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National Rivers Authority
Anglian Region

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Anglian Region
Regional Biology Laboratory Microbiological Survey
19th & 20th August 1992

THE WASH GRID SURVEY

Objectives

1. To carry out a bacteriological survey covering the Wash Grid 1991 sampling sites, utilising the Sea Vigil for sample processing.
2. To test the laboratory procedures, techniques and equipment under aseptic conditions on board the Sea Vigil.
3. To test the sampling methods and equipment.
4. To develop techniques capable of qualitatively assessing water quality in terms of bacteriological content in the Wash.
5. Determination of time schedules for future assessment of Wash surveys.

Procedure

Field Preparation

The Sea Vigil was loaded with laboratory equipment on Tuesday 18th August for use in the field on the 19th and 20th August. Any outstanding equipment required for the survey was transported to Boston docks by the personnel carrying out the survey, on the first day of the survey (19th August).

Survey Preparation

Prior to the survey, a meeting was held on 11th August 1992 in order to discuss the criteria for a survey of the Wash. During the meeting the following issues were covered, each one relating to the key objectives above :-

1. Sample collection sites.
2. Organisms to be sought eg. total coliforms.
3. Dilutions and volumes of sample to be analysed.
4. Factors to be measured such as salinity, DO (% saturation).

It was decided that 66 samples should be collected (see map 1 for details of these sites) and analysed over the two day period (excluding controls).
It was decided that a series of dilutions was not required for this survey, but to filter a 50ml volume per sample per organism sought would be sufficient. A qualitative assessment was considered adequate in order to satisfy the objectives of this survey.

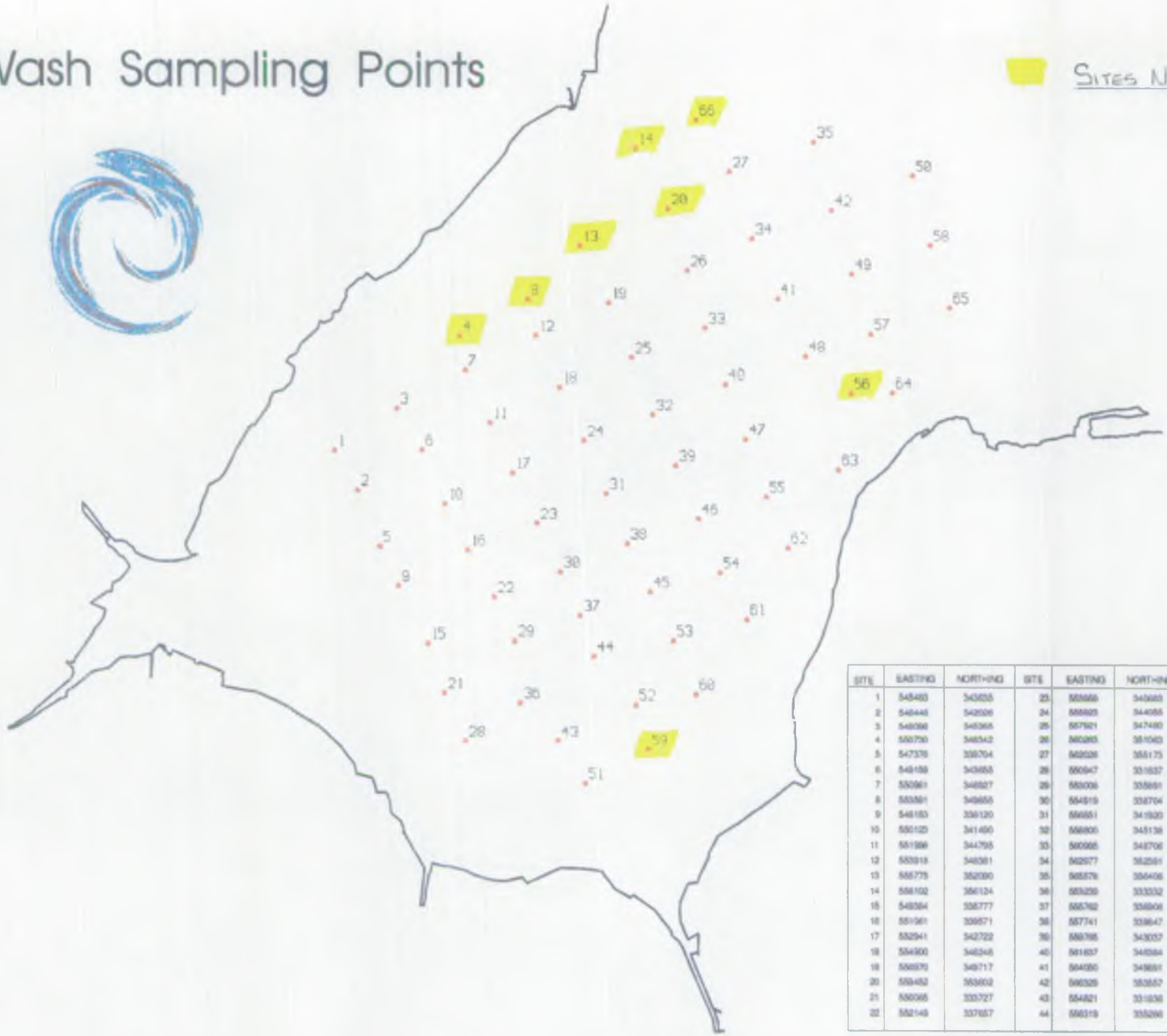
Equipment & Materials - see checklist, Appendix 3. (ref.Witham & Welland Report July 1992)

ENVIRONMENT AGENCY

102594

The Wash Sampling Points

Sites Not Sampled



SITE	EASTING	NORTHING	SITE	EASTING	NORTHING	SITE	EASTING	NORTHING
1	548483	343655	23	555668	340683	45	559884	337907
2	548446	342026	24	556825	344055	46	560715	340672
3	548066	346366	25	557921	347480	47	562957	344124
4	549790	348542	26	560265	351043	48	565252	347520
5	547376	339704	27	562028	353175	49	567166	350927
6	549159	343655	28	560947	331637	50	569725	355066
7	550961	346927	29	563006	335691	51	555966	329966
8	553561	349655	30	564519	338704	52	558087	333271
9	548183	336120	31	566651	341920	53	559644	335920
10	550122	341490	32	568905	345138	54	561611	338676
11	551996	344795	33	560966	348706	55	563560	341803
12	553918	348381	34	562977	352581	56	567131	346056
13	555775	352090	35	565576	356406	57	567961	348446
14	556102	356124	36	563259	333232	58	570481	352128
15	548364	336777	37	565762	336806	59	568815	331435
16	551361	339571	38	567741	339647	60	560589	333684
17	552941	342722	39	569766	343057	61	562743	336766
18	554900	346248	40	561657	346584	62	564481	339701
19	556970	349717	41	564060	349651	63	566065	342697
20	559452	353602	42	566329	353557	64	568965	346068
21	560366	333727	43	564821	331636	65	571261	348658
22	552149	337657	44	566319	335266	66	560633	357291

Laboratory Preparation

The following was prepared prior to sample collection and analysis.

a. The media was prepared at the Regional Microbiology Laboratory, autoclaved and poured according to the manufactures instructions. These were stored in the fridge at 4 C until the day of the survey.
b. All agar plates were fully labelled.
c. All equipment, such as sample bottles and laboratory glassware were autoclaved.
d. The benches in the laboratory on the Sea Vigil were swabbed with disinfectant.
e. The equipment on board Sea Vigil was assembled and set out ready for sample handling.

