THE CONTAMINATION OF OYSTERS.

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(With 1 chart.)

TOPOGRAPHY AND TIDES.

The Harbour of Poole lies a few miles to the West of Bournemouth. It has an area approximately of 10,000 acres, but there is only a very narrow passage between its waters and the open sea. The width of this bottle-neck entrance is about 250 yards. The last published Admiralty chart gives the rate of the incoming tide through this narrow entrance at $3\frac{1}{4}$ knots, and of the outgoing tide at 4 knots. The local opinion is that these official rates err on the moderate side. The rapid ebb and flow of the tides has an influence in the bacteriology of the oysters within the harbour.

The presence of the Isle of Wight to the eastward causes Poole Harbour and Bournemouth Bay to have four tides instead of the usual two. About three-quarters of an hour after the first high water the tide begins to ebb; but about $2\frac{3}{4}$ hours later it is high again. The first flood tide begins $4\frac{1}{2}$ hours before high water.

OYSTERS.

For many years past the oysters dredged from Poole Harbour and from Bournemouth Bay have been contaminated. The reports of various Medical Officers have associated these oysters with cases of Enteric Fever. One of the present writers has known of Enteric Fever which occurred after eating Poole or Bournemouth oysters: no other possible cause of the disease could be ascertained in these cases. Samples of oysters have been taken infrequently and at irregular intervals in the past and examined bacteriologically: they were found by various bacteriologists to be polluted, more or less heavily, with *B. coli*, and sometimes with *Streptococci* and *B. welchii* (Table I). At the present time Poole oysters cannot be dredged except for relaying, and this ban upon them is of considerable financial loss to the district.

THE PRESENT ENQUIRY.

Early in 1914 one of us (A.T.N.) was appointed to the post of Medical Officer of Health to the Borough and Port of Poole, and before the outbreak of war he had begun some research work on the subject of the pollution of the harbour water and of the oysters, hoping by this to settle once and for all, by a thorough investigation, many details concerning the oyster pollution, which had been unsatisfactorily dealt with in the past by other observers in a partial manner and unconvincingly. This earlier research was necessarily interrupted, but on his return to the Borough the investigations were continued. The enquiry was directed towards:

1. The source of pollution of the oysters.
2. The nature of the infecting micro-organisms.
3. The discovery, if possible, of a suitable relaying place; and
4. In the absence of this, the effect of sterilisation by chlorinated sea-water.

Towards the cost of this research the Borough Council of Poole made a contribution of five pounds, and the Southern Sea Fisheries Board a grant of a similar amount. All the work referred to in this paper was done at the Poole Borough Laboratory; and we should like here to express our appreciation of the help given to us by our Laboratory Attendant, S. Marshall, who prepared most of our culture media, and aided us generally in the work.

TECHNIQUE.

Samples both of sea-water and of minced oysters were plated in neutral-red-bile-salts-peptone-lactose-agar. Both single strength and double strength were used—the latter for the plates of 10 c.c. and upwards. Of the sea-water we plated varying quantities from 0·1 c.c. to 15·0 c.c.; and examined in MacConkey Lactose tubes quantities from 20 c.c. to 100 c.c. We should like here to insist that plate cultivation gives more accurate results than can be obtained by the use of liquid media. If a 20 c.c. tube shows acid and gas it cannot accurately be estimated exactly how many lactose fermenters were present: if, however, four plates each of 5 c.c. are made, then the number of lactose fermenters can be seen and correctly counted. Gradually, as the work progressed, we used more plates and fewer tubes. Apart from this, our technique was that usually employed in the examination of samples of drinking water, as far as the sea-water was concerned. When dealing with the oysters we cleansed the outsides of the shells by thorough scrubbing under running water. The oysters were then opened with a sterilised knife, and the contents finely minced before removal from the concave shell. Each oyster was then emulsified in 100 c.c. of sterile saline. In dealing with oysters which were presumably polluted, plates of 0·5 c.c., 1 c.c. and 5 c.c. of the emulsion were made. When examining oysters after relaying we used six plates each of 5 c.c. and two plates each of 10 c.c. Saline emulsion corresponding to half an oyster was therefore plated and examined. On several occasions we checked our plate results by inoculating a series of MacConkey Lactose tubes.

THE SOURCE OF POLLUTION OF THE OYSTERS.

The first oysters examined, taken from a bank just inside the harbour mouth, showed the presence of many lactose fermenters—about 700 per oyster, quite enough in our opinion to condemn them. (See Table I.) We had then to decide from what quarter this pollution came; did it originate within the harbour of Poole, or was it brought in from the outside?

Table I.

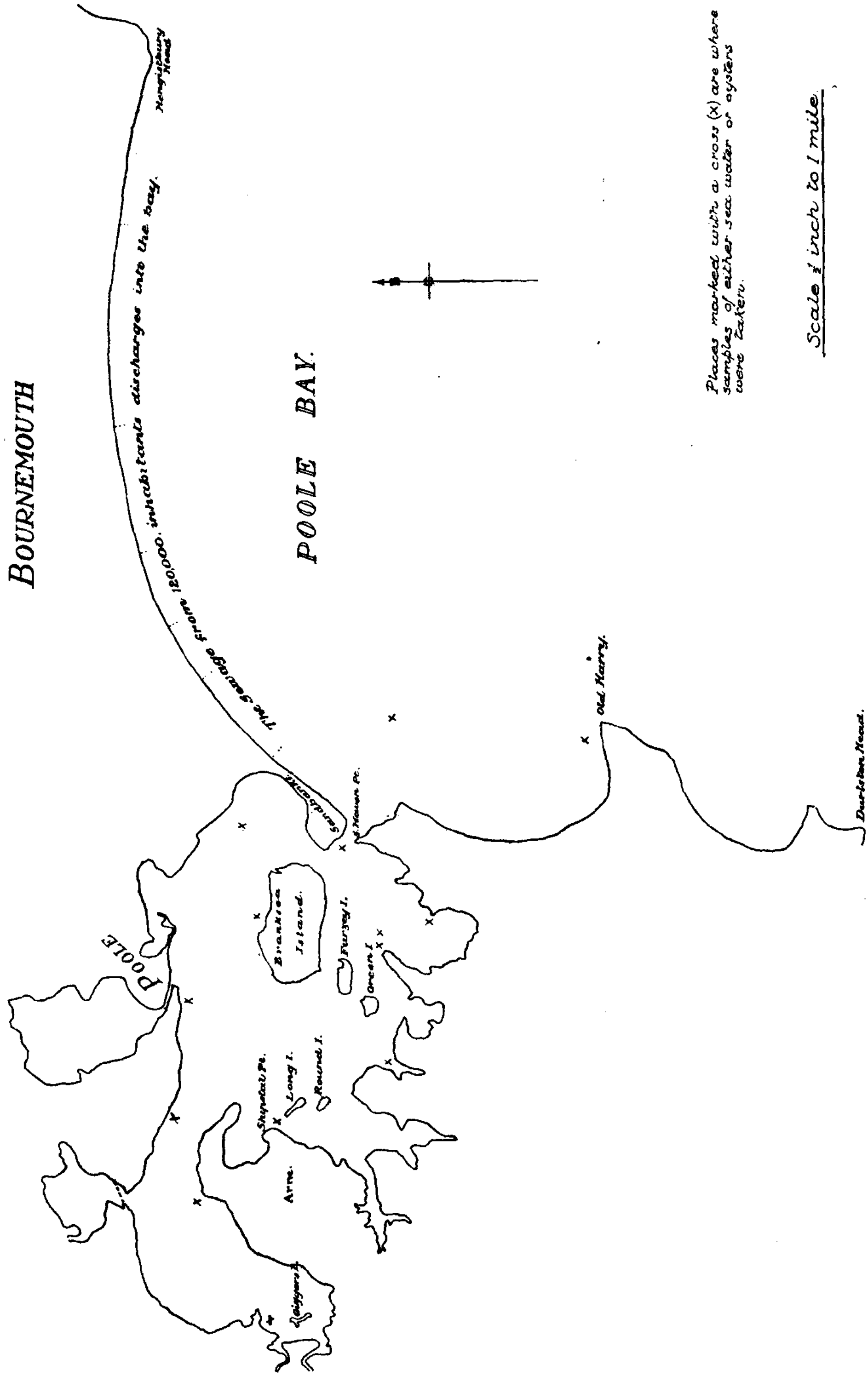
Poole and Bournemouth Bay Oysters, 1914–1919.

Date	Place	Bacteriological findings
May, 1914	Poole Harbour	700 lactose fermenters per oyster
June, 1914	Poole Harbour	*Streptococci* and *B. welchii* in $\frac{1}{100}$ of an oyster
June, 1914	Poole Harbour	1500 lactose fermenters per oyster
May, 1915	Poole Harbour	3000 lactose fermenters per oyster
Nov. 1915	Poole Harbour	*B. coli* and *streptococci* in $\frac{1}{5}$ c.c.
Sept. 1919	Poole Harbour	2400 lactose fermenters per oyster
Oct. 1919	Bournemouth Bay	1500 lactose fermenters per oyster

To settle this question we took half hourly samples of the water at the bottle-neck entrance to the harbour from Sandbanks Pier (see chart). The samples were taken throughout several tides. They were obtained in sterilised bottles from a depth of three feet below the surface by means of a specially designed plunger. The samples were sent by bicycle or motor-car to the laboratory four miles away, and were plated and tubed within a few hours of being taken. The first results were a surprise to us, and the experiment was therefore repeated over two other tides. The second series of plates however showed results which were generally constant—sufficiently so, at any rate, to prove without any doubt that the first incoming tide brought many lactose fermenters into the harbour; that on the second tide not so many were brought in, and that the water at outgoing tides was comparatively clean and free from micro-organisms (Table II). The pollution of the oyster beds therefore came in from the open sea; and not from the Town of Poole or from the rivers that flow into the top of the Harbour.

A knowledge of the local sewage outfalls and of the tides in the bay will explain how these faecal organisms can make their way in from the open sea. All the sewers of Bournemouth and of Poole discharge into the sea, and not into the harbour: the sewage is carried out in the ebb tide in the direction of the headland known as Old Harry. On arrival in this neighbourhood it is met by the strong incoming tide and pushed into Poole harbour. Subsidence of micro-organisms and certainly of macroscopical pieces of sewage takes place here, and this explains the finding that the water leaving the harbour is cleaner than that which comes in.

Having satisfied ourselves by these water samples, taken at Sandbanks, that the pollution came in on the first high tide, we next proceeded to ascertain how the water in different parts of the harbour was affected. We followed up



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the Main Channel taking samples at Salterns, at Hamworthy and off Russell Quay during different stages of the tide: these were infected, but, speaking generally, not so heavily as the water at Sandbanks. We found the same in the South Channel at Redhorn and at Goathorn, and in the Wych Channel opposite the Ower (Table II).

At these places we did not repeat our observations over several days, nor take so many samples—there seemed to be no need since none of the results were at variance with our earlier observations at Sandbanks.

IDENTITY OF MICRO-ORGANISMS.

We were not able to make a prolonged and thorough investigation of the lactose fermenting organisms which we isolated from the sea-water and from oysters. One of us (J.M.S.) had only four months to spend on non-military employment in this country before his return to Australia, and it was possible therefore systematically to examine only a small proportion of the organisms

Table II.

Showing number of lactose fermenting organisms in 10 c.c. of the sea-water at various places at different stages of the tide.

State of tide	Hours after low water	Sandbanks Pier	Sandbanks Pier	Sandbanks Pier	Salterns	Hamworthy	Russell Quay	South Channel	Redhorn	Goathorn	Wych	Ower	Shipstall
Low water	0	0	1	0	–	–	–	–	–	–	–	–	–
Incoming tide	½	0	2	1	–	–	0	–	–	–	–	–	0
	1	0	3	0	–	–	0	–	–	–	–	0	0
	1½	1	2	1	–	–	1	–	1	–	1	0	0
	2	14	0	12	10	–	0	–	1	–	3	0	0
	2½	38	6	20	12	10	20	–	0	–	10	0	0
	3	56	14	24	12	30	44	–	26	–	12	0	0
	3½	86	20	32	14	16	8	–	14	–	–	0	0
	4	100	48	48	26	–	70	–	–	–	–	0	0
1st high water	4½	82	12	8	2	–	15	2	–	–	–	–	0
	5	6	1	2	–	–	7	1	–	–	–	–	0
	5½	2	0	1	–	–	0	28	–	1	–	–	0
	6	0	0	0	–	–	0	10	–	1	–	–	0
	6½	0	0	0	–	–	0	5	1	4	–	–	0
	7	0	0	0	–	–	0	–	0	1	–	–	0
	7½	0	0	0	–	–	0	–	0	–	–	–	0
2nd high water	8	0	0	0	–	–	1	–	1	–	–	0	0
Outgoing tide	8½	1	1	0	–	–	0	–	–	–	–	0	0
	9	1	0	1	–	–	3	–	–	–	–	1	0
	9½	0	1	0	–	–	1	–	–	–	–	0	0
	10	2	2	1	–	–	0	–	–	–	–	0	0
	10½	3	0	0	–	–	1	–	–	–	–	0	0
	11	1	1	0	–	–	0	–	–	–	–	–	0
	11½	0	0	0	–	–	0	–	–	–	–	–	0
Low water	12	0	0	0	–	–	–	–	–	–	–	–	0

Note.—Although no lactose fermenters were found in any quantity of 10 c.c. of Shipstall water examined, yet acid and gas were produced in 100 c.c. tubes. No change was however found in 75 c.c. tubes.

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that we found during our routine examination of the plates and tubes. In all, 27 lactose fermenting organisms were subcultured. Their morphology was considered and they were examined regarding their behaviour to Gram's stain. They were tested also for motility. Their fermentation reactions were examined in peptone water containing lactose, saccharose, glucose and dulcite. Their Indole Reactions and their Vosges-Proskauer Reactions were deterred. It will be seen from Table III that the majority of these organisms proved to be "coliform" in nature.

Table III.

Lactose fermenting organisms in sea-water.

	Morphology	Glucose	Lactose	Saccharose	Dulcite	Indole	Litmus milk	Motility
1.	Gram-bacillus	A & G	A & G	A & G	A & G	+	A & C	+
2.	,,	A & G	A & G	–	A & G	+	A & C	–
3.	,,	A & G	A & G	–	–	–	A	?
4.	,,	A & G	A & G	A & G	A & G	+	A & C	–
5.	,,	A & G	A & G	A & G	A & G	+	A & C	+
6.	Gram + coccus	–	A & G	–	–	–	A	–
7.	Gram-bacillus	A & G	A & G	A & G	–	+	A & C	–
8.	,,	A & G	A & G	A & G	A & G	+	A & C	–
9.	,,	A & G	A & G	A & G	A & G	–	A & C	+
10.	,,	A & G	A & G	A & G	–	+	A & C	+
11.	,,	A & G	A & G	A & G	A & G	+	A & C	+
12.	,,	A & G	A & G	–	A & G	+	A	–
13.	,,	A & G	A & G	0	A & G	+	0	+
14.	,,	A & G	A & G	A & G	A & G	+	0	–
15.	,,	A & G	A & G	–	A & G	+	0	–
16.	,,	A & G	A & G	0	A & G	–	0	?
17.	,,	A & G	A & G	A & G	A & G	–	0	+
18.	,,	A & G	A & G	–	A & G	+	0	–
19.	Gram-bacillus	A & G	A & G	0	A	–	0	–
20.	Gram + coccus	A	A	0	A	0	0	–
21.	Gram-bacillus	A	A	–	A	–	0	–
22.	,,	A & G	A & G	A & G	A & G	–	0	+
23.	,,	A & G	A & G	–	A	–	0	+
24.	,,	A	A & G	0	A & G	+	0	–
25.	,,	A	A	–	A	–	0	+
26.	Gram + coccus	A	A & G	–	A	–	0	–
27.	Gram-bacillus	A & G	A & G	–	A & G	–	0	–

Note.—A & G = acid and gas. + = Indole production. – = no change. 0 = not examined. A = acid only. A & C = acid and clot. Nos. 1–12 are organisms obtained from sea-water in 1914. Nos. 13–27 are organisms recovered from sea-water and oysters in 1919. None of the organisms except No. 3 gave a positive Vosges-Proskauer Reaction.

It has been shown by other workers that intestinal organisms die rapidly in sea-water, and that a period of four or five days is sufficient even for a heavily infected sea-water to become practically sterilised.

The presence, therefore, of these lactose fermenters of the faecal or "coliform" type showed that the pollution was recent. Not much interest attaches to the non-lactose fermenters which we isolated and subcultured. As is usual in enquiries of this nature, no *B. typhosus* or para-typhoid organisms were isolated.

A SUITABLE RELAYING PLACE.

It seemed to us hardly likely that we should find a place anywhere within the harbour where the water was comparatively clean and suitable for the relaying and cleansing of oysters. If such a place was to be found it must be somewhere as far as possible from the harbour entrance; some place where the tides were not very swift, so that sedimentation and purification might have had time to take place before the water arrived at this hypothetical relaying ground. We thought that Redhorn or the top of the Wareham Channel might possibly be fairly clean, but they proved to be otherwise. Samples taken off Ower Farm and off Arne near Shipstall Point were however clean or nearly so—*B. coli* being present in 100 c.c. tubes but not in 75 c.c. tubes and five 10 c.c. plates being free from lactose fermenters (Table II). Owing to the difficulties of navigation, Ower is not a very accessible place, so we decided to relay some oysters off Shipstall Point near Arne. This place is situated at the top of the Wych Channel about three miles from the open sea. Between Arne and the sea lies Branksea Island and the water coming up to Arne takes a winding course around this: in addition, between Arne and the sea there are many acres of mud flats covered by rank weed and grass, which are awash with water when the tide is high: these practically form a filter for the sea-water. They slow the rate of the tide and must necessarily act as a mechanical filter to some of the water passing from the sea towards Arne.

Again, as this spot is so far from the open sea it is improbable that the water which forms any one high tide was actually in the open sea during the low water preceding that high tide—in other words if a single gallon of sewage contaminated water from the neighbourhood of Old Harry could be followed into Poole Harbour it would take several tides to find its way up to Shipstall Point. Indeed the actual movement of the water in these upper reaches is only slight, and this has been demonstrated by tide-floats. Something almost comparable to "storage" takes place in these places that are more remote from the sea, and self purification of the water is the natural outcome of this.

These natural conditions no doubt explain the fact that off Shipstall Point near Arne the sea-water was reasonably free from lactose fermenting organisms, and gave us a hope that the place might be suitable for the relaying of oysters. Twelve oysters were therefore dredged from the main channel. Six of them were brought straightway to the Laboratory and the remaining six were relaid in a buoyed net off Arne. The first half dozen were polluted, but not heavily; the half dozen which were relaid at Arne for seven days were clean with an average of only two lactose fermenting organisms per oyster. In view of this we decided not to pursue at present the intended part of our research which dealt with sterilisation by means of chlorine; but rather to accumulate as many facts as possible regarding the relaying at Arne.

The experiment was therefore repeated on several occasions, and always with the same results—namely that the oysters before relaying were dirty and after relaying were clean—sometimes almost sterile (Table IV).

The greatest care was taken in the minute examination of these relaid oysters and a large number of plates of varying quantities were made. On one occasion for instance, 16 plates each of 2 c.c. were made from one oyster—practically one-third of the oyster was plated in small quantities—and only 13 lactose fermenters were found, corresponding to a total of 40 per oyster. On another occasion 0·9 part of an oyster was plated, giving a count of 12 lactose fermenters per oyster. On another occasion 25 c.c. of the emulsion were tubed in quantities of 1 c.c. in each of 25 tubes: acid and gas and a coliform organism were present in one tube—a total of four per oyster. In all 42 oysters after relaying were examined. The highest count observed was

Table IV.

Results of relaying oysters at Shipstall.

Before relaying		After relaying		
Date	Lactose fermenters per oyster	Date	Lactose fermenters per oyster	
22. 9. 19	100	30. 9. 19	2	
8. 10. 19	1600	18. 10. 19	14	
8. 10. 19	1200	18. 10. 19	10	
23. 10. 19	400	30. 10. 19	40	
—	—	4. 11. 19	10	(*a*)
4. 11. 19	400	12. 11. 19	15	(*b*)
4. 11. 19	700	12. 11. 19	40	
12. 11. 19	2580	20. 11. 19	17	(*c*)
12. 11. 19	1050	20. 11. 19	12	(*c*)
22. 11. 19	590	2. 12. 19	3	(*d*)

(*a*) These oysters were found off Arne. They had not been laid there by us.
(*b*) 5 oysters mixed. Quantities equal to one-third of an oyster plated.
(*c*) 5 oysters mixed. Quantities equal to nine-tenths of an oyster plated.
(*d*) 5 oysters mixed. Quantities equal to one-half of an oyster plated.

40 *B. coli* per oyster: the lowest was 2 per oyster. The average was 16 per oyster. As the result of these findings we have recommended that Shipstall Point off Arne is a safe place for the relaying of oysters; and have been able to give an assurance that oysters which have been relaid there for a week will be of reasonable bacteriological purity.

CONCLUSION.

1. The contamination of the water in Poole Harbour comes in with the flood tide from the open sea, and does not originate within the Harbour.

2. The nearer the sea and the more rapid the current, the greater is the pollution of the Harbour water.

3. The large oyster beds near the harbour entrance and in the main channel are polluted, and the oysters in them contain many organisms derived from sewage.

4. These oysters can be cleansed by relaying off Shipstall Point near Arne where the water is comparatively free from sewage organisms.