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FINAL REPORT OF THE CONTROLLED COHORT INVESTIGATIONS INTO THE HEALTH EFFECTS OF BATHING IN SEWAGE CONTAMINATED COASTAL WATERS

January 1993

NOT TO BE QUOTED WITHOUT PERMISSION

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This report is submitted in completion of the Department of the Environment Contract PECD 7/7/377 (Phase III) and Department for Health / Welsh Office / NRA project No. 228 between the Water Research Centre and the Centre for Research University College, University of Wales, Lampeter Information Centre Head Office

Class No

Accession No ANTO

Executive Summary

Background

This report explains a healthy volunteer experiment designed to quantify the possible health effects of bathing in sewage polluted sea waters. The research protocol was similar to a controlled clinical trial; volunteers were randomised into bather and non-bather groups and subjected to interviews and medical examinations before and after exposure in UK sea waters passing the EC Bathing Water Directive (76/160/EEC). Water quality was measured in great spatial and temporal detail allowing precise allocation of water quality exposure indices to individual bathers. This is the first time a healthy volunteer experiment has been accomplished in the examination of health effects of environmental pollution.

Part I The Southend-on-Sea Controlled cohort study

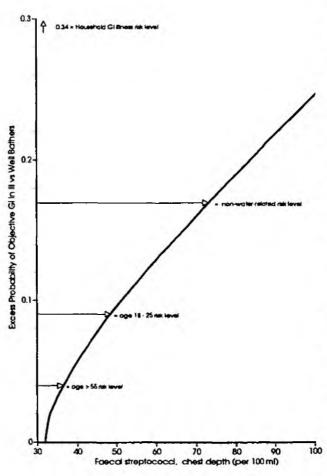
- 1. Water quality passed the mandatory criteria of Directive 76/160/EEC on the study day.
- 2. At one week post-exposure bathers reported significantly higher rates of ear infections, ear/eye infections, gastrointestinal symptoms and skin symptoms.
- 3. Dietary habits did not affect the result for gastrointestinal symptoms at one week post-exposure.
- 4. At three weeks post-exposure significant elevations in sore throats, ear infections and ear/eye infections were reported.
- 5. Ingestion of seawater had a significant effect on gastrointestinal symptom reporting.
- Significant differences were not detected in the results of clinical analyses of ear and throat swabs or faecal samples.

Part II An investigation of dose-response relationships between water quality and gastroenteritis

- 1. Categorical and multiple logistic regression procedures were used to identify relationships between water quality and gastroenteritis and to assess the validity of pooling the data from all four controlled cohort studies.
- 2. A single significant dose response relationship was identified between faecal streptococci (per 100 ml) at chest depth and gastroenteritis (p < 0.001). The relationship was independent of site studied.
- 3. Non-water-related risk factors did not confound the relationship and no significant interaction between confounders and the water quality index was found.
- 4. The threshold of risk was objectively defined as 32 faecal streptococci per 100 ml at chest depth. The resulting model (p = 0.012) is shown opposite.

Policy implications

- The model allows the prediction of the probability of gastroenteritis (i.e. the risk of illness) at a given faecal streptococci level. This probability can be compared with the risk of illness attributable to the other risk factors such as household illness.
- The results clearly Indicate that the current mandatory standards specified in Directive (76/160/EEC) may not be appropriate. Consideration should be given to changing both the recommended sampling depth and the microbial indicators used to assess compliance of UK marine waters.
- The results provide the necessary scientific information for the construction of standards or objectives for marine recreational waters as used by 'normal' adult bathers.



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1. Summary

The first section of this report (Part I) details the results of the 1992 controlled cohort investigation at Southend-on-Sea, Thorpe Bay. This was the final part of the second phase of studies designed to explore the possible health effects of bathing in UK coastal waters. This study was the fourth of a series of studies initiated at Langland Bay in 1989. The methodology followed that implemented at Moreton, Wirral, in 1990 and Southsea near Portsmouth in 1991. The site was selected to (I) provide water quality within the European *Imperative* standards for total and faecal coliforms in bathing waters, as defined in Directive (76/160/EEC), and (II) provide a large enough population for volunteer recruitment. Water quality at Southend-on-Sea, Thorpe Bay, complied with the mandatory bacteriological criteria of Directive (76/160/EEC) during the 1992 bathing season.

Interviews with volunteers prior to exposure collected data in five main areas: (I) social and demographic criteria, (II) health, including chronic illness and symptoms in the previous three weeks, (III) use of prescription drugs, smoking and alcohol use, (IV) leisure and recreational use of water and (V) the condition of ears and throats.

