLAM PRODUCING AREAS IN DIGBY AND ANNAPOLIS COUNTIES, N.S., 1947.

Digby and Digby Raquette: Clams are found in scattered areas along the harbour front, in Digby Basin, and in the Digby Raquette flats to the north of the town. Heavy sewage pollution could be expected from the town sewage system, and from the various fish packing plants.

The Joggins: The area known as "the Joggins" begins at a distance of one-half mile to the southward of the Digby wharves, and comprises two basins, named, respectively, "the Little Joggins", and "the Grand Joggins". There was no evidence of immediate sewage contamination in these areas, but when it is considered that Digby, with a winter population of about 1,500 people, increased in summer to about 3,000 through the addition of summer visitors, discharges sewage into the waterfront through some five municipal sewers and many private ones, pollution would, with an incoming tide, be carried southwards directly over the two Joggins. The Joggins flats are extensive, and some diggers do operate there, in spite of the obvious danger of contamination.

Smith's Cove: Situated six or seven miles eastward from Digby, the small Smith's Cove flats are not exploited to any great extent. Two summer hotels built on the cottage system (Harbour View Inn and Mountain Gap Inn) are in close contiguity to the cove, but are provided with cesspools built in sandy soil. The contents of these would soak through a sufficiency of soil that would purify any effluent that might reach the shoreline.

Bear River: The Bear River empties into the Annapolis Basin through a channel in the broad, sand-mud clam flats. Some sewage may enter the river above the flats, from summer cottages, and flow down over the flats, but all such summer homes have cesspools or privies, and no sewage is thrown directly into the river. The river flows swiftly, has a good scouring action, and, with the extreme rise in tide level, should give a thorough mixture with very high dilution of any possible contamination.

Table VI (a) - Digby, N.S.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
Sept. 8	Water	Digby Basin; N. Shore at Mouth	170	45
	Clams		2,200	130
Sept. 8	Water	Digby Raquette	350	78
	Clams		2,200	330
Sept. 6	Water	The Joggins, E. Bank, above Bridge	350	78
	Clams		5,400	230
Sept. 6	Water	Bear River	23	0
	Clams		330	230
Sept. 6	Water	Smith's Cove	49	0
	Clams		230	78

Goat Island: Situated just north of Clementsport, N.S., the Goat Island flats are heavily dug; the area may be considered perfectly safe from a sanitary standpoint. There are

no dwellings in the immediate vicinity of the flats. Above this point no clams are dug.

The town of Annapolis Royal is situated about seven miles further eastward; what small amount of sewage flows into the basin at that point is relatively of little import, and should be quickly diluted by the great volume of tidal water.

Thorne Cove: Situated almost directly across Annapolis Basin from Clementsport, Thorne Cove flats are extensive and heavily worked. A small stream flows through the flats, and may carry surface run-off from surrounding farmlands. Except for this, there is little chance of direct pollution.

H.M.C.S. Cornwallis, Deepbrook, N.S. : Patchy clam populations are found on the rocky beach immediately behind the naval training centre. This area has been closed to the taking of clams, since large quantities of sewage reached the basin in wartime; now, when only a maintenance staff is retained at the station, the amount of sewage reaching the clam flats may be negligible.

Weymouth North : The village of Weymouth North is situated at the mouth of the Sissiboo River; clam flats extend along both sides of the river near its emergence into St. Mary's Bay, a large body of water some five miles in breadth at this point. There are few inhabitants in the immediate vicinity. The river flows swiftly in a well-defined channel, and the strong tides afford rapid dilution for any raw sewage entering the river from the town of Weymouth some two miles to the south.

Weymouth : A small flat on the west bank of the Sissiboo River, just below the town of Weymouth, is dug commercially to some extent. The close proximity of this flat to the town would indicate that dangerous sewage pollution is possible, and indeed probable.

Table VI (b) - Annapolis, N.S., and Weymouth, N.S.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
Sept. 4	Water	Goat Island	26	0
	Clams		1,100	2,400
	Water (stream)	Thorne Cove, east	240	0
	Clams		5,400	>16,000
Sept. 9	Water (sea)	Thorne Cove, west	7.8	0
	Clams		490	20
	Water	Weymouth North	13	0
	Clams		3,500	130
	Water	Weymouth	>1,600	20
	Clams		1,700	330
Sept. 11	Water	Goat Island	0	0
	Clams		490	45
	Water	H.M.C.S. Cornwallis	11	0
	Clams		270	0

Belliveau's Cove: Flats of about one hundred and fifty

acres in extent are located at Belliveau's Cove on St. Mary's Bay. Clam populations are patchy and scattered throughout the flats. Sanitary conditions, in general, should be good.

Grosses Coques : Small clam flats are found here on the shores of St. Mary's Bay. Sanitary conditions seem to be above reproach. No clams are dug for export between this point and the town of Yarmouth.

Table VI(c) - Belliveau's Cove, N.S., and Grosses Coques, N.S.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
Sept. 9	Water	Belliveau's Cove	0	0
	Clams		310	45
	Water	Grosses Coques	4.5	0
	Clams		230	20

Observations, Digby and Annapolis Counties, N.S., 1947.

The coliform and fecal streptococci M.P.N. values obtained during the survey of clam producing areas in Digby and Annapolis Counties in September, 1947, indicated several potentially dangerous areas. In general, M.P.N. values for clams and water from these areas were considerably lower than similar values obtained from the sampling of Charlotte County, N.B., during June and July, 1947; to what extent this could be attributed to the effect of seasonal hydrographic changes is not known. A similar sampling, conducted at the same time as the Charlotte County, N.B. survey, might possibly have shown higher fecal bacterial loads in clams and sea water from these areas.

64.3 per cent of the water samples analysed during these investigations gave coliform M.P.N.'s of less than 50, while 71.4 per cent contained no fecal streptococci. 78.4 per cent of the clam samples tested gave coliform M.P.N.'s of less than 2,400, while 85.7 per cent gave fecal streptococci M.P.N.'s of 350 or less.

SURVEY OF CLAM PRODUCING AREAS IN HALIFAX COUNTY, N.S., 1947.

Musquodoboit Harbour and Ostrea Lake: Ostrea Lake is situated about six miles south of the head of Musquodoboit Harbour, and represents the extreme lower portion of this harbour. From the mouth of the Harbour, the main channel runs in a north-easterly direction, and then turns to the north-west. Clam flats are located along the eastern and western sides of the main channel, which runs down the north-western side of the harbour. There are no clams in the upper four miles of the harbour. Near the mouth of the harbour the main channel runs in a north-easterly direction, and here clams are dug only on the western side; the eastern shore flats would be exposed only during a very low tide.

Ostrea Lake settlement is located on the eastern shore; the countryside is well wooded, very rocky, and sparsely populated. Drainage to the lake would include surface runoff from farms along the eastern shore, but there is little sewage discharged directly to the lake. There are a few dwellings along the Musquodoboit River four miles above the northern clam flats; the amount of sewage entering the harbour would be extremely small, and should not materially affect the clam producing areas.

The general characteristics of all of the seven clam flats sampled in Musquodoboit Harbour were similar, with no obvious sources of pollution visible in any case, except that large numbers of seabirds were present, especially on the Flat Island (Birdrock) and Indian Point flats. All samples were taken on October 1, 1947.

Table VII (a) - Musquodoboit Harbour, N.S.

Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
Water	Sedge Bank Flats	0	0
Clams		5,400	1,700
Water	Cheticumchee Flats	13	0
Clams		3,500	1,100
Water	Flat Island (Birdrock) Flats	0	0
Clams		5,400	790
Water	Indian Point Flats	2	0
Clams		16,000	790
Water	Francis Nose Flats	0	0
Clams		3,500	330
Water	Coke Flats	2	0
Clams		2,400	230
Water	Baker's Island Flats	4.5	0
Clams		1,700	130

Clam Harbour : Clam Harbour is situated approximately fifty miles east of the city of Halifax. The harbour runs north and south for a distance of three and one-half miles; clams are found along the western and eastern sides of the main channel, which extends down the western shore of the harbour. A secondary gravel road extends down the eastern side of the harbour, with houses located along the way. A small settlement, Clam Harbour, is situated at the head of the harbour. Mary's River enters here. The areas along the western shore from which clams are fished are far enough removed from the eastern shore settlement so that any surface drainage there would have no effect. Flats near the head of the harbour may be receiving pollution from the cannery and the small settlement situated there.

Petpeswick Harbour : Petpeswick Harbour is located about forty miles east of Halifax, and extends a distance of six miles north of the harbour mouth. The clam flats are located above and below the narrows; those below the narrows are situated along the western side of the harbour, and are one-quarter of a mile from the eastern shore, while the flats above the narrows are approximately two hundred yards from the eastern shore.

The Little River, which is fed by Pacey Lake, enters the head of the harbour about two miles above the western clam flats; there are no settlements along this river. Scattered dwellings are located along a secondary gravel road which extends down the eastern shore of the harbour. The countryside is rocky and heavily wooded in some sections. Drainage to the harbour would

include surface run-off from farmlands, but there is little, if any, sewage discharged directly to the harbour.

Table VII (b)

Survey of Clam Producing Flats in Clam Harbour, N.S., Sept. 24, 1947.

Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
Water	Lower West Cove	49	0
Clams		2,400	790
Water	Muggy's Cove	0	0
Clams		490	490
Water	Label's Point	14	4.5
Clams		>16,000	220
Water	Indian Cove	33	0
Clams		5,400	93
Water	Middle Ground Flats	27	0
Clams		>16,000	270
Water	Off the Point Flats	13	0
Clams		2,400	270
Water	Upper West Cove	33	4.5
Clams		>16,000	270

Table VII (c)

Survey of Clam Flats, Petpeswick Harbour, N.S., Sept. 29, 1947.

Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
Water	The Cove Flats	0	3.7
Clams		78	1,100
Water	Upper Cove (The Dyke) Flats	0	68
Clams		790	1,100
Water	Lower Turn Flats	2	4.5
Clams		130	1,400
Water	Long Cove Flats	0	2
Clams		130	45
Water	Upper Turn Flats	0	4.5
Clams		78	45

Chezzetcook Harbour : Chezzetcook Harbour is located about thirty miles east of Halifax, and extends about five miles north of the harbour mouth. The principal clam flats are located on the many islands found in the central portion of the harbour. There are small settlements at Head of Chezzetcook, East Chezzetcook, and Grand Desert, but the homes are built well back from the shore, and are equipped with outdoor privies; there is little danger of sewage pollution from these sources reaching the flats. Sanitary conditions are generally good.

Table VII (d)

Survey of Clam Flats, Chezzetcook Harbour, N.S., Sept. 25, 1947.

Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
Water	Murphy's Point Flats	7.8	0
Clams		16,000	790
Water	Indian Island Flats	7.8	0
Clams		16,000	1,300
Water	Conrad Island Flats	4.5	13
Clams		>16,000	790
Water	Gaetz Island Flats	2	0
Clams		>16,000	790
Water	Ferguson Island Flats	2	0
Clams		16,000	78
Water	Red Island Flats	4.5	0
Clams		5,400	270
Water	Black Point Flats	2	0
Clams		3,500	220

Cole Harbour : Cole Harbour is located about six miles east of Dartmouth, N.S.; clam flats are found in the lower section of the harbour, south of the Canadian National Railway trestle, in an area that was formerly dykeland. The surrounding county property

is heavily wooded and rocky, and there are no dwellings located along the shoreline except for twenty small summer cottages, equipped with privies, situated on a rocky point in the southern end of the harbour; it is felt that little raw sewage from this source reaches the clam flats.

The Halifax County Home is situated on the upper north-west side of the harbour, well back from the shoreline, on high land. Sewage disposal is by septic tank and tile disposal bed; the amount of sewage discharged into the harbour should be negligible.

Table VII (e)

Survey of Clam Flats, Cole Harbour, N.S., September 26, 1947.

Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
Water	South-West Flats	11	0
Clams		3,500	9,200
Water	Middle-Ground Flats	0	0
Clams		>16,000	>16,000
Water	South-East Flats	2	0
Clams		5,400	5,400
Water	West of C.N.R. Trestle	2	0
Clams		5,400	5,400

Observations, Halifax County, N.S., 1947.

The clam producing areas of Halifax County are all rocky, heavily wooded, and sparsely settled; the danger of heavy sewage pollution reaching the flats in any instance would seem remote. It is therefore surprising to note that seventy per cent of the clam samples from these areas gave coliform M.P.N.'s of more than 2,400. Only thirty-three per cent gave fecal streptococci M.P.N.'s of more than 790. Water samples taken from all the Halifax County areas showed low M.P.N.'s, and in all cases met the standards of the United States Public Health Service.

The high M.P.N. values obtained from clams are difficult to explain in terms of direct sewage pollution. The low M.P.N.'s obtained from water samples also indicate that sewage contamination of the flats from a distant source is doubtful.

The Bacterial Flora of Clams from "Clean" and Polluted areas.

A study of the bacterial flora in clams from "clean" and polluted areas was proposed. St. Andrews Harbour, N.B., provided an area where sewage pollution was known to be heavy (Tables I, II, IV, and V). Dick Island and Hog Island, two heavily wooded, rocky islands in Bocabec Bay, N.B., were selected as areas having no sources of pollution except seabirds, and were sampled on June 17 and July 28, 1947. Malpeque Bay, P.E.I., an area considered to be free from sewage pollution, was selected as suitable and sampled on July 21 and July 23, 1948. Results obtained through bacteriological analysis of clam and water samples from these areas appear in Table VIII.

Table VIII (a)

Survey of Dick Island and Hog Island, Bocabec Bay, N.B., June 17 and July 28, 1947.

Specimen	Area	Coliform M.P.N.
Water	Dick Island, West shore Flats	33
Clams		>16,000
Water	Dick Island, North shore Flats	26
Clams		16,000
Water	Dick Island, East shore Flats	110
Clams		9,200
Water	Hog Island, North shore Flats	34
Clams		>16,000
Water	Hog Island, East shore Flats	170
Clams		>16,000
Water	Hog Island, South shore Flats	11
Clams		>16,000
Water	Hog Island, West shore Flats	11
Clams		16,000
Water	Dick Island, East shore Flats (July 28)	240
Clams		9,200
Water	Dick Island, West shore Flats (July 28)	350
Clams		24,000

Observations : Dick Island and Hog Island were found to be the nesting grounds for large flocks of seagulls; coliform M.P.N.'s obtained from clam and water samples were so high as to preclude

any further sampling of the islands as a coliform-free area.

Table VIII (b)

Clam Survey, Malpeque Bay, P.E.I., July, 1948.

Date	Specimen	Area	Coliform M.P.N.	Fecal Streptococci M.P.N.
July 21	Water	Bentinck Cove, Flat No.1	0	0
	Clams		790	110
	Water	Bentinck Cove, Flat No.2	23	13
	Clams		330	210
	Water	Mouth of Plat River	7.8	4.5
	Clams		16,000	3,500
	Water	Burnt Point	2	2
	Clams		220	45
	Water	Taylor's Point	1,600	4.5
	Clams		>16,000	230
July 23	Water	Mouth of Plat River	11	4.5
	Clams		9,200	210
	Water	Burnt Point	4.5	2
	Clams		790	460
	Water	Mouth of Grand River	0	4.5
	Clams		16,000	130

Observations : Only six clam producing flats were discovered in the Malpeque Bay area; apparently, with the disappearance of the eelgrass, most of the flats became silted over or washed

away, so that the only remaining producing beds are located near the mouths of small streams; they cannot be considered entirely free of contamination. The clams are small, and their shells are so thin and fragile as to make aseptic shucking difficult.

All but one of the water samples analysed contained some fecal bacteria; this precluded any possibility of considering these areas as entirely pollution-free. M.P.N. values recorded for clams were relatively high; in all probability, this was due to the seasonal effect induced by the high water temperatures recorded in Malpeque Bay at the time of sampling. It is evident that Sanitary Engineering surveys alone are inadequate in assessing the sanitary condition of soft shell clams from a producing area.

Classification of Coli-Aerogenes Group Organisms Isolated from Clam and Water Samples, 1947 and 1948.

Considerable controversy has arisen over the validity of the Standard Methods test for the presence of members of the coli-aerogenes group as an index of fecal pollution. An attempt was made during the investigations to discover to what extent definitely fecal (Escherichia coli) types were associated with high coliform M.P.N.'s in clams, and to what extent these M.P.N. values could be attributed to Aerobacter aerogenes and intermediate types, which some investigators contend may not be of fecal origin. Coliform bacteria isolated from clam and water samples were classified according to their "IMVIC" reactions; two hundred and three such isolations were made from areas in Charlotte

County, N.B., one hundred from Digby and Annapolis Counties, N.S., and seventy from Halifax County, N.S., in 1947. Two hundred and sixty-eight cultures isolated from Charlotte County, N.B. samples were classified in 1948. (See Tables IX to XV incl.).

Observations : In 1947, sixty per cent of the coliform bacteria isolated from Charlotte County, fifty-seven per cent from Digby and Annapolis Counties, and seventy per cent from Halifax County were classified as Aerobacter types. In some cases, specimens isolated from areas judged to be free from all obvious sewage pollution, but which still gave high coliform M.P.N. values, proved to be all Aerobacter types. There was, however, no consistent correlation between the fecal streptococci M.P.N.'s and the incidence of fecal and non-fecal coliform types.

Charlotte County, N.B.: The St. Andrews Harbour area, which was known to be heavily polluted by direct sewage contamination, showed a high incidence of E.coli. Clams from Pocologan Harbour, an area not obviously heavily contaminated, also showed a majority of fecal types, although isolations from two samples of water from the same area were all Aerobacter types. Dick Island, an uninhabited island in Bocabec Bay previously considered as a "clean" area, also showed a high incidence of E.coli, in all probability due to the tremendous numbers of seabirds that frequent the island. Coliform bacteria isolated from Oak Bay and Chamcook Harbour clams in October, 1947, were all Aerobacter types.

Digby and Annapolis Counties, N.S. : Thorne Cove and Goat Island are free from heavy sewage pollution, and both coliform

TABLE IX

COLIFORM ISOLATIONS

CHARLOTTE COUNTY, N.B., JULY, 1947.