Field Procedure

Field equipment supplied by the Regional Microbiology Laboratory :-

Labelled sterile 300ml glass bottles
Sample sheets
Clipboards
Pencils and waterproof pens
Labguard microbial handsoap
Bottles of deionised water
Safety clothes and lifejacket

Field Staff Five staff participated in the field work :-
- One from the Regional Biology Laboratory, taking samples and carrying out the analysis.
- One from the Marine section, assisting in sample collection and analysis.
- One from the Chemistry laboratory, taking meter readings eg. temperature, salinity etc.
- Two crew from Sea Vigil, navigation and boat handling.

Tide Table Information (BST)

Date		Boston		Tabs Head		Fosdyke	
Wed 19th	HW	1002	2226	1003	2227	1034	2258
August	LW	0522	1736	0504	1718		
Thurs 20th	HW	1034	2258				
August	LW	0545	1801				

SCHEDULE

Wednesday 19th August

Left Aqua House at 8:00 and arrived at Boston Docks 9:00. After half an hours induction on safety and boat handling, set sail at 9:30. The weather was sunny and sea exceptionally calm.
The first sample was collected at 11:22

Sampling

Each sample was collected in accordance with The Bacteriological Examination of Drinking Water Supplies 1982, Report 71 using a 300ml sterile glass bottle labelled with the sample number. At each sample site a sterile glass bottle was attached to a long weighted rope. A 30cm length marker was made on the rope to indicate when the bottle would be at the correct depth to take the sample.
To take each sample the bottle top was removed and retained in one hand. The rope was then lowered below the surface of the water to a depth of about 30cm and the bottle filled to about 1 inch below the neck. The bottle was attached to the rope so that the neck would slightly tilt and the bottle fill with water. The bottle was then removed and the top replaced immediately. Each sample was then taken into the laboratory on Sea Vigil for analysis.
Whilst each sample was being collected the following information was simultaneously recorded on a sample sheet :-

a. Time of sample collection
b. Location of sample site (for reference see adjoining map)
c. Temperature of the water (C)
d. Salinity
e. Dissolved Oxygen (% saturation)
f. Weather conditions
g. Water clarity

Time Schedule

The following is a breakdown of the amount of time spent performing the field survey :-

Time taken at each sample point - approx. 5 minutes
Time taken for travel between sites - 5 mins - 45 mins
Time taken for the fieldwork - 14 hours
Total time taken for the survey including travel to and from the laboratory - 16 hours (fieldwork plus 1 hour travel each way).

Laboratory Procedure

On board Sea Vigil the following laboratory procedure of analysis took place:- (for further reference see Witham and Welland Report, 7th July 1992. In accordance with Bacteriological Examination of Drinking Water Supplies 1982 - Report 71.)

- The media was removed from the plastic crates and all the plates labelled with the appropriate information.
- The filtration pump was plugged in and switched on at the socket.
- One membrane was carefully placed onto the filtration grid using flamed forceps. The magnetic calibrated funnel was then replaced.
- The vacuum pump was then turned on and the system allowed to run for 5 seconds in order to clear any airways. The valve in operation was fully opened.
- Each sample bottle in turn was shaken, and the contents of the bottle poured into the calibrated filter funnel to fill upto the 50ml graduation.
- The pump was then switched off and the magnetic funnel removed. Using a pair

of sterile forceps the membrane was removed and placed onto a solid agar plate. The nature of the agar depending upon the organism to be sought.

- This procedure was followed for every sample collected and organism sought, and all samples were analysed immediately upon collection at all sample sites.
- One set of controls using 50ml of Ringers was filtered after every tenth sample collected.
- The petri dishes were then inverted, placed into a plastic box and incubated at the required temperature for a set period of time depending upon the organism.
- Due to a lack of magnetic filter funnels, 5 were recycled all day.
  This procedure was followed by rinsing thoroughly with deionised water and by boiling individual funnels in a pan of water for 1-2 minutes. This gave adequate sterilisation for re use instead of having to return to the main laboratory to autoclave the equipment.
  The funnels were then allowed to cool, and be used upon demand.

Three incubators on board Sea Vigil were available for use by our team. In order for batches of samples to have their incubation periods staggered, each of the three incubators were set at a different temperature.
ie. One at 30 C, 37 C and 44 C.
After the first phase of incubation of 4 hours at 30 C, the plates were separated into one of the other two incubators for their second phase of growth.

The period of time which was required for the incubation of each organism cultivated was as follows, along with the corresponding temperature settings :-

Faecal streptococci	- 4 hrs at 30 C - 44 hrs at 44 C
Total coliforms	- 4 hrs at 30 C - 14 hrs at 37 C
Faecal coliforms	- 14 hrs at 44 C

This enabled 28 sites to be covered during the first day leading to two batches of plates being incubated at varying times of the day.

Sea Vigil docked Boston at 22:55 and the team arrived back at the laboratory at 24:00 (midnight), leaving one person on board Sea Vigil to monitor the incubation of the plates.

<u>Thursday 20th August</u>

We left Kingfisher House at 7:30 and arrived at Boston Docks at approximately 8:50. We then sailed out of Boston docks at 9:15 and collected the first sample at 9:20 at Freddy Grays. The weather was raining with a light breeze.
After incubation, the petri dishes from the day before were removed from the incubators and the colonies formed counted using an illuminated colony counter. The exception being for faecal streptococci plates which had a total incubation time of 48 hours.

Faecal coliforms &	- only the bright yellow
Total coliforms	colonies were counted.
Faecal streptococci	- only the red colonies were counted.

The procedure of sample collection and analysis was the same as the previous day, other than the following exceptions :-

1. Due to rougher weather conditions, new autoclaved filter funnels were used until they ran out, after which a number of these were recycled as previously described.
2. Due to a lack of sample bottles in the field, the same procedure as for the filter funnels was followed ie. rinsed with deionised water and boiled for 1-2 minutes (see Appendix 2). It was necessary to sterilise some sample bottles. The same method was used as described for the filter funnels.
3. Due to weekend restrictions, the indicator organism faecal streptococci was not grown due to the extensive period of incubation required.

Two batches of samples were once again incubated at varying times of the day. This meant that 38 samples could be analysed.

Over the period of 2 days, a total of 7 sets of controls were prepared.

Analysis of the Results

Figure 1 illustrates the difference between the total and faecal coliform counts. At all 66 sites the total coliform results were greater than the faecal coliform results. The relationship is more clearly expressed in Table. 1 when compared to the faecal streptococci results. Here it is shown that two sample sites had insignificant counts of faecal streptococci; this organism was not detected at any other sites sampled in the survey.

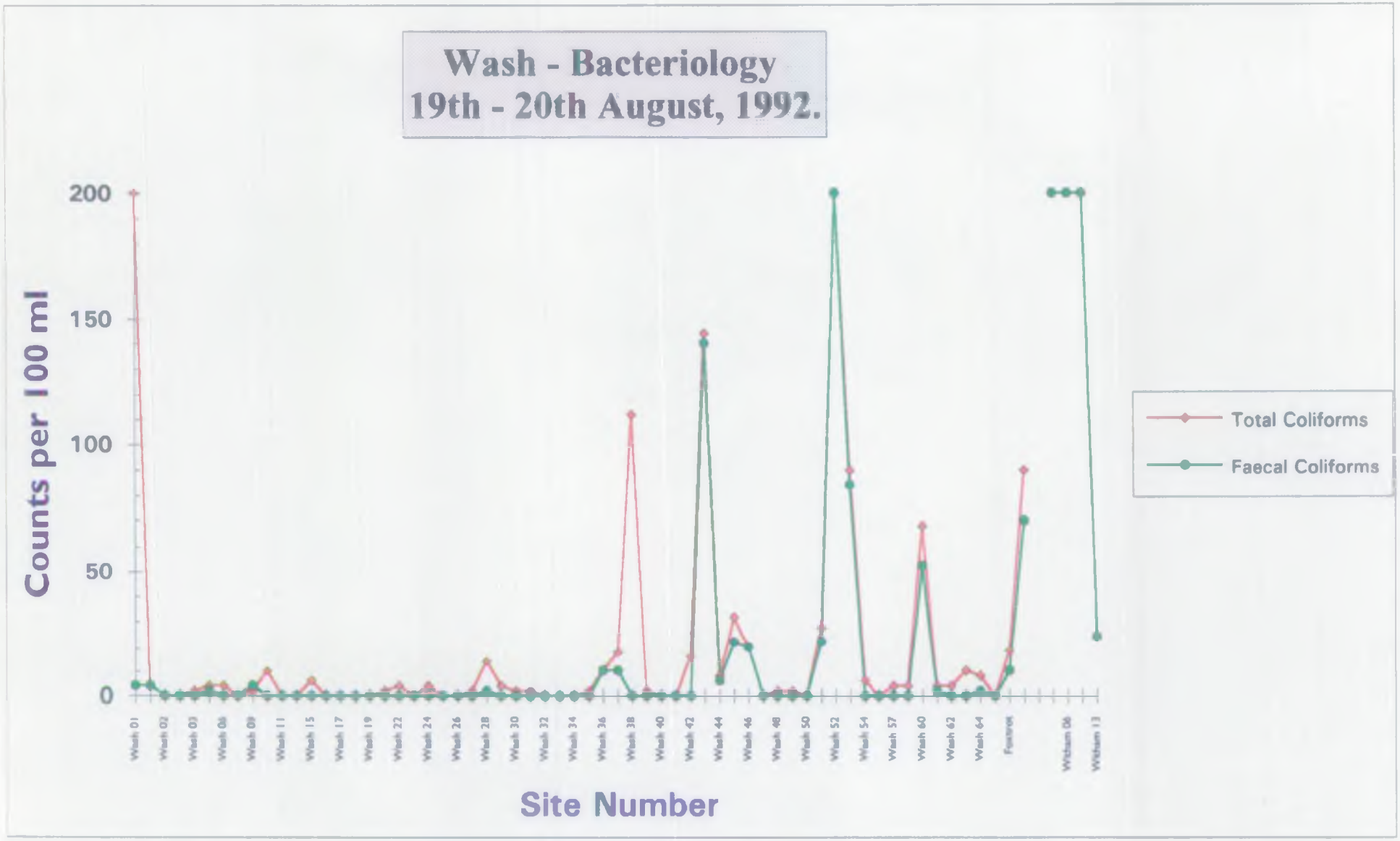
As expected the faecal streptococci results are less than the faecal coliform counts. Therefore the presumptive analysis highlights a drop in counts from total coliform to faecal coliform to faecal streptococci. This trend has also been identified in Report 71. The total counts found also meet with the standards set for bathing beaches.

Figure 2 shows that the River Witham has a higher concentration of bacteria than the Wash. Higher bacterial counts are expected in rivers and lower counts in sea water.
The high counts at these sites may be caused by Boston sewage treatment works which discharges to the Witham downstream of Slippery Gowt. The trend is still high at No. 9 Beacon which is downstream of the sewage works, and decreases with distance away from the works as the effluent is diluted by the receiving water. The high counts at these sites, upstream of the sewage works could be caused by a number of factors. The first could be tidal back wash from the sewage works. The sample was taken at Boston docks, when high water was approaching, therefore saline water was flowing upstream towards Boston carrying sewage effluent in the current. The remainder of this flow could have been collected when the sample was taken after the tide had changed. Alternatively

FIGURE 1

WA_BACT1.XLC

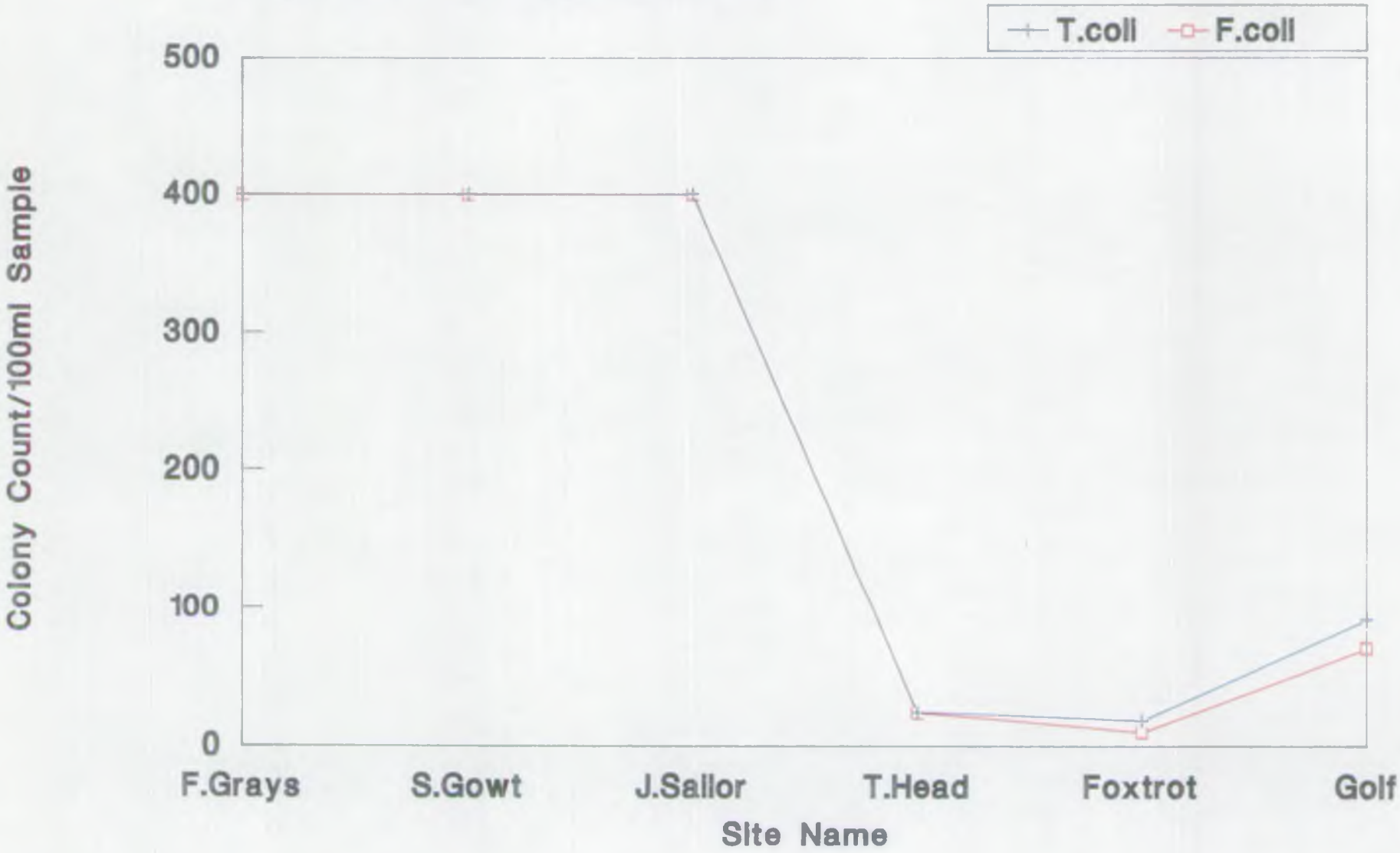
Printed 4/9/92



Analysis on-board 'Sea Vigil'.

WASH SURVEY ON BOARD 'SEA VIGIL'

Figure 2 - Analysis of Data



the high counts could have been caused by discharges upstream of the site such as Boston docks and other surrounding industries. Samples taken further upstream towards Boston would confirm this hypothesis.
Figures 3 and 4 illustrate the concentration of bacteria in the River Witham, the associated estuary, and the Wash. Each line in the figure corresponds to a contour line representing a number of colony counts per 100ml. ie. a contour line of 10 refers to a plate count giving a total number of 10 bacteria per 100ml of water sample.
The closer the contour lines to one another, the greater the concentration of bacteria.

Both figures 3 and 4 indicate that there is a high level of total and faecal coliforms in the areas surrounding the River Witham and Great Ouse estuaries. This is to be expected since the Boston and the Kings Lynn sewage treatment works lie further upstream on these rivers.

Table 1 illustrates the data collected throughout the Wash Survey. Wash sites 01 and 03 are shown to have been sampled and analysed twice. Although there are no significant differences between data obtained for Wash sites 03, there is a significant difference at Wash sites 01.

Table 1 illustrates that for the first day of sampling Wash site 01 had a total coliform count of 200+, whereas for the second day of sampling Wash site 01 had a total coliform count of 4. Although there are great differences between these two figures, there are several factors that require consideration:

1) The two samples analysed from Wash 01 were taken at different turns of the tide and on different days.
2) The whole cycle is varies every day. ie. no two consecutive days have the same cycle.

Wash site 01 which is downstream of the sewage works was sampled and analysed at 11:22 on the first day of the survey (1 hour and 20 minutes after high tide at Boston). The data in Table 1 illustrates how the total and faecal coliform counts decrease with distance away from the works as the effluent is diluted by the receiving water.
Site 01 was sampled and analysed 41 minutes before low water, the following day. Both the total and the faecal coliform counts obtained were very low.
The difference between results could lie in that the effluent from the sewage works discharged at high water from Boston would have already gone out to sea. Therefore by the time the sample was taken, the level of faecal contamination would be significantly low at this site.

The results as a whole show a general trend of decreasing faecal pollution as sites further out to sea were sampled. This was to be expected since any sewage effluents discharged from either Boston or Kings Lynn would become more diluted as taken out with the tide.

WASH Survey : August 1992 – Total Coliforms

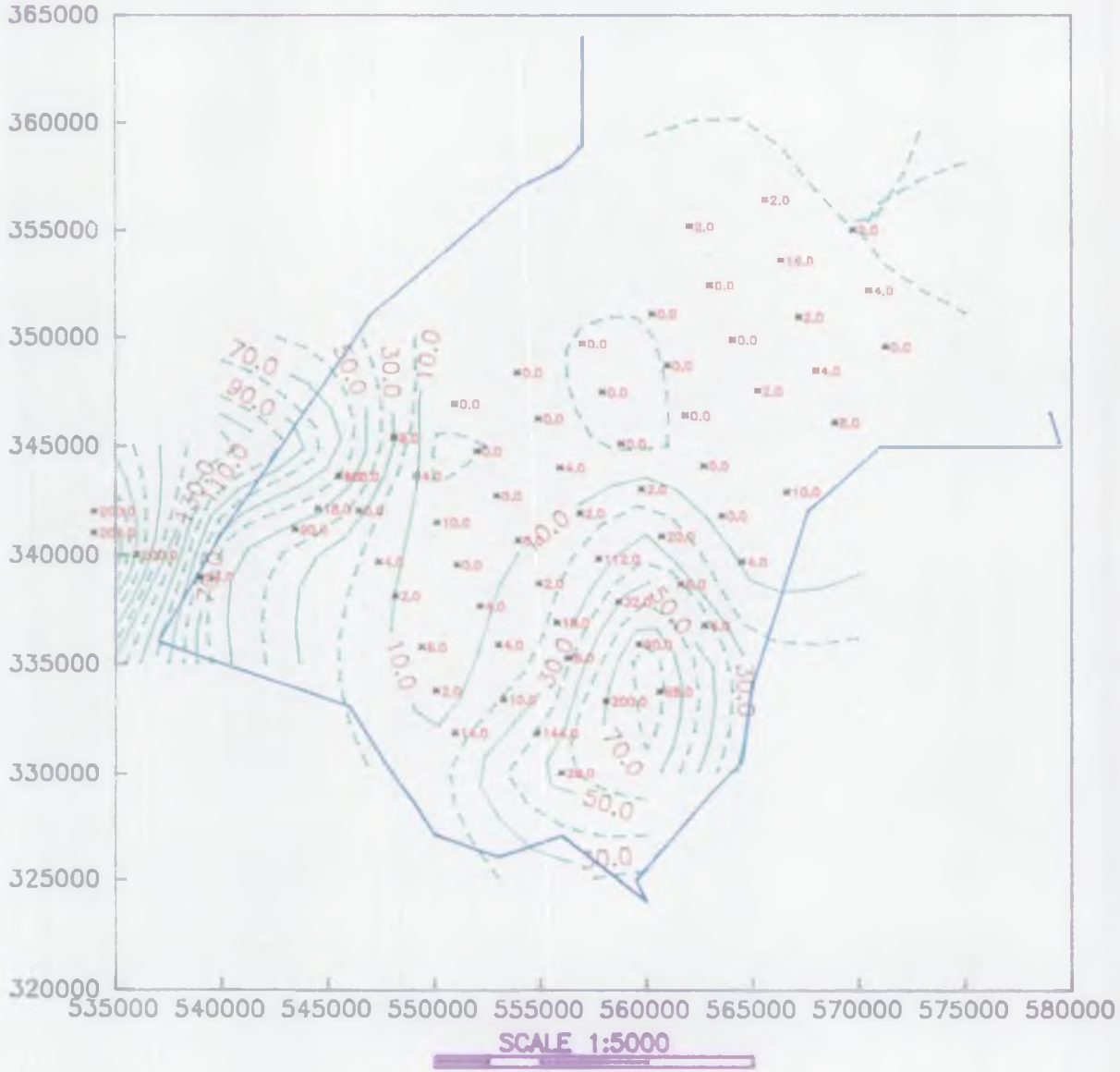
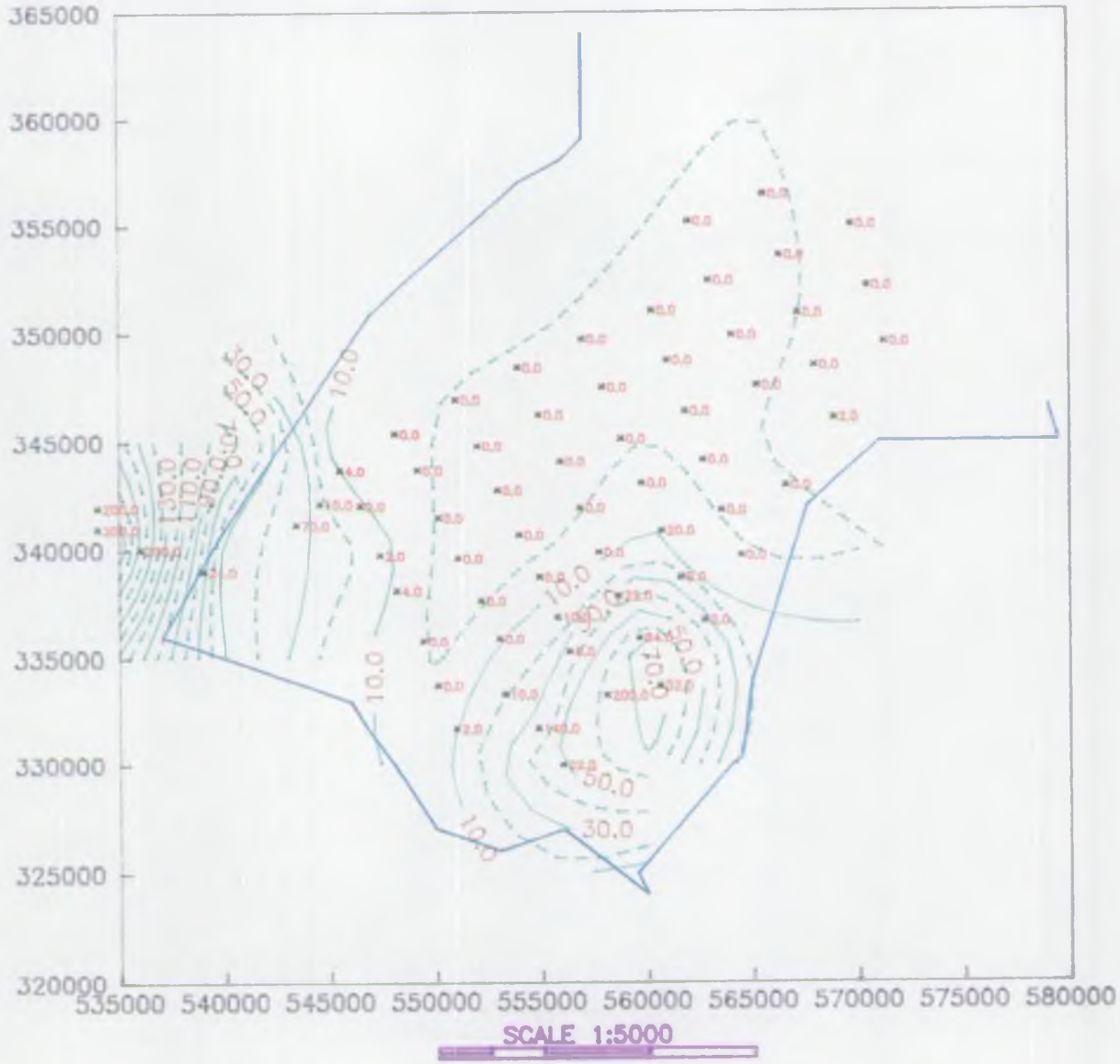


FIGURE 4

WASH Survey : August 1992 – Faecal Coliforms



TABLES 1

WA_BACT1.XLS

Analysis on board 'Sea Vigil'.

Printed 4/9/92

Date of Analysis	Time	Site Name	Latitude	Longitude	Easting	Northing	Sample Point Name	Total Coliforms	Faecal Coliforms	Faecal Streps
19-Aug-92	11:22	Wash 01	52 58.20 N	0 10.00 E	545483	343635	WASH SITE 01 - LOWER ROAD (FREISTON)	200	4	0
20-Aug-92	19:20	Wash 01	52 58.20 N	0 10.00 E	545483	343635	WASH SITE 01 - LOWER ROAD (FREISTON)	4	4	
20-Aug-92	10:25	Wash 02	52 57.31 N	0 10.82 E	546446	342026	WASH SITE 02 - ROGER SAND (W)	0	0	
19-Aug-92	11:37	Wash 03	52 59.10 N	0 12.38 E	548098	345385	WASH SITE 03 - LOWER ROAD (WRANGLE)	0	0	0
20-Aug-92	19:10	Wash 03	52 59.10 N	0 12.38 E	548098	345385	WASH SITE 03 - LOWER ROAD (WRANGLE)	2	0	
20-Aug-92	10:44	Wash 05	52 56.05 N	0 11.58 E	547376	339704	WASH SITE 05 - GAT CHANNEL (MID)	4	2	
19-Aug-92	11:50	Wash 06	52 58.15 N	0 13.29 E	549159	343655	WASH SITE 06 - ROGER SAND (N)	4	0	0
20-Aug-92	18:57	Wash 07	52 59.88 N	0 14.99 E	550961	346927	WASH SITE 07 - BOSTON DEEP (S)	0	0	
20-Aug-92	10:48	Wash 09	52 55.18 N	0 12.24 E	548153	338120	WASH SITE 09 - GAT SAND (E)	2	4	
19-Aug-92	12:03	Wash 10	52 56.97 N	0 14.09 E	550123	341490	WASH SITE 10 - ROGER SAND (E)	10	0	0
20-Aug-92	15:35	Wash 11	52 58.71 N	0 15.85 E	551998	344795	WASH SITE 11 - W OF THE ANTS	0	0	
20-Aug-92	18:44	Wash 12	53 00.61 N	0 17.66 E	553918	348381	WASH SITE 12 - LONG SAND (NW)	0	0	
20-Aug-92	11:00	Wash 15	52 53.90 N	0 13.27 E	549384	335777	WASH SITE 15 - WISBECH CHANNEL (N)	6	0	
19-Aug-92	12:14	Wash 16	52 55.91 N	0 14.86 E	551061	339571	WASH SITE 16 - GAT CHANNEL (E)	0	0	0
20-Aug-92	15:18	Wash 17	52 57.58 N	0 16.64 E	552941	342722	WASH SITE 17 - BOSTON ROADS	0	0	
19-Aug-92	14:40	Wash 18	52 59.45 N	0 18.48 E	554900	346248	WASH SITE 18 - LONG SAND (SE)	0	0	0
20-Aug-92	18:27	Wash 19	53 01.29 N	0 20.43 E	556970	349717	WASH SITE 19 - PARLOUR CHANNEL (W)	0	0	
20-Aug-92	11:09	Wash 21	52 52.79 N	0 13.82 E	550065	333727	WASH SITE 21 - WISBECH CHANNEL (MOUT	2	0	
19-Aug-92	12:25	Wash 22	52 54.87 N	0 15.78 E	552149	337657	WASH SITE 22 - BAR FLAT	4	0	0
20-Aug-92	15:00	Wash 23	52 56.46 N	0 17.48 E	553959	340683	WASH SITE 23 - N OF BAR FLAT BUOY	0	0	
19-Aug-92	14:29	Wash 24	52 58.25 N	0 19.33 E	555923	344055	WASH SITE 24 - E OF ROARING MIDDLE BU	4	0	0
19-Aug-92	14:55	Wash 25	53 00.05 N	0 21.22 E	557921	347460	WASH SITE 25 - LYNN DEEPS (W)	0	0	0

Bacteriological Survey - The Wash : August, 1992

Date of Analysis	Time	Site Name	Latitude	Longitude	Easting	Northing	Sample Point Name	Total Coliforms	Faecal Coliforms	Faecal Streps
20-Aug-92	18:11	Wash 26	53 01.95 N	0 23.41 E	560263	351063	WASH SITE 26 - PARLOUR CHANNEL (NE)	0	0	
20-Aug-92	17:50	Wash 27	53 04.14 N	0 25.12 E	562026	355173	WASH SITE 27 - WEST OF LYNN KNOCK	2	0	
20-Aug-92	11:18	Wash 28	52 51.75 N	0 14.55 E	550947	331837	WASH SITE 28 - OUTER WESTMARK KNOCI	14	2	
19-Aug-92	12:35	Wash 29	52 53.90 N	0 16.50 E	553008	335891	WASH SITE 29 - OLD LYNN CHANNEL (MID	4	0	0
20-Aug-92	14:46	Wash 30	52 55.38 N	0 18.28 E	554919	338704	WASH SITE 30 - ROARING MIDDLE (N)	2	0	
19-Aug-92	14:19	Wash 31	52 57.08 N	0 20.10 E	556851	341920	WASH SITE 31 - CENTRAL WASH (MID)	2	0	2
20-Aug-92	16:00	Wash 32	52 58.79 N	0 21.94 E	558800	345138	WASH SITE 32 - NE OF ROARING MIDDLE B	0	0	
19-Aug-92	15:35	Wash 33	53 00.66 N	0 24.00 E	560995	348706	WASH SITE 33 - THE WELL / LYNN DEEPS	0	0	0
20-Aug-92	17:36	Wash 34	53 02.62 N	0 25.88 E	562977	352391	WASH SITE 34 - THE WELL / NORTH WELL	0	0	
19-Aug-92	17:19	Wash 35	53 04.74 N	0 28.33 E	565578	356408	WASH SITE 35 - EAST OF LYNN KNOCK	2	0	0
19-Aug-92	12:52	Wash 36	52 52.51 N	0 16.63 E	553239	333332	WASH SITE 36 - THIEF SAND (N)	10	10	0
20-Aug-92	14:35	Wash 37	52 54.40 N	0 18.98 E	555762	336906	WASH SITE 37 - TEETOTAL CHANNEL (N)	18	10	
19-Aug-92	14:10	Wash 38	52 55.95 N	0 20.84 E	557741	339847	WASH SITE 38 - W OF NO 1 BELL BUOY (N C	112	0	14
20-Aug-92	16:14	Wash 39	52 57.64 N	0 22.74 E	559765	343037	WASH SITE 39 - CENTRAL WASH (MID)	2	0	
19-Aug-92	15:48	Wash 40	52 59.40 N	0 24.69 E	561837	346384	WASH SITE 40 - E OF LYNN DEEP	0	0	0
20-Aug-92	17:25	Wash 41	53 01.25 N	0 26.77 E	564050	349891	WASH SITE 41 - E OF THE WELL	0	0	
19-Aug-92	17:05	Wash 42	53 03.19 N	0 28.91 E	566329	353557	WASH SITE 42 - NE OF NORTH WELL BUOY	16	0	0
20-Aug-92	11:36	Wash 43	52 51.69 N	0 18.00 E	554821	331836	WASH SITE 43 - TEETOTAL CHANNEL (S)	144	140	
20-Aug-92	14:27	Wash 44	52 53.52 N	0 19.44 E	556319	335286	WASH SITE 44 - SEAL SAND (N)	8	6	
19-Aug-92	13:59	Wash 45	52 54.86 N	0 21.61 E	558684	337867	WASH SITE 45 - DASELEYS SLED (N)	32	22	0
20-Aug-92	16:25	Wash 46	52 56.45 N	0 23.51 E	560715	340872	WASH SITE 46 - SW OF SUNK BUOY (W CAR	20	20	
19-Aug-92	16:00	Wash 47	52 58.17 N	0 25.39 E	562697	344124	WASH SITE 47 - WEST OF SUNK SAND	0	0	0

Analysis on board 'Sea Vigil'.

Date of Analysis	Time	Site Name	Latitude	Longitude	Easting	Northing	Sample Point Name	Total Coliforms	Faecal Coliforms	Faecal Streps
20-Aug-92	17:13	Wash 48	52 59.95 N	0 27.75 E	565232	347520	WASH SITE 48 - NORTH OF SUNK SAND	2	0	
19-Aug-92	16:50	Wash 49	53 01.75 N	0 29.58 E	567166	350927	WASH SITE 49 - SW OF WOOLPACK BUOY	2	0	0
19-Aug-92	17:40	Wash 50	53 03.90 N	0 32.00 E	569725	355006	WASH SITE 50 - NE OF WOOLPACK BUOY	0	0	0
20-Aug-92	11:47	Wash 51	52 50.66 N	0 18.96 E	555966	329986	WASH SITE 51 - DASELEYS SAND (SW)	28	22	
20-Aug-92	13:44	Wash 52	52 52.40 N	0 20.95 E	558087	333271	WASH SITE 52 - SEAL SAND (E)	200	200	
19-Aug-92	13:44	Wash 53	52 53.80 N	0 22.41 E	559646	335920	WASH SITE 53 - OLD BELL MIDDLE (E)	90	84	0
20-Aug-92	12:54	Wash 54	52 55.25 N	0 24.25 E	561611	338676	WASH SITE 54 - S OF SUNK BUOY (W CARD	6	0	
20-Aug-92	16:42	Wash 55	52 56.90 N	0 26.08 E	563560	341803	WASH SITE 55 - SW OF SUNK SAND	0	0	
19-Aug-92	16:38	Wash 57	53 00.40 N	0 30.22 E	567961	348449	WASH SITE 57 - MIDDLE BANK	4	0	0
19-Aug-92	17:55	Wash 58	53 02.34 N	0 32.56 E	570461	352125	WASH SITE 58 - THE SLEDWAY (W)	4	0	0
20-Aug-92	12:25	Wash 60	52 52.59 N	0 23.20 E	560599	333694	WASH SITE 60 - CORK HOLE	68	52	
20-Aug-92	12:45	Wash 61	52 54.20 N	0 25.20 E	562740	336766	WASH SITE 61 - 3.6 KM O/S HEACHAM	4	2	
19-Aug-92	20:03	Wash 62	52 55.75 N	0 26.85 E	564491	339701	WASH SITE 62 - 2.1 KM O/S HUNSTANTON (S	4	0	0
19-Aug-92	19:07	Wash 63	52 57.43 N	0 28.84 E	566605	342897	WASH SITE 63 - 1.0 KM O/S ST EDMUNDS PT	10	0	0
19-Aug-92	18:50	Wash 64	52 59.10 N	0 30.95 E	568865	346069	WASH SITE 64 - 1.6 KM O/S GORE POINT	8	2	0
19-Aug-92	18:10	Wash 65	53 00.94 N	0 33.20 E	571261	349555	WASH SITE 65 - NORTH OF GORE MIDDLE	0	0	0
20-Aug-92	19:32	Foxtrot	52 57.40 N	0 09.10 E	544520	342122	WASH - FOXTROT BUOY, LOWER ROAD	18	10	
20-Aug-92	19:29	Golf	52 56.90 N	0 08.10 E	543428	341162	WASH - GOLF BUOY, LOWER ROAD	90	70	
20-Aug-92	09:20	Witham 05			534000	342000	WITHAM SQ 05 - FREDDY GRAYS	200	200	
20-Aug-92	09:32	Witham 06			534000	341000	WITHAM SQ 06 - SLIPPERY GOWT	200	200	
20-Aug-92	09:27	Witham 09			536000	340000	WITHAM SQ 09 - JOLLY SAILOR	200	200	
20-Aug-92	09:57	Witham 13			539000	339000	WITHAM SQ 13 - TABS HEAD	24	24	

Recommendations

1. Purchase of a Ultra Violet cabinet
   The boiling of filter funnels is not advisable for the following reasons :-
   a. After boiling several times, a coat of limescale builds up on the surface and the rubber sealing around the base of the funnel begins to crumble.
   b. It is not good practice and is unsafe to continue with this procedure especially during bad weather conditions.

2. To find alternative flaming method for aseptic technique :-
   a. There is no gas supply on Sea Vigil for safety reasons due to mixing of fumes in the atmosphere leading to a possible explosion.
   b. Present flaming includes the use of spirit burners utilising methylated spirits. This procedure is very dangerous during bad weather conditions. The motion of the boat leads to spillages and the possibility of a small fire. A hot air gun is to be considered as a possible solution to this problem.

3. Purchase a small refrigerator. Agar plates must be kept at 4 C and can not be allowed to stay out of a fridge for long periods of time. Wastage of plates was high due to mould growth. This had accumulated after almost one week out of the fridge. A decrease in wastage can only be achieved by purchasing a fridge for Sea Vigil.

4. Purchase a number of small plastic boxes capable of holding approximately 20 plates/samples. This would allow batches of samples to have staggered incubation periods, and would also cut down on 'lost' space inside each incubator.

5. The planning of future surveys should have the following considered :-
   a. A parallel run with the main laboratory. This would allow comparisons of data and methods between different analysis environments.
   b. To carry out a similar survey of the wash, concentrating on the problem sites which have been indicated from these results.
   c. To analyse a 100ml sample instead of a 50ml sample. This enables an accurate estimation to be made at every site without involving calculations.

6. **Long days.** Considerations have to be made for possible overnight stays either on board Sea Vigil or by booking accommodation in the town nearest to the docks. Optimal use has to be made of all time available for analysis on Sea Vigil, therefore the length of the working day can not be shortened, due to reliance on the tide. However overnight stays at the docks does decrease travelling time for the personnel involved, and hence is an essential consideration for future surveys and cannot be over looked.

7. **Planning**
   Any possible staggering of samples has to be considered prior to a survey. Therefore it is essential that the number of samples to be collected during a survey is previously planned, for allowance to be made for the overlapping of batch incubation times. It is also important to consider if staff will be available at the end of the second phase of incubation to count

the plates.

8. **Controls** - A number of controls should be used in order to identify areas of error in the methodology. Therefore it is recommended :-

   a. Positive control - analysis of a stock culture.
   b. Negative control - a standard volume of sterile Ringers diluent would be used to replace the sample.

9. **Quality Controls**
   a. Carry out the presumptive analysis immediately after sample collection to help minimise any bacterial die off.
   b. Run sample blanks.
   c. Ensure that equipment and media are sterile prior to sample analysis. I-nclusion of Browne tubes and autoclave tape.
   d. Participation in the Public Health Laboratory Service (PHLS). Water Microbiological Assessment Scheme.
   e. Put forward an application for NAMAS accreditation.
   f. Carry out the presumptive analysis ;
      i) immediately on receipt of the sample.
      ii) 6 hours after receipt of the sample.
      Variation in the data will then indicate the affect of delayed analysis on the result.
   g. Arrange for other microbiologists ie. those on board Sea Vigil, those in the mobile lab and those in the main laboratory at Aqua House to carry out the same analysis on the same samples, and compare results.
   h. To have a trial run of MLS agar and MLS broth, to see if there is any difference in colony growth on a liquid or a solid medium.
      If there is a difference then further investigations will have to be made.
   i. To standardise procedures and to implement them not only in the main laboratory, but also in the mobile laboratory and Sea Vigil.

10. To produce a standard checklist indicating the equipment that would be needed for use on each survey, and the numbers of each piece required. The checklist could be incorporated into routine use in the main laboratory, as well as being essential during Sea Vigil and Mobile laboratory work. This would ensure that nothing would be forgotten. Once again forward planning is required.

11. When possible, processing of a sample should begin at once. If storage is unavoidable, the sample should be kept cool to minimise change. It is important to remember that once a sample is placed in a clean container it will start to change microbiologically as well as chemically. Sometimes these changes are irrelevant and cannot be avoided in certain circumstances, but at all other times they must be taken into account.

12. Always shake the bottle containing the sample before processing it. It is always desirable to break up clumps of bacteria as far as possible, and to attempt to seperate the organisms from the particles of suspended matter on which a high proportion of them are growing or to which they are simply growing. This leads to easier more efficient plate counting.

13. The data collected from the Unicomarine survey in 1991 should be reported upon so that future surveys can compare results.

APPENDIX 1

Raw Data

Wednesday 19th August		Colony Counts per 100ml Sample		
Site No.	**Time**	**Total Coliforms**	**Faecal Coliforms**	**Faecal Streps**
1	11:22	>400	4	0
3	11:37	0	0	0
6	11:50	4	0	0
10	12:03	10	0	0
16	12:14	0	0	0
22	12:25	4	0	0
29	12:35	4	0	0
36	12:52	10	10	0
53	13:44	90	84	0
CONTROL 1.	13:50	0	0	0
45	13:59	32	22	0
38	14:10	112	0	14
31	14:19	2	0	2
24	14:29	4	0	0
18	14:40	0	0	0
25	14:55	0	0	0
33	15:35	0	0	0
40	15:48	0	0	0
47	16:00	0	0	0
57	16:38	4	0	0
CONTROL 2.	16:44	0	0	0
49	16:50	2	0	0
42	17:05	16	0	0
35	17:19	2	0	0
50	17:40	0	0	0
58	17:55	4	0	0
65	18:10	0	0	0
64	18:50	8	2	0
63	19:07	10	0	0
62	20:03	4	0	0
CONTROL 3.	20:10	0	0	0

APPENDIX 2

Thursday 20th August — Colony Count per 100ml Sample

Site No.	Time	Total Coliforms	Faecal Coliforms
Freddy Grays	9:20	>400	>400
Slippery Gowt	9:27	>400	>400
No.9 Beacon	9:32	>400	>400
Tabs Head	9:57	24	24
2	10:25	0	0
5	10:44	4	2
9	10:48	2	4
15	11:00	6	0
21	11:09	2	0
28	11:18	14	2
CONTROL 4.	11:24	0	0
43	11:36	144	140
51	11:47	28	22
60	12:25	68	52
61	12:45	4	2
54	12:54	6	0
* 52	13:44	>200	>200
44	14:27	8	6
37	14:35	18	10
30	14:46	2	0
23	15:00	0	0
CONTROL 5.	15:11	0	0
17	15:18	0	0
11	15:35	0	0
32	16:00	0	0
39	16:14	2	0
46	16:25	20	20
55	16:42	0	0
48	17:13	2	0
41	17:25	0	0
34	17:36	0	0
27	17:50	2	0
CONTROL 6.	17:59	0	0
26	18:11	0	0
19	18:27	0	0
12	18:44	0	0
7	18:57	0	0
3	19:10	2	0
1	19:20	4	4
2A	19:29	18	10
1A	19:32	90	70
CONTROL 7.	19:41	0	0

* Indicates that all samples from here on, were collected in a pan boiled bottle.

APPENDIX 3

Survey : Wash Grid - Sea Light

Date : 19/20 Aug

CHECKLIST

No.	ITEM	CHECK	NOTES
49	Sample Bottles		
MEDIA			
120	Slanetz & Bartely Plates		
500	MLSA/B Plates		
2000	mls Ringers Soln		
	mls Lactose Peptone Water		
EQUIPMENT : - PRESUMPTIVE ANALYSIS			
2	Manifolds		
2	Vaccum Pump		
4	Conical Flasks		
2	Wire Cage		
6	Lengths of Rubber Tubing		
	Petri Dishes		
	Absorbant Pads		
60X	Filtration Membranes		
48	Universal Bottles		
30	Magnetic Funnels		
	Automated Pipettor		
	Packs of Pipette tips		Size :
1Pack	Pipette Filler 10mls		
1Pack	Pipette Filler 1mls		
2	Sterile Forceps		
Bottle	Alcohol		
2	Watch Glasses		
2	Bunsen Burners		
5	Measuring Cylinders		
4	Marker Pens		
ALL	Plastic Incubation Boxes		
2	Universal Bottles Racks		
1 bottle	Paper Towels & Handsoap		

[ ]	Boiling Pan	[ ]	
[2]	Metal Tongs	[ ]	
[2]	Heat Proof Gloves	[ ]	
[2]	StopClock	[ ]	
[2]	Laboratory Coats	[ ]	
[ ]	Stools	[ ]	
[ ]	Trolley	[ ]	
[2]	Bench Mats	[ ]	
[ ]	Matches	[ ]	
[ ]	Glass Beakers	[ ]	
[ ]	Water Butt	[ ]	
[Bottle]	Distilled/Deionised Water	[ ]	
[ ]	Disinfectant	[ ]	
[Box]	Plastic Gloves	[ ]	
[ ]	Incubators/Portable	[ ]	
[1]	Colony Counter	[ ]	

FIELWORK EQUIPMENT

[ ]	Face Masks	[ ]	
[2]	Long Gloves	[ ]	
[6]	Plastic Crate	[ ]	
[15]	Sample & Data Sheets	[ ]	
[1]	Clipboards	[ ]	
[4]	Pencils & Waterproof Pens	[ ]	
[1set]	Waterproof/MUSTA Wet Gear	[ ]	
[1pair]	Wellies	[ ]	
[2]	Life Jackets	[ ]	
[4]	Waste Disposal Bags	[ ]	
[ ]	Cool Box for Transportation	[ ]	
[ ]	Sampling Equipment eg. rope	[ ]	
[ ]	Mobile Phone for Lab Contact	[ ]	
[ ]	Lockable Safety Cabinet	[ ]	
[1]	Recording Results Book	[ ]	
[ ]	Measuring Meters	[ ]	

EQUIPMENT : - CONFIRMATORY ANALYSIS

|___|	Waterbaths	|___|	_____
|___|	Universal Bottles	|___|	_____
|___|	Durham Tubes	|___|	_____
|___|	Universal Racks	|___|	_____
|___|	Sterile loops/Disposible	|___|	_____

Equipment & Material Assembly : _____

Equipment Checklisted By : ----------

Date : ---------- **Signed :** _____