Saturday 4th July was the exposure day. The volunteers were assigned randomly to one of two groups; (I) exposed (bather) or (II) control (non-bather). This randomisation produced no evidence of social and demographic bias between the two groups. On the exposure day a short interview provided information on recent illness and diet. Packed lunches were provided for the volunteers. Bathers took a supervised dip in a defined bathing area and were asked to immerse their heads three times.

The study period was overcast with some drizzle and sea temperature was 19°C. Water samples were taken from the bathing area at three depths at each of four equally spaced positions along a 60 m stretch of the foreshore. Sampling took place at half hourly intervals between 13.30 and 16.30 BST. Water quality during the study period showed compliance with EC *Imperative* levels for total and faecal coliform organisms. Samples also passed the *Guide* criteria for faecal coliforms and faecal streptococci but failed the *Guide* standard for total coliforms. *Cryptosporidia* spp., *Salmonella* spp., enterovirus and rotavirus were not detected in samples analysed for these parameters. The geometric mean faecal coliform count was 134 100 mt⁻¹ (n=84).

Volunteers were given a post-exposure interview and medical examination on the Friday or Saturday of the week following the study day. Faecal samples were submitted for bacteriological and virological analyses and ear and throat swabs were taken from volunteers at this stage. A total of 365 volunteers completed this post-exposure stage from an initial recruitment of over 1,000. Three weeks after the study day participants received a postal questionnaire and final faecal specimen pot. Postal questionnaire returns totalled 350.

Statistical analysis of the results of ear and throat swabs for bacterial parameters revealed no significant differences between the exposed and control groups. Only two throat swabs showed a positive virus identification; one from each group. Faecal sample analysis produced no positive results.

On the exposure day the bather group reported significantly more loose motions (Relative risk 2.81, 95% CI 1.20-6.62) and diarrhoea (RR 9.27, 95% CI 1.17-73.33).

At one week post-exposure the bather group reported significantly higher rates of ear infections (RR 5.10, 95% CI 1.48-17.60), ear / eye infection (RR 2.86, 95%CI 1.22-6.73), gastrointestinal symptoms (RR 1.81, 95% CI 1.22-2.71) and skin symptoms (RR 2.50, 95% CI 1.16-5.38).

The three week post-exposure questionnaire results showed significantly more of the following reported symptoms in the exposure group: sore throats (RR 1.61, 95% CI 1.08-2.41), ear infections (RR 3.94, 95% CI 1.49-10.44) and ear / eye infections (RR 2.78, 95% CI 1.37-5.64).

The effects of swallowing seawater on gastrointestinal symptoms were such that bathers who swallowed seawater reported significantly more appetite loss, diarrhoea and any gastrointestinal symptom than the control group at both post-exposure intervals. A comparison between bathers who did not ingest and the control group revealed no significant differences.

The effects of dietary habits on gastrointestinal symptom reporting were examined by stratified analyses. The bather group were found to consume more of the following: mayonnaise on the exposure day, bought sandwiches at one week post-exposure and seafood at both times. Examination of the exposure day symptoms revealed that bather vv non-bather elevation of loose motions and diarrhoea became non-significant when seafood consumption was controlled for. This was not the case when mayonnaise consumption was the control variable. At one week post-exposure, controlling for either bought sandwiches or seafood did not affect the significant bather vv non-bather differential for reporting of any gastrointestinal symptom.

Part II of this report deals with the analysis of all four controlled cohort investigations: Langland Bay 1989, Moreton 1990, Southsea 1991 and Southend-on-Sea.

A statistical comparison of water quality from the four site investigations revealed that geometric mean faecal streptococci concentration at 30 cm and chest depths was the only bacterial parameter to show no significant difference between the four investigations. The crude results of bather vv non-bather comparisons at each site show several similarities between the studies. All studies showed some evidence of significantly elevated post-exposure gastrointestinal illness in the exposed group. The studies also revealed some elevation in the exposed group of symptoms such as ear infections, sore throats and skin symptoms.