Specimen	Source	Coliform M.P.N. per 100 ml.	COLI-AEROGENES GROUP - REACTION CLASSIFICATION					
			Total # Specimens	Escherichia coli	Intermediates (Atypical E. coli)	Aerobacter cloacae	Intermediates (Atypical A.aerogenes)	Aerobacter aerogenes
Water	Pocologan Harbour - West -	540	16					16
Water	Pocologan Harbour - East -	>1,600	17					17
Clams	Pocologan Harbour - East -	200	12	6		4		2
Water	St. Andrews Harbour Station # 4.	>1,600	15	9	2	1		3
Clams	St. Andrews Harbour Station # 4.	>1,600,000	20	19		1		
Water	Bocabec R.	>1,600	8	1				7
Water	Bocabec Bay (Dick I - East)	240	2				2	
Water	Bocabec Bay (Dick I - West)	350	8	5				3
Clams	Dick I - East	9,200	18	14				4
Clams	Dick I - West	24,000	17	10			1	6

TABLE X

COLI-AEROGENES GROUP - REACTION CLASSIFICATION

CHARLOTTE COUNTY, N.B., OCTOBER, 1947.

Specimen	Source	Coliform M.P.N.	Fecal Streptococci M.P.N.	Total # Isolations	Escherichia coli	Aerobacter cloacae	Intermediates (Atypical A.aerogenes)	Aerobacter aerogenes
Clams	St. Andrews Harbour (Station # 2)	5,400	9,200	10	1	1		8
Clams	St. Andrew's Harbour (Station # 8)	9,200	3,500	10	4		6	
Clams	Oak Bay	2,400	1,400	10			10	
Clams	Chamcook Bay	1,100	700	10			10	
Shellstock Clams	Crow Harbour (Shaw & Ellis Co., Pocologan, N.B.)	300	140	10			10	
Shucked Meats (Unwashed)	Pocologan Harbour (Shaw & Ellis Co., Pocologan, N.B.)	330	700	10	10			
Washed & Packed Clam Meats	Oak Bay (Chamcook Packers, Chamcook, N.B.)	790	1,100	10				10

TABLE XI

COLI-AEROGENES GROUP - REACTION CLASSIFICATION. CLAM SPECIMENS.

DIGBY & ANNAPOLIS COUNTIES, N.S. - SEPT., 1947.

- Source -	Coliform M.P.N.	Fecal Streptococci M.P.N.	Total # Specimens	Escherichia coli	Intermediates (Atypical E. coli)	Aerobacter cloacae	Aerobacter aerogenes
Sissiboo R. at North Weymouth	3,500	130	15				15
Sissiboo R. at Town of Weymouth	1,700	330	15	2	4		9
Belliveau's Cove	310	45	15			15	
Sissiboo R. at Town of Weymouth (Shellstock, A.L. Baker & Son, Yarmouth)	1,100	330	15	8	2		5
Thorne Cove	490	20	20	14			6
Goat Island	490	45	20	13		1	6

TABLE XII

COLI - AEROGENES GROUP - REACTION CLASSIFICATION

HALIFAX COUNTY, N.S., SEPT., 1947.

SPECIMEN	SOURCE	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.	TOTAL NO. SPECIMENS ISOLATED	ESCHERICHIA COLI	AEROBACTER AEROGENES
Clams	Petpeswick Harbour (Upper Cove)	790	1,100	10		10
Shucked Meats (Washed & packed)	Musquodoboit Harbour (Baker & Co., Ostrea Lake)	170	0	10	5	5
Clams	Musquodoboit Harbour (Sedge Bank)	5,400	1,700	10	2	8
Clams	Musquodoboit Harbour (Indian Point)	>16,000	790	10		10
Clams	Musquodoboit Harbour (Francis Nose)	3,500	330	10		10
Shellstock Clams	Cole Harbour (General Seafoods, Ostrea Lake)	1,300	78	10	4	6
Shucked Meats (Unwashed)	Ostrea Lake (General Seafoods, Ostrea Lake)	230	78	10	10	

TABLE XIII

- 1948 -

COLI- AEROGENES GROUP - REACTION CLASSIFICATION

SPECIMEN and DATE	SOURCE	SANITARY SURVEY	COLIFORM M.P.N. per 100 ml.	FECAL STREPTO-COCCI M.P.N. per 100 ml.	TOTAL # of ISOLATIONS	ESCHERICHIA COLI	INTERMEDIATE STRAINS	AEROBACTER AEROGENES
CLAMS JULY 14	ST. ANDREWS HARBOUR	DIRECT AND HEAVY SEWAGE POLLUTION	16,000	16,000	10	6	2	2
CLAMS AUGUST 19			92,000	24,000	15	2	-	13
WATER OCTOBER 21	STATION #4		240	79	15	-	4	11
CLAMS OCTOBER 21			9,200	2,400	15	-	-	15
WATER OCTOBER 21	ST. ANDREWS HARBOUR		170	49	15	15	-	-
CLAMS OCTOBER 21	STATION #2	Fairly heavy Sewage pollution	2,400	700	10	10	-	-
CLAMS July 14	ST. ANDREWS HARBOUR	Doubtful Sanitary Quality	310	460	6	1	3	2
CLAMS OCTOBER 21	STATION #8	Drainage From St. Andrews Garbage Dump.	230	68	15	11	-	4

TABLE XIV

1948

SOURCE	SANITARY SURVEY	SPECIMEN and DATE	COLIFORM M.P.N. per 100 ml.	FECAL STREPTO-COCCI M.P.N. per 100 ml.	COLI-AEROGENES GROUP - REACTION CLASSIFICATION: TOTAL NO. of ISOLATIONS	ESCHERICHIA COLI	INTERMEDIATE STRAINS	AEROBACTER AEROGENES
BOCABEC (BIG) BAY	GOOD - SEWAGE POLLUTION REMOTE	CLAMS JULY 14	78	20	7	1	3	3
		CLAMS AUG. 19	330	20	3	-	3	-
		STREAM WATER OCT. 21	920	7.8	15	-	-	15
		CLAMS OCT. 21	230	68	15	-	11	4
POTTERY BRIDGE	FAIR - SLIGHT	CLAMS JULY 14	790	78	7	7	-	-
ST. ANDREWS, N.B.	SEWAGE POLLUTION	CLAMS OCT. 21	230	20	10	10	-	-
NIGER REEF, ST. ANDREWS, N.B.	FAIR - SLIGHT SEWAGE POLLUTION	CLAMS OCT. 21	330	45	10	8	2	-
ST. ANDREWS HARBOUR STATION #6	FAIR/SLIGHT SEWAGE POLLUTION	CLAMS OCT. 21	68	45	15	15	-	-

TABLE XV

- 1948 -

COLI - AEROGENES GROUP - REACTION CLASSIFICATION

SOURCE	SANITARY SURVEY	SPECIMEN and DATE	COLIFORM M.P.N. per 100 ml.	FECAL STREPTO-COCCI per 100 ml.	TOTAL # of ISOLATIONS	ESCHERICHIA COLI	INTERMEDIATE STRAINS	AEROBACTER AEROGENES
BAR ROAD,	GOOD - SEWAGE POLLUTION	CLAMS JULY 14	78	0	10	10	-	-
CHAMCOOK	REMOTE	CLAMS AUG. 19	460	130	3	2	1	-
		CLAMS OCT. 21	490	45	10	10	-	-
OAK BAY, (COOKSON'S ISLAND)	FAIR - SLIGHT SEWAGE POLLUTION	CLAMS AUG. 19	700	110	10	1	-	9
		CLAMS OCT. 21	330	20	10	10	-	-
DIGDEQUASH RIVER	GOOD - SEWAGE POLLUTION	CLAMS AUG 19	790	130	12	6	6	-
	REMOTE	CLAMS OCT. 21	130	68	15	-	-	15
BOCABEC RIVER	DOUBTFUL SANITARY QUALITY	WATER OCT. 21	130	0	10	-	10	-

and fecal streptococci M.P.N.'s were low, yet the incidence of E.coli was high in both cases. The Sissiboo River clam flats at the town of Weymouth receive direct sewage pollution from the town, and here the incidence of E.coli was high; down the river, at Weymouth North, isolations of coliform organisms proved to be all Aerobacter aerogenes. Fifteen isolations from clams from Belliveau's Cove, where sewage pollution is negligible, were all Aerobacter cloacae.

Halifax County, N.S. : E.coli was isolated from a sample of Cole Harbour clams, and from samples of shucked clam meats in two shucking plants at Ostrea Lake, but in all of these instances M.P.N. values were very low. Isolations from clams dug in Petpeswick and Musquodoboit Harbours, where no sewage pollution is apparent, were nearly all Aerobacter aerogenes.

Charlotte County, N.B., 1948. : Of the two hundred and sixty-eight cultures isolated in 1948 from Charlotte County clams and sea water, and classified according to their "IMVIC" reactions, one hundred and twenty-six (47 per cent) proved to be typical E.coli, ninety-seven (36.2 per cent) were typical A.aerogenes, and forty-five (16.8 per cent) were classified as intermediate types. No consistent correlation could be demonstrated between fecal streptococci numbers and the incidence of fecal and non-fecal coliform types in clams from areas subjected to various amounts of pollution, but it is possible that a more direct relationship exists between the incidence of E.coli and the fecal streptococci numbers in sea water.

TABLE XVI

RELATIONSHIP BETWEEN COLIFORM AND FECAL STREPTOCOCCI M.P.N.'S.

FOR CLAM SAMPLES, 1947

COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.
>16,000	>16,000	5,400	790	1,300	1,700	230	2,400
	11,000		270		1,300		210
	790		93		430		78
	790	3,500	9,200		310		78
	790		1,100		230		78
	270		330		78		
	270		330	1,100	2,400		45
	220		220		2,400		45
16,000	1,300		130		700		20
	790	2,400	2,300		700		0
	430		1,400		330		0
	78		790	790	1,500	180	430
9,200	9,200		390		1,100		
	5,400		270		330	170	0
	930		230		330	140	78
	90	2,200	330	700	330	130	1,400
	78		130	490	490		45
5,400	>16,000	1,700	1,300		45		
	5,400		1,300		45	110	230
	5,400		640		20	78	1,100
	1,700		490	330	490		45
	390		490		230		45
	230		430		220		
	230		330		140	20	0
					78		
			130		45	0	45

TABLE XVII

RELATIONSHIP BETWEEN COLIFORM AND FECAL STREPTOCOCCI M.P.N.'S.

FOR WATER SAMPLES, 1947

COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.	NO. of SPECI-MENS	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.	NO. of SPECI-MENS	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.	NO. of SPECI-MENS
>1600	2,400	2	49	0	4	7.8	4.5	1
	1,500	1	46	13	1		2	1
	20	2	33	4.5	1		0	5
540	40	1		0	2	4.5	13	1
350	78	2	27	0	1		2	1
	0	1	26	0	1		0	4
240	30	1	23	4.9	1	2	4.5	1
	0	3		4.5	1		0	9
170	45	1		0	1	0	68	1
130	0	1	21	13	1		4.5	1
79	14	1	13	4.5	1		3.7	1
	4.5	1		2	1		2	1
70	33	1		0	5		0	11
49	20	1	11	0	2			

TABLE XVIII

Relationship Between Coliform and Fecal Streptococci M.P.N.'s from Water Samples, and Sanitary Survey Evaluation. 1948.

SANITARY SURVEY	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.	# of SPECIMENS
	240	0	1
GOOD:	70	11	1
	33	2	1
SEWAGE	27	2	1
POLLUTION	26	0	1
REMOTE	23	0	1
	11	0	2
	7.8	0	4
	6.8	4.5	1
		2	1
	4.5	2	3
		0	3
	2	0	2
	0	0	8

SANITARY SURVEY	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.	# of SPECIMENS
FAIR:	280	43	1
	240	13	1
	170	0	1
SLIGHT	130	13	1
SEWAGE	79	13	1
POLLUTION		4.5	1
	49	4.5	1
	31	78	1
	22	49	1
		0	1
	17	0	3
	6.8	0	1
	4.5	0	1
	3.7	0	1
	2	0	1
	0	0	2

SANITARY SURVEY	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.	# of SPECIMENS
POOR:	9,200	1,100	1
		920	1
DIRECT	3,500	330	1
AND	2,400	230	1
HEAVY	>1,600	>1,600	1
SEWAGE		11	1
POLLUTION		4.5	1
	1,600	540	1
	920	110	1
		23	1
	540	350	1
	240	79	1
	170	49	1
		13	1
	130	23	2
		0	1
	110	3	1
		0	1
	22	4.5	1
	13	0	1
	4	7.8	1

TABLE XIX

Relationship Between Coliform and Fecal Streptococci M.P.N.'s from Clam Samples, and Sanitary Survey Evaluation, 1948

SANITARY SURVEY	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.	# of SPECIMENS
GOOD: SEWAGE POLLUTION REMOTE	1,300	78	1
	790	130	1
		78	2
	490	130	1
		110	1
		45	1
	460	130	1
		0	1
	330	110	1
		78	3
		20	1
	230	430	1
		230	1
		130	1
		68	1
		39	1
		20	1
		0	2
	170	45	1
		0	1
	130	78	1
		68	1
		20	1
	110	0	1
	78	20	2
		0	3
	68	0	1
	45	45	2
		20	2
		0	4
	0	0	3
FAIR: SLIGHT SEWAGE POLLUTION	5,400	390	1
	2,400	700	1
		230	1
	1,700	430	1
		330	1
	1,400	170	1
	1,300	230	1
		82	1
	1,100	330	1
	790	330	1
		45	1
	700	430	1
		110	2
	490	230	1
	330	140	1
		45	1
		20	1
	78	78	1
	68	45	1
POOR: DIRECT AND HEAVY DEWAGE POLLUTION	92,000	24,000	1
	>16,000	>16,000	1
		2,100	1
		790	1
	16,000	16,000	1
		9,200	1
	9,200	5,400	1
		2,400	1
	5,400	1,300	1
		700	1
	2,400	700	1
	1,700	230	2
	1,300	110	1
	1,100	790	1
	790	230	1
	330	430	2
		130	1
		45	1
	230	230	1
		68	1

Relationship Between Coliform and Fecal Streptococci Most Probable Numbers from Clam and Water Samples, and Their Relationship to the Sanitary Quality of the Producing Area.

During September and October, 1947, and during the whole of the 1948 investigation, tests for both coliform bacteria and fecal streptococci were carried out on all water and clam samples analysed; comparative M.P.N. values obtained, in relation to the sanitary quality of the producing area as determined by Sanitary Engineering methods of evaluation, appear in Tables XVI, XVII, XVIII, and XIX.

Observations : During the 1947 survey, little correlation between coliform and fecal streptococci M.P.N. values for clams and water could be shown. A much closer correlation between coliform and fecal streptococci values and the sanitary quality of the producing area was observed during the 1948 investigations; while the fecal streptococci content of both clams and sea water was markedly lower than their coliform content, in most cases both indices gave a fairly accurate evaluation of the sanitary quality of the producing areas. Where Sanitary Engineering Surveys have shown either absence of sewage pollution, or heavy and direct sewage pollution, coliform and fecal streptococci numbers were, with four or five exceptions, in close agreement; in areas where slight sewage contamination was known to be present, the two indices showed fair agreement. It would seem necessary to investigate the few exceptions, because it is possible that one of the two indices may be more efficient in assessing the potential hazard

of a pollution source not noted during Sanitary Engineering Surveys; there was, however, no opportunity of studying these exceptions to elucidate the value of either index. Both the coliform and fecal streptococci indices would appear to be significant only when water temperatures over the clam flats remain relatively low.

It is possible that 1. the survival time of the enterococci in sea water more closely approximates that of the enteric pathogens than that of the coliform group, and 2. the enterococci may not multiply to as great an extent within the clam as coliforms do during seasonal periods of high water temperatures. Until the truth or fallacy of these postulations can be definitely proven, however, the test for fecal streptococci as an index of pollution for soft shell clams would seem to have little advantage over the coliform index.

Investigation of Some Aspects of the Commercial Packing of Clam Meats.

During the 1947 and 1948 studies, twelve plants packing shucked clam meats for export were inspected, and samples of shellstock clams, wash water, and clam meats both before and after washing and packing, were obtained for bacteriological examination. In 1947, when high water temperatures were general, a large percentage of the shellstock clams processed by the shucking plants gave coliform M.P.N. values far in excess of the 2,400 per 100 ml. limit suggested by the United States Public Health Service; in most cases, plant practices did nothing to reduce this heavy load

of coliform organisms, and, in some cases, increased it. It was therefore apparent that some procedure which would effectively reduce the fecal bacterial content of shucked soft shell clams was necessary before all clams, from areas considered free from dangerous pollution, could meet bacteriological export requirements, especially during the seasonal period of high water temperatures.

The Bactericidal Effect of Dipping Shellstock Clams in Boiling Water for Short Periods of Time.

Several of the clam shucking plants inspected during the surveys followed the practice of dipping the washed shellstock clams in cauldrons of boiling or near-boiling water for periods up to twenty seconds. Following this treatment, the "shocked" clams are cooled by immersion in cold, fresh water, and shucked in the usual manner. This treatment causes the adductor muscle to relax, and the mantle becomes soft and loose, facilitating easy and speedy shucking.

The process was not carried out with any idea of reducing bacterial numbers; it was noticed, however, that meats from clams treated in this manner showed a marked reduction in the numbers of coliform bacteria and fecal streptococci. Samples of (1) shellstock, and (2) the same shellstock after the heat treatment, were obtained wherever possible for testing. (Table XX).

Experiments were conducted during February, 1948, using highly polluted clams from St. Andrews Harbour, N.B., which were shipped to the Laboratory of Hygiene, Ottawa, for testing. (Table XXI).

TABLE XX

Reduction in Fecal Bacterial Content of Soft Shell Clams Caused by Commercial "Shocking" Process, 1947

Source	Specimen	Treatment	Coliform M.P.N.	Fecal Streptococci M.P.N.
Jarvis & Co. St. Andrews, N.B.	Clams (washed)	nil	1,100	2,400
	Shucked meats	"shocked" 30 sec. at 180°F, cooled, shucked.	230	130
Chamcook Packers, Chamcook, N.B.	Clams (washed)	nil	1,300	1,700
	Shucked meats	"shocked" 15 sec. at 190°F, cooled, shucked.	330	260
A.L. Baker & Son, Yarmouth, N.S.	Clams (washed)	nil	1,100	330
	Shucked meats	"shocked" 20 sec. at 190°F, cooled, shucked.	140 230	78 45
Northern Food Products, Grand Desert, N.S.	Clams (washed)	nil	9,200	780
	Shucked meats	"shocked" 30 sec. at 190°F, cooled, shucked.	110	230
Baker & Co., Ostrea Lake, N.S.	Clams (washed)	nil	490	45
	Shucked meats	"shocked" 30 sec. at 180°F, cooled, shucked.	20	0
General Seafoods, Ltd., Ostrea Lake, N.S.	Clams (washed)	nil	1,300	78
	Shucked meats	"shocked" 30 sec. at 180°F, cooled, shucked.	230	210
St. Andrews Harbour Station #4	Clams (washed)	nil	5,400	3,500
	Shucked meats	"shocked" 20 sec. (190°F) under lab. conditions.	790	330
St. Andrews Harbour Station #8	Clams (washed)	nil	5,400	3,500
	Shucked	"shocked" 20 sec. (190°F) under lab. CONDITIONS.	330	230

During the 1948 summer investigations, nine similar experiments were conducted in the mobile laboratory, using clams from areas subjected to varying degrees of sewage pollution. (Table XXII (a).

Two experiments were conducted at the shucking plant of Chamcook Packers, Chamcook, N.B. In the first experiment, duplicate baskets of shellstock clams from Oak Bay were dipped in water held at 99°C. (1) for the usual three second dipping period, and (2) for a longer, ten second heat treatment; both lots were then shucked, washed, and packed in the usual commercial manner by the plant operators, duplicate samples of each withdrawn for immediate testing, and the remainder refrigerated for seven days. Duplicate samples were withdrawn at intervals throughout this period for testing. Results obtained from this experiment, and those from a second similar experiment, in which Oak Bay clams were "dipped" three seconds in water held at 99°C. before shucking, washing, packing, and refrigeration, appear in Table XXII (b).

Experiments Conducted at the Laboratory of Hygiene, Ottawa, Ont., February, 1948.

One half-bushel of clams was packed in rockweed and shipped to the Laboratory of Hygiene, Ottawa; clams were dug from the St. Andrews Harbour, N.B. flats on February 13, and tests were conducted on February 17.

Three samples were analysed in the usual manner, and six additional samples were heated in a 99°C. waterbath for periods ranging from ten to sixty seconds.

TABLE XXII(a)

Reduction in Fecal Bacterial Content of Soft Shell Clams Caused by the "Shocking" Process 1948.

Source	Treatment		Coliform M.P.N.	Fecal Streptococci M.P.N.
St. Andrews Harbour Station #4	Nil		1,300	790
	"shocked"	10 seconds at 99°C	330	45
	"	20 " " "	330	20
	"	30 " " "	330	20
St. Andrews Harbour Station #4	Nil		>16,000	790
	"shocked"	10 seconds at 95°C	2,400	230
	"	20 " " "	2,400	330
	"	30 " " "	1,300	330
Bar Road, (Chamcook)	Nil		490	230
	"shocked"	5 seconds at 99°C	230	40
	"	10 " " "	78	68
St. Andrews Harbour Station #4	Nil		14,000	16,000
	"shocked"	5 seconds at 99°C	1,300	1,300
	"	10 " " "	1,300	490
Chamcook Packers, Chamcook, N.B.	Nil		4,600	2,400
	"shocked"	3 seconds at 99°C	220	230
	"	10 " " "	130	170
St. Andrews Harbour, Station #4	Nil		9,200	2,400
	"shocked"	3 seconds at 97°C	330	230
	"	10 " " "	230	230
St. Andrews Harbour Station #2	Nil		2,400	700
	"shocked"	3 seconds at 97°C	790	230
	"	10 " " "	330	78
Bar Road, (Chamcook)	Nil		490	45
	"shocked"	3 seconds at 99°C	330	20
	"	10 " " "	230	20
Oak Bay, (Cookson's Island)	Nil		330	20
	"shocked"	3 seconds at 99°C	230	20
	"	10 " " "	78	0

TABLE XXII(b)

Refrigeration of "Shocked" Clam Meats.

Chamcook Packers, Chamcook, N.B.

SPECIMEN	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.
Shellstock (Oak Bay) - "meats" only	4,600	2,400
Above, "Shocked" 3 seconds at 99°C.	220	230
As Above: Refrigerated 24 hours	790	140
" " : " 48 hours	1,100	110
" " : " 72 hours	790	230
" " : " 96 hours	93	140
" " : " 144 hours	220	490
" " : " 168 hours	45	120
As Above: "Shocked" 10 seconds at 99°C.	130	170
As Above: Refrigerated 24 hours	490	170
" " : " 48 hours	790	110
" " : " 72 hours	1,700	210
" " : " 96 hours	45	220
" " : " 144 hours	230	140
" " : " 168 hours	68	160
Shellstock (Oak Bay) - "meats" only	1,700	330
Above, "Shocked" 3 seconds at 99°C.	110	78
As Above: Refrigerated 24 hours	230	110
" " : " 48 hours	330	210
" " : " 72 hours	230	78
" " : " 96 hours	230	110

Table XXI (a)

Specimen	Treatment	Coliform M.P.N.
Shellstock	As on arrival	>16,000
Shellstock	As on arrival	>16,000
Shellstock	Stored one week in refrigerator	70,000
Clam meats	"Shocked" 10 seconds at 99°C.	2,400
Clam meats	"Shocked" 15 seconds at 99°C.	2,400
Clam meats	"Shocked" 20 seconds at 99°C.	2,400
Clam meats	"Shocked" 30 seconds at 99°C.	1,700
Clam meats	"Shocked" 45 seconds at 99°C.	2,400
Clam meats	"Shocked" 60 seconds at 99°C.	2,400

Obviously the reduction in coliform numbers is significant in the "shocking" treatment at 99°C. The reduction after thirty to sixty seconds heat treatment does not appear to be significantly greater than after ten to twenty seconds "shocking" at 99°C.

The blended clam slurry and decimal dilutions were streaked on plates of McConkey's agar, Bismuth Sulphite agar, and S.S. agar, and inoculated into Tetrathionate broth, for incubation at 37°C. After 24 hours incubation, the Tetrathionate enrichment cultures were streaked on the same agar media. All colonies thought to be non-lactose-fermenters were picked off the plates for inoculation into beef heart infusion broth. After 24 hours incubation at 37°C., transfers were made to Tryptone Glucose

Extract agar plates. After 24 hours incubation at 37°C., discrete colonies were picked to Tryptone Glucose Extract agar slopes. The resulting cultures were used to inoculate the various "IMVIC" and carbohydrate media necessary for classifying the organisms isolated.

No Salmonellae were isolated. Ten cultures proved, culturally and biochemically, to be Shigella types, five were Alkaligenes faecalis, and two were Proteus morgani.

One half-bushel of clams from the Pottery Bridge flats, one-quarter mile west of Niger Reef, St. Andrews, N.B., were shipped to Ottawa on February 24, 1948. Tests were conducted on February 27; two samples were tested immediately, while six additional samples were heated in a 99°C. water bath for 10 to 60 seconds.

Table XXI (b)

Treatment	Coliform M.P.N.	Fecal Streptococci M.P.N.
As on arrival (Duplicates)	2,400	230
As on arrival (Duplicates)	2,400	230
Shocked 10 seconds at 99°C.	230	45
Shocked 15 seconds at 99°C.	78	78
Shocked 20 seconds at 99°C.	130	20
Shocked 30 seconds at 99°C.	130	20
Shocked 45 seconds at 99°C.	130	0
Shocked 60 seconds at 99°C.	45	45

Effect of the "Shock" Treatment on Salmonella typhosa.

Duplicate samples of clams were thoroughly washed and dried, and each clam inoculated by injection with hypodermic syringe of .25 ml. of a twenty-hour culture of Salmonella typhosa in beef heart infusion broth. Twelve inoculated clams were then shucked aseptically into a sterile Waring blendor, an equal amount of sterile one per cent saline added, and blended. A second sample of twelve inoculated clams was dipped for 30 seconds in a 99°C. water bath, and tested in the same manner as the unheated sample. Serial dilutions of the resulting slurries were plated on Bismuth Sulphite agar in .5, .1, .01, .001, .0001, .00001, and .000001 dilutions; incubation was at 37°C., for 48 hours.

Table XXI (c)

Treatment	Numbers of Sal. typhosa per ml. on Bismuth Sulphite agar.
Inoculated; shucked and tested immediately	2,300,000
Inoculated; "shocked" 30 seconds at 99°C. before shucking and testing.	410,000

Observations : The "shock" process caused an apparent 82.2 per cent reduction of the viable Sal. typhosa organisms injected into the clams. Whether this method of inoculation would give the same results as normal contamination through the branchial siphon is questionable, and further experimentation is certainly necessary.

In all of the experiments reported, a short-term dipping of the shellstock clams in near-boiling water resulted in a reduction of coliform and fecal streptococci numbers. A more marked reduction was effected when the original fecal bacterial content of the clams was high than when the original M.P.N. values was very low. A very short period of immersion in the hot water is effective; the reduction obtained after three to ten seconds immersion does not appear to be significantly less than that recorded after longer periods of heat treatment.

The commercial three second dipping procedure caused a significant reduction in fecal bacterial content; the reduction in M.P.N. values when clams were dipped in water held at 99 C. for ten seconds was not significantly greater than that observed after the three second dipping period. There was no significant increase in the M.P.N. values in "shocked" clam meats during the seven day refrigeration period.

The short period of immersion in hot water involved in this process does not seem to impair the keeping qualities of the packed, refrigerated clam meats. Sterilization of the shells by the "shocking" process also reduces the amount of surface contamination carried to the shucking tables on the shellstock clams. The treatment also facilitates shucking, apparently without materially affecting the palatability of the product, and for this reason is already in use in some Canadian shucking plants.

If a satisfactory procedure, which could in some manner express the results of Sanitary Engineering surveys and bacteriological tests in terms of potential public health hazard, could be devised, the clam problem which we see today would not exist. Such is not the case, and it is apparent from our studies that the simple determination of the coliform index may not be related at all times to real public health problems for this product. Therefore it would appear that a treatment somewhat similar to the pasteurization of milk, when used for clams taken from areas which are deemed safe according to Sanitary Engineering standards of evaluation, would provide additional factors of safety. The problem resolves itself into one of how it should be applied, and what precautions should be enforced in the shucking plants to make this process a practical one.

Bacteriological Analysis of Clam Meats and Clam Liquors.

Duplicate samples of clams from various sources were (1) shucked and tested in the usual manner, and (2) shucked, and the shell liquor drained off aseptically to a sterile bottle; then both liquors and drained meats were tested in the usual manner for coliform and fecal streptococci content. Results obtained from eight such tests appear in Table XXIII.

Observations : In four cases, the clam liquors proved to be entirely free from fecal bacteria, and in all cases M.P.N. values obtained for shell liquor were markedly lower than those for whole clams and drained meats. M.P.N.'s for drained clam meats were, in all cases, considerably higher than for whole clams. It is thus

TABLE XXIII

Bacteriological Analysis of Clam Meats and Clam Liquors.

SOURCE	SPECIMEN	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.
St. Andrews Hbr., Station # 4.	Sea Water	9,200	920
	Clams	>16,000	2,400
	Meats only	54,000	9,200
	Liquors only	9,200	330
Chamcook Packers Co. (Oak Bay clams)	Clams	2,400	1,100
	Meats only	4,600	2,400
	Liquors only	0	0
St. Andrews Hbr., Station # 4.	Sea Water	540	350
	Clams	5,400	3,500
	Meats only	>16,000	16,000
	Liquors only	1,700	490
Bar Road, Chamcook.	Clams	330	45
	Meats only	490	230
	Liquors only	0	0
Bar Road, Chamcook.	Clams	330	78
	Meats only	1,300	270
	Liquors only	0	0
Pottery Bridge, St. Andrews.	Sea Water	22	4
	Clams	230	78
	Meats only	490	230
	Liquors only	45	20
Oak Bay, West Cove.	Sea Water	17	0
	Clams	700	110
	Meats only	2,400	270
	Liquors only	20	0

TABLE XXIV

First Flushing Experiment.

St. Andrews Harbour (Station #4) Clams Held in Biological Station Tank.

(June 14 - June 26, 1948)

HOURS IN TANK	COLIFORM M.P.N.	FECAL STREPTOCOCCI M.P.N.
0	3,500	3,500
24	1,700	1,400
31	3,500	1,100
48	1,300	230
72	790	230
79	1,700	790
96	790	110
103	490	78
120	330	78
144	330	45
168	700	20
175	330	20
192	790	45
199	790	20
216	130	40
223	230	0
240	130	0
247	130	0
264	230	0
288	230	0

Samples of sea water flowing through the tank were withdrawn at intervals and tested for fecal bacterial content.

Observations : The clams appeared to be feeding in a normal manner during the holding period in the tank; their siphons protruded, and fecal strings were thrown out in large quantities.

In the first experiment, a considerable reduction in both coliform and fecal streptococci numbers was noted during the holding period; the reduction of fecal streptococci numbers was more decisive. The reduction in coliform M.P.N.'s recorded during the second experiment was less significant, probably because the "flushing" water was more heavily contaminated with coliform organisms during this period. Fecal streptococci M.P.N.'s were markedly reduced, as in the first experiment; no fecal streptococci were isolated from the "flushing" water at any time.

DISCUSSION

The 1947 study of the bacteriology of Mya arenaria in Charlotte County, N.B., indicated that a serious public health problem may exist. The fecal bacterial content of soft shell clams reached alarming proportions in most of the Charlotte County areas in June and July, 1947; this increase in coliform numbers was in no way related to the Sanitary Engineering evaluation of the sanitary quality of the areas concerned, and no relationship between the source of water and the bacterial contamination of clams could be demonstrated. In October, 1947, however, a considerable reduction in coliform numbers was observed; it was evident that the bacterial flora of Mya is subject to seasonal fluctuations induced by changing hydrographic or meteorological conditions, and that the Standard Methods test for coliform bacteria may not accurately evaluate the sanitary quality of a producing area at all times.

Similar investigations carried out in Charlotte County in 1948 indicate that there is a marked year-to-year variation in the effect of seasonal conditions influencing the fecal bacterial content of soft shell clams; coliform and fecal streptococci M.P.N. values remained relatively low during June and July, 1948, and a general increase in the fecal bacterial content of clams did not occur until late August.

It is considered probable that there is a close relationship between water temperature and the coliform content of soft shell clams. Water temperatures recorded at St. Andrew's during May to November, 1948, were consistently lower than those for the same period in 1947; as long as the temperature of the water over the clam flats remained low, coliform M.P.N. values were a significant index of sewage contamination, but when water temperatures reached a critical "threshold" level, multiplication of coliform bacteria within the clams occurred. The resultant increase in coliform numbers was without sanitary significance, since it could occur in the absence of further sewage pollution. Studies by Hachey and McLellan (1948) at St. Andrews, N.B., indicate the possibility that there is a cycle including approximately four years of sub-normal water temperatures and four years of supra-normal water temperatures. During years when supra-normal water temperatures are general, it is considered probable that an increase in coliform numbers similar to that observed in 1947 will occur in soft shell clams. The coliform and fecal streptococci indices for soft shell clams would appear to be significant only when water temperatures remain relatively low.

The value of the fecal streptococcal test is estimated variously by different workers. Some regard it as of considerable value, whereas others are of the opinion that it adds little to the information yielded by the test for coliform bacteria.

It is not known definitely whether fecal streptococci survive on the average for a shorter or longer time in water than coliform bacteria; until this is known, the interpretation of the results of the fecal streptococcal test, particularly when negative, must remain doubtful.

During the present investigations, little advantage was seen in the test for fecal streptococci as an index of pollution, although fair correlation was obtained in 1948 between coliform and fecal streptococci numbers and the known sanitary condition of the water. No correlation was obtained either in 1947 or 1948 between fecal streptococci and the incidence of so-called fecal and non-fecal coliform types in soft shell clams.

Attempts were made to study the nature of coliform bacteria isolated during the investigations; six hundred and forty coliform cultures were isolated and classified according to their "IMVIC" reactions. While areas known to be heavily polluted by fecal material showed a high incidence of Escherichia coli, no absolute relationship between the source of water and the type of coliform contamination of the clam could be demonstrated. There is little or no evidence that coliform bacteria multiply on fresh grasses or grains, or in soil. On the other hand, all types of coliform bacteria may occur in feces. Since coliform bacteria are constantly present in the intestine, usually in numbers greatly exceeding those of pathogenic intestinal bacilli, and since their death rate in sea water is slower than that of

organisms of the enteric group, it follows that whenever typhoid or paratyphoid bacilli, for example, gain access to a clam producing area through excretal pollution, they are always accompanied by the natural organisms inhabiting the intestine. It may be concluded that the finding of coliform bacteria, however atypical, in water or clams, shows that more or less recent excretal pollution has probably occurred; this, though not constituting in itself conclusive evidence of danger, is nevertheless sufficient to indicate that the shellfish may be potentially dangerous. It is therefore believed that, when water temperatures in the producing areas remain low, the Standard Methods test for the presence of coliform bacteria will serve as a reliable index of sewage contamination of sea water and soft shell clams. Much more study of a qualitative character is needed before the practical value of routine differentiation in the coliform group can be demonstrated.

It is apparent that some procedure which would effectively reduce the fecal bacterial content of shucked soft shell clams is necessary before all clams from areas considered free from all dangerous pollution could meet bacteriological export requirements, especially during the seasonal period of high water temperatures. Two methods of reducing the bacterial content of soft shell clams were studied during the present investigation. Of particular interest is the hot dipping process already in use in some Canadian shucking plants. Wire baskets of soft shell

clams are immersed in scalding water at a temperature of 180°F. or higher for three to ten seconds. The heat causes a separation of the membranes covering the external surfaces of the mantle and siphons, rendering the shucking and cleaning much easier; this was the primary intent of the dipping process. Control experiments at 99°C. demonstrated a reduction of as much as ninety per cent of the coliform M.P.N. of clams. The percentage reduction increased as the coliform numbers of the shellstock clams increased.

The mode of action of this reduction in bacterial content is difficult to explain, particularly after considering experiments which demonstrate that the greatest concentration of bacteria lies in the body of the clam, the bacterial concentration of the shell liquor being of the same order as the surrounding sea water, and that the penetration of heat is not deep into the clams, since they are still alive after dipping. The most reasonable explanation is that it is a "shocking" process, causing sudden discharge of some of the intestinal contents out into the hot water. Further work is necessary to establish the validity of this theory.

Many problems of control present themselves. There may be some difficulty in maintaining a constant scalding temperature in the dipping tanks used by the small shucking plants. It is possible that clams from heavily polluted areas might be passed through the process; this brings up the question of comparative

ratios of coliforms to pathogens at various levels of pollution, and, until further knowledge is obtained on this question, the advisability of using the process for the cleansing of all soft shell clams is certainly open to question. Although the hot water in the dipping tanks has always been found to be free from bacterial contamination, there is an accumulation of mud and other materials which might be defined as filth; this has particular significance in the regulations of the United States Food and Drug Administration. There is also some evidence which indicates that clams so treated are rendered more susceptible to spoilage because of a breakdown in their external surfaces.

In spite of such possible imperfections, it is believed that the hot water dipping process may be of great value during seasonal periods of high water temperatures. If a satisfactory procedure, which could in some manner express the results of Sanitary Engineering surveys and bacteriological tests in terms of potential public health hazard, could be devised, the clam problem which we see today would not exist. Such is not the case, and it is apparent from our studies that the simple determination of coliform bacteria may not be related at all times to real public health problems for this product. Thus it would appear that the "shock" treatment, when used exclusively for clams from areas deemed safe according to Sanitary Engineering standards of evaluation, would provide additional factors of safety.

SUMMARY

1. A general bacteriological survey of the major soft shell clam producing areas in Charlotte County, N.B., and Digby, Annapolis, and Halifax Counties, N.S. was conducted during June to October, 1947.

2. A periodic bacteriological survey of clams and sea water from twelve key stations in Charlotte County, N.B. was conducted during the summer of 1948. Results obtained during this period differed very markedly from those recorded during the 1947 survey. Coliform M.P.N.'s recorded during the 1947 survey reached a maximum early in July and remained at a high level throughout the summer; in 1948, however, coliform M.P.N. values remained relatively low throughout the summer survey period, although indications were that a general increase in the fecal bacterial content of clams occurred in late August.

It is considered probable that a close relationship exists between water temperature and the fecal bacterial content of soft shell clams. As long as water temperatures remain low, coliform M.P.N. values obtained from clams will be an accurate index of pollution reaching the flats, but when the temperature of water over these flats reaches a critical threshold level, multiplication of fecal bacteria within the clam may occur; the resultant increase in coliform numbers would not be of sanitary significance, since it could occur in the absence of further sewage pollution.

3. Studies were conducted to determine whether the bacterial

numbers in mud and silt bore any relationship to bacterial numbers found in clams. It was impossible to demonstrate any close relationship.

4. During the 1947 survey, little correlation between coliform and fecal streptococci M.P.N. values for clams and water could be shown; in 1948, however, better agreement between the two indices of sewage pollution and the sanitary quality of the producing areas was noted. Both the coliform and fecal streptococci indices would appear to be significant only when water temperatures remain relatively low. From data presently at hand, the test for fecal streptococci as an index of pollution of soft shell clams would seem to have little advantage over the coliform index.

5. Attempts were made to study the nature of the coliform bacteria isolated during the surveys, and to relate them to other indices of fecal pollution; 640 isolated colonies were classified according to their "IMVIC" reactions. There was, however, no consistent correlation between the fecal streptococci M.P.N.'s and the incidence of fecal and non-fecal coliform types in clams.

6. Malpeque Bay, P.E.I., and the islands of Bocabec Bay, N.B. were found to be unsuitable for the study of soft shell clams entirely removed from any possibility of direct fecal contamination; these supposedly "clean" areas were not entirely free from bacteria of fecal origin. It is evident that Sanitary Engineering surveys alone are inadequate in assessing the sanitary quality of a producing area.

7. It was discovered that a very large proportion of the fecal

bacteria found in the soft shell clam are concentrated in the meats, and that coliform and fecal streptococci M.P.N. values for clam liquor closely approximate those of the sea water over the clam flats. The clam liquor therefore has a diluting effect when shell clams are analysed for bacterial content; since no shell fluid is present when commercially packed clam meats are tested, it might be advisable to test only drained meats in all cases where shellstock clams are analysed bacteriologically.

8. Results of experiments carried out during the present investigations would indicate that the "shock" treatment of clams by dipping in near-boiling water for 3 to 10 seconds before shucking appears to cause a marked reduction in coliform and fecal streptococci numbers. The short period of immersion in hot water involved in the process does not seem to impair the keeping qualities of the packed refrigerated clam meats.

It would appear that this treatment, when used for clams taken from areas which are deemed safe according to Sanitary Engineering standards of evaluation, would provide additional factors of safety. The problem resolves itself into one of how it should be applied, and what precautions should be enforced in shucking plants to make this process a practical one.

9. A considerable reduction in the fecal bacterial content of clams was obtained by flushing in large tanks with a continuous flow of sea water. It is possible that a modification of this method of self-purification of moderately polluted soft shell clams may be of commercial value.

REFERENCES

(1) Anonymous.

1949. First typhoid case in seven years. Jour. Amer. Med. Assoc., 139: 317.

(2) Alder, J., and Hancock, A.

1851. On the branchial currents in Pholas and Myas. Ann. Mag. Nat. Hist., 8: second series.

(3) Bardsley, D. A.

1934. The distribution and sanitary significance of B. coli, B. lactis aerogenes, and intermediate types of coliform bacilli in water, soil, feces and ice cream. Jour. Hyg., 34: 38-43.

(4) Beard, P. J., and Meadowcroft, N. F.

1935. Survival and rate of death of intestinal bacteria in sea water. Amer. Jour. Pub. Health, 25: 1023-1026.

(5) Belding, D. L.

1930. The soft shell clam fishery of Massachusetts. Commonwealth of Massachusetts, Dept. Conservation Bull., 1-65.

(6) Bigger, J. W.

1937. The growth of coliform bacilli in water. Jour. Path. and Bact., 44: 167.

(7) Brown, H. J., and Gibbons, N. E.

1949. Enterococci as an index of fecal contamination in egg products. Can. Jour. Pub. Health, 40: 30.

(8) Browne, W. W.

1917. The presence of B. coli and B. welchii groups in the intestinal tracts of fish. Jour. Bact., 2: 417-422.

(9) Buchbinder, L., Osler, A. G., and Steffen, G. I.

1948. Studies in enterococcal food poisoning. U.S. Pub. Health Repts., 63: 109-118.

(10) California State Bureau of Sanitary Engineering.

1943. Report on a pollution survey of Santa Monica beaches in 1942. Cal. State Printing Office, Sacramento.

(11) Carpenter, L. V., Setter, L. R., and Weinberg, M.

1938. Chloramine treatment of sea water. Amer. Jour. Pub. Health, 28: 929-934.

(12) Connecticut State Board of Health.

1927. An account of an outbreak of typhoid fever caused by the eating of raw clams. Ann. Rept. Conn. State Board of Health, p.128.

(13) Dangler, G., and Steffen, G. I.

1948. Studies on enterococcal food poisoning. Jour. Milk and Food Technol., 11: 242-243.

(14) Darby, C. W., and Mallmann, W. L.

1939. Studies on media for coliform organisms. Jour. Amer. Water Works Assoc., 31: 689.

(15) DeGiaxia, A.

1889. Ueber das Verhalten einiger pathogener Micro-organismen im Meerwasser. Zeitschr. f. Hyg., 6: 162-225.

(16) Dienert, F., and Guillerd, A.

1940. Etude de la pollution de l'eau de mer par le deversement des eaux d'egouts. Ann. d'Hyg. Pub. Ser., 5: 209-217.

(17) Dodgson, R. W.

1928. Report on mussel purification. His Majesty's Stationery Office, London.

(18) Dodgson, R. W.

1937. Shellfish and the Public Health. Pub. Health, 50: 279-284.

(19) Dodgson, R. W.

1938. Shellfish and the Public Health. Proc. Roy. Soc. Med., 31: 925-934.

(20) Eliot, C.

1926. Observations on the colon-aerogenes group from the oyster. Amer. Jour. Hyg., 6: 777-783.

(21) Frankland, P., and Frankland, A.

1894. Microorganisms in water. Longmans, Green, and Co., 532 p.

(22) Gard, S., and Nilsson, H.

1942. The classification of the coli-aerogenes group with special reference to the bacteriological analysis of water. Nordisk Hyg. Tidskr., 23: 199-227.

(23) Gibbons, N. E.

1934. Lactose fermenting bacteria from the intestinal contents of marine fish. Contr. Can. Biol. Fish. N.S., 8: 291-300.

(24) Green, C.E., and Beard, P.J.

1938. Survival of E. typhi in sewage treatment plant processes.
Amer. Jour. Pub. Health, 28: 762-770.

(25) Griffiths, F. P.

1937. A review of the bacteriology of fresh marine fishery products.
Food Research, 2: 121-134.

(26) Griffiths, F. P., and Fuller, J. E.

1936. Detection and significance of E. coli in commercial fish and fillets.
Amer. Jour. Pub. Health, 26: 259-264.

(27) Hachey, H. B., and McLellan, H. J.

1948. Trends and cycles in surface temperatures of the Canadian Atlantic.
Jour. Fish. Res. Bd., 7: 355-362.

(28) Hajna, A. A.

1935. Isolating typhoid bacilli in sewage.
Municipal Sanitation, 6: 234.

(29) Hajna, A. A., and Perry, C. A.

1943. Comparative study of presumptive and confirmative media for bacteria of the coliform group and for the streptococci.
Amer. Jour. Pub. Health, 33: 550-556.

(30) Harold, C. H. H.

1934. Twenty-ninth Ann. Rept. Met. Water Bd.
London.

(31) Heukelekian, H., and Schulhoff, H. B.

1935. Studies on survival of B. typhosus in surface waters and sewage.
New Jersey Agr. Exp. Sta. Bull., No. 589.

(32) Hoskins, J. K.

1933. The most probable numbers of B. coli in water analysis.
Jour. Amer. Water Works Assoc., 25: 867-877.

(33) Hoskins, J. K.

1934. Most probable numbers for the evaluation of coli-aerogenes tests by the fermentation tube method.
U.S. Pub. Health Repts., 49: 393-405.

(34) Houston, A. C.

1900. On the value of examinations of water for streptococci and staphylococci with a view to detection of its recent contamination with animal organic matter.
Supplement to the twenty-ninth Ann. Rept. of the Local Govt. Bd., containing the report of the Medical Officer for 1900, p.458.

(35) Hunter, A. C.

1939. Uses and limitations of the coliform group in sanitary control of food production.
Food Res., 4: 531-538.

(36) Hunter, A. C., and Harrison, C. W.

1928. Bacteriology and chemistry of oysters, with special reference to regulatory control and production, handling, and shipment.
U.S. Dept. Agr. Tech. Bull., 64: 1-75.

(37) Jordan, E. O.

1925. The viability of typhoid bacilli in shell oysters.
Jour. Amer. Med. Assoc., 84: 1402-1403.

(38) Kehr, R. W., and Butterfield, C. T.

1943. Notes on the relation between coliforms and enteric pathogens.
U.S. Pub. Health Repts., 58: 589-606.

(39) Knowlton, W. T.

1929. B. coli surveys, Los Angeles ocean outfall. California Sewage Works Jour., 2: 150-152.

(40) Krassilnikov, N. A.

1938. The bactericidal action of sea water. Mikrobiologiia, 7: 329-334.

(41) Krumweide, C., Park, W. H., Cooper, G., Grund, M., Tyler, C. H., and Rosenstein, C.

1926. Effect of storage and changing sea water on contaminated oysters. Amer. Jour. Pub. Health, 16: 263-268.

(42) Lawes, J. P., and Andrews, F. W.

1894. Report on the results of investigations of the microorganisms of sewage. Report to London County Council.

(43) Levine, M.

1921. Bacteria fermenting lactose and their significance in water analysis. Iowa State College of Agriculture and Mech. Arts. Bull., No. 62.

(44) Lloyd, B.

1930. Bacteria of the Clyde Sea area: a quantitative investigation. Jour. Marine Biol. Assoc., 16: 879-907.

(45) London Metropolitan Water Board.

1938. Annual reports on the results of chemical and bacteriological examination of the London waters, 1927-1938 incl.

(46) Loosanoff, V. L.

1946. Commercial clams of the Atlantic Coast of the U.S.A. U.S. Fish and Wildlife Service, Fishery Leaflet 13.

(47) Ludewig, E.

1949. City of New York, Department of Health. Personal communication.

(48) MacConkey, A.

1905. Lactose fermenting bacteria in feces. Jour. Hyg., 5: 333.

(49) MacConkey, A.

1909. Further observations on the differentiation of lactose fermenting bacteria, with special reference to those of intestinal origin. Jour. Hyg., 5: 86.

(50) Medcof, J. C., Leim, A. H., Needler, A. B., Needler, A.W.H., Gibbard, J., and Naubert, J.

1947. Paralytic shellfish poisoning on the Canadian Atlantic Coast. Fish. Res. Bd. of Can. Bull., No. 75.

(51) Mom, C. P., and Schaeffer, C. O.

1940. Typhoid bacteria in sewage and in sludge. Sewage Works Jour., 12: 715.

(52) Newcombe, C. L.

1935. Growth of Mya arenaria in the Bay of Fundy region. Can. Jour. Res., D. 13: 97-137.

(53) Osler, A. G., Buchbinder, L., and Steffen, G. I.

1948. Experimental enterococcal food poisoning in man. Proc. Soc. Exp. Biol. and Med., 67: 456-459.

(54) Ostrolenk, M., and Hunter, A. C.

1946. The distribution of enteric streptococci. Jour. Bact., 51: 6.

(55) Ostrolenk, M., Kramer, N., and Cleverdon, R. C.

1947. Comparative studies of enterococci and E. coli as indices of pollution. Jour. Bact., 53: 197.

(56) Parr, L. W.

1935. Sanitary significance of the succession of colon-aerogenes organisms in feces. Proc. Soc. Exp. Biol. and Med., 32: 580-583.

(57) Parr, L. W.

1938. Organisms involved in the pollution of water from long stored feces. Amer. Jour. Pub. Health, 28: 445-450.

(58) Payne, V.

1938. Comparison of modified Eijkman medium and standard lactose broth in the examination of oysters, clams, and shellfish waters. U.S. Pub. Health Repts., 56: 2058-2064.

(59) Perry, C.A.

1930. Thesis - Bacteriological examination of oysters. Johns Hopkins University, School of Hygiene and Pub. Health, 118.

(60) Perry, C. A.

1939. A summary of studies on pollution in shellfish. Food Res., 4: 381.

(61) Platt, A. E.

1935. The viability of B. coli and B. aerogenes in water. A method for the rapid enumeration of these organisms. Jour. Hyg., 35: 437-448.

(62) Prescott, S. C., and Winslow, C. E. A.

1931. Elements of water bacteriology. John Wiley and Sons, New York, 5th ed., p.219.

(63) Renn, C. E.

1946. Unpublished data.
(Harvard University)

(64) Ruchhoft, C. C.

1934. Studies on the longevity of B. typhosus in sewage sludge.
Sewage Works Jour., 6: 1054.

(65) Ruchhoft, C. C., Kallas, J. G., Chinn, B., and Coulter, E. W.

1931. Coli-aerogenes differentiation in water analysis; biochemically differential tests and their interpretation.
Jour. Bact., 22: 125-181.

(66) Sandholzer, L. A.

1942. A comparison of presumptive tests for coliform bacteria in sea water and shellfish.
Jour. Bact., 43: 47-48.

(67) Savage, W. G., and Wood, D. R.

1917. The vitality and viability of streptococci in water.
Jour. Hyg., 16: 227-239.

(68) Sherman, J. M.

1937. The streptococci.
Bact. Reviews, 1: 3.

(69) Soper, G. A.

1909. The discharge of sewage into tidal waters.
Jour. Amer. Med. Assoc., 52: 1221-1224.

(70) Stafford, J.

1901. The clam fishery of Passamaquoddy Bay.
Contrib. Can. Biol., 3: 19-40.

(79) Weston, A. D.

1938. Disposal of sewage into the Atlantic Ocean. Modern Sewage Disposal, Federation of Sewage Works Assoc., New York, 209-218.

(80) Weymouth, F. W.

1930. The edible clams, mussels, and scallops of California. State of Cal. Fish. Bull., No. 4.

(81) White, J. C., and Sherman, J. M.

1944. Occurrence of enterococci in milk. Jour. Bact., 48: 262.

(82) Wilson, G. S.

1935. Shellfish sanitation. Med. Res. Council, Special Report Series, No.206.

(83) Wilson, W. J.

1928. Isolation of B. typhosus from sewage and shellfish. Brit. Med. Jour., 1: 1061.

(84) Wilson, W. J.

1933. Isolation of enteric bacilli from sewage and water, and its bearing on epidemiology. Brit. Med. Jour., 2: 560.

(85) Wilson, W. J., and Blair, E. M.

1931. Further experience of the bismuth sulfite media in the isolation of B. typhosus and B. paratyphosus from feces, sewage, and water. Jour. Hyg., 31: 138-161.

86) Winslow, C. E. A., and Hunnewell, M. P.

1902. Streptococci characteristic of sewage and sewage polluted waters. Science N.S., 15: 827.

(87) Winter, C. E., and Sandholzer, L. A.

1946. Isolation of enterococci from natural sources.
Jour. Bact., 51: 27.

(88) Yonge, C. M.

1923. Mechanism of feeding, digestion, and assimilation in the lamellibranch Mya.
Brit. Jour. Exp. Biol., 1: 15-63.

(89) ZoBell, C. E.

1936. Bactericidal action of sea water.
Proc. Soc. Exp. Biol. Med., 34: 113-116.

(90) ZoBell, C. E.

1941. The occurrence of coliform bacteria in oceanic water.
Jour. Bact., 42: 248.

(91) ZoBell, C. E.

1946. Marine Microbiology.
Chronica Botanica Co., Waltham, Mass.
(Chapter XVl, p.182, Sanitary aspects)