The combined data set for the studies was used to investigate possible dose-response relationships between indices of water quality and gastrointestinal illness using categorical analyses and multiple logistic regression. This illness was chosen because it has been most often examined in international epidemiological studies seeking to inform policy on recreational water standards. Illness was defined as follows:

- (i) Subjective gastroenteritis (any combination of diarrhoea, indigestion, vomiting or nausea)
- (II) Objective gastroenteritis (any case of vomiting or diarrhoea or any subjective gastroenteritis accompanied by fever)

Individuals with a predisposition to gastroenteritis and bathers without exposure data were excluded. Illness was examined categorically against water quality. Water quality was classified into exposure categories on the basis of existing standards. Where standards were not available, median values from all four studies were used as cutpoints. The analysis looked for statistically significant trends between bathers and non-bathers and between bathers exposed to different levels of bacterial concentration. The only indicator to show a significant trend in illness with water quality, and therefore a potential dose-response relationship, was faecal streptococci at chest depth. The trend was highly significant ($\rho < 0.001$) for objective gastroenteritis with or without the non-bather group and marginal for the subjective category when the non-bather group was excluded ($\rho = 0.056$).

Non-water related risk factors for gastroenteritis were defined as potential confounders of dose-response relationships. These included variables such as age, gender, frequency of diarrhoea, use of medicines, dietary habits and household illness.

Dose response relationships were investigated on a site by site basis using four categories of faecal streptococci exposure (per 100 ml, at chest depth): (I) unexposed (control), (II) 0-34, (III) 35-70 and (IV) 70+. Subjective gastroenteritis showed the only significant difference (p = 0.007) between sites in one category, 0-34 faecal streptococci per 100 ml. Given this result the data sets were pooled.

Significant differences in non-water-related risk factors between the bathers and non-bathers were identified by contingency table analysis. Potential confounding of the significant trend in gastroenteritis with faecal streptococci density (chest depth) by significant non-water-related risk factors was then assessed using multiple logistic regression. Comparison of crude and adjusted odds ratios showed no evidence of confounding.

The faecal streptococci density at which gastroenteritis in bathers exceeded that in non-bathers was then identified by splitting the exposure index into 20 unit intervals. Subjective and objective gastroenteritis was found to be significantly elevated in bathers exposed more than 39 faecal streptococci per 100 ml at chest depth

Logistic regression was used to assess any confounding effect of the non-water-related risk factors on the relationship between gastroenteritis and the 20 unit classes of faecal streptococci (chest depth) among ill vv well bathers. A set of indicator variables were used to identify any site specificity in the relationship between illness and water quality. A degree of confounding was apparent for subjective gastroenteritis only. No significant interaction was found between potential confounders and the water quality index. Dose-response relationships were not site specific.

The effects of duration of exposure were examined using analysis of variance. The analysis revealed no significant differences in exposure time by illness status or indicator density.

Logistic modelling was used to (I) define the threshold of risk for objective gastroenteritis and (II) produce a continuous model for use in risk assessment. Objective gastroenteritis among ill bathers vv well bathers was the outcome variable and faecal streptococci density, modelled as a continuous variable, the main effect. In the above analysis the 20-39 faecal streptococci per 100 ml exposure category was found to be the highest group showing no excess risk. The median value of chest depth faecal streptococci density in this category was 32 per 100 ml. Models were therefore computed for bathers exposed to less than 32 and 32 or more faecal streptococci per 100 ml at chest depth. The model below 32 per 100 ml was not significant. The model above this threshold was significant (p = 0.012). The predicted probability of objective gastroenteritis at the threshold value was 0.087 which was close to that observed among non-bathers (0.097). The continuous model produced allowed a comparison of risk from exposure to bathing in sewage contaminated water with the risk associated with nonwater related factors identified in the study. The form of the dose-response relationship and related non-water-related risk factors are shown in Figure 1.

2. Introduction

This report contains (1) details of the controlled cohort study which took place at Southend-on-Sea, Thorpe Bay, during the 1992 bathing season and (II) the results of a combined analysis of data from four comparable epidemiological studies undertaken in two consecutive phases. The first phase was a pilot study at Langland Bay, Swansea, 1989 (Jones et al., 1991a; Pike et al., 1990). The second phase of research took place at the following locations: Moreton, Wirral, 1990 (Jones et al., 1990) and Southsea near Portsmouth, 1991 (Jones et al., 1991b) and Southend-on-Sea, 1992. The Southend-on-Sea Beach study was the third and final study of the second phase of epidemiological investigations into the possible health effects of bathing in sewage polluted waters. The epidemiological design of the studies follows the suggestions of the World Health Organisation (WHO, 1972), that researchers should, as far as possible, attempt to implement an experiment closely resembling a randomised clinical trial. The main aims of this method are (I) to acquire the best possible data on exposure to water pollution by detailed spatial and temporal monitoring of the bathing area during the period of human exposure; (II) to obtain data on potential confounding factors which might contribute to illness e.g. dietary habits; (II) to investigate whether clinical microbiological confirmation of reported symptoms can be achieved by laboratory analysis of specimens provided by study participants and finally (IV) to compute statistical relationships linking seawater quality and measures of illness outcome which can be used to inform the policy debate concerning recreational water quality standards. studies were contracted to the Centre for Research into Environment and Health (CREH) at the University of Wales with overall management by the Water Research Centre The following agencies were responsible for funding this research programme; the Department of Environment (DoE), the National Rivers Authority (NRA), the Welsh Office (WO) and the Department for Health (DfH).

PART I

The Southend-on-Sea (Thorpe Bay) Controlled Cohort Study 1992

3. The Southend-on-Sea, Thorpe Bay, controlled cohort study 1992

3.1 Study Design and Methodology

3.1.1 Site Selection, Recruitment and Interview Arrangements

Thorpe Bay (NRA location: 11800, NGR: TQ 911847) was chosen from three EC designated beaches in the vicinity of Southend-on-Sea as the study location by the NRA. The site was chosen with the logistics of volunteer recruitment in mind, the large urban population of Southend-on-Sea and neighbouring towns on the Thames estuary providing a large pool of prospective volunteers. Water quality at the site has a history of compliance with the EC Directive 76/160/EEC *Imperative* standards for total and faecal coliforms in bathing waters (EEC, 1976). A study date of 4th July was chosen to allow for a repeat later in the bathing season given poor weather on the test day.

This final study was designed to be directly comparable with the studies from the previous two bathing seasons (Moreton and Southsea). The methodology therefore followed the same protocol (Jones *et al.*, 1990; Jones *et al.*, 1991b). Ethical approval for the study design was received by the DoE in 1989 from the Royal College of Physicians Committee for Research on Healthy Volunteers. Information sheets for volunteers and recruiters were designed in accordance with the recommendations of this committee (RCP, 1986). The study received local ethical clearance sought by the Director of Public Health from the Research Ethics Committee, Southend-on-Sea.

The study at Southend-on-Sea aimed to gather information from approximately 400 healthy adult volunteers over the age of 18. Initially, 1020 volunteers were recruited to account for the drop out experienced in previous studies. Volunteers had read and signed an agreement to take part and supplied relevant contact details of themselves and their general practitioners (Appendix I).

This recruitment phase was organised by CREH and Oxford Conferences Ltd (OC). A field team of 8 trained recruiters contacted prospective volunteers in the Royals Shopping Centre, in the town centre and along the sea front at Southend-on-Sea. Displays showing information from the previous studies were placed in the shopping centre and reader friendly information sheets, giving details of the study, were distributed liberally. Southend-on-Sea Borough Council (SBC) gave both clerical support and office space to the team. A 24 hour answer phone service was installed for prospective volunteers. Local press and media coverage through the recruitment phase was handled by Mrs C. Pownall (OC), Dr David Kay (CREH) and Mr Mike Pressling (SBC). Recruitment took place over the four weeks prior to the study day.

The volunteer details were input to a data base compiled at the CREH office in Wales. Volunteers were then contacted by telephone, or letter, to arrange an appointment for interview. Interview times were confirmed by letter and each volunteer's general practitioner was notified of their patient's involvement (Appendix I).

Southend-on-Sea Borough Council provided a large committee room at the civic office building for the pre-exposure interviews (Green questionnaire, Appendix II) which took place on the 2nd and 3rd of July. This room also contained suitably screened areas for medical interviews. Volunteers logged in at a reception desk and received information about the next stages of the project at an exit desk. The team of trained interviewers was assembled by SBC Community Services Department and CREH. Staff also included medical and scientific personnel from the Welsh Unit of the Communicable Diseases Surveillance Centre (CDSC) of the Public Health Laboratory Service (PHLS). A total of 413 volunteers completed the pre-exposure interview stage. Out of these, six were advised not to take part either on medical grounds or for reasons such as refusal to adhere to the randomisation at the beach. At the medical interviews,

volunteers received instructions on when and how to take their faecal sample the following week.

Randomisation of the interviewed volunteers into the bather and non-bather groups took place at the close of interviews on the evening prior to the study day. Volunteers were then allocated supervisor numbers specified on lists printed for each volunteer, a blue list of bathers and a red list of non-bathing controls. In addition, volunteers who had made prior arrangements to have the pre-exposure interview at the beach were randomised into two groups and appropriate lists produced. Poster size lists were also printed ready for posting in prominent locations on the exposure day.

3.1.2 Site Preparation

Figure 3.1 shows details of the study site. Portakabins were used for administration and medical interviews. A marquee was also available for on site pre-exposure interviews. The CREH mobile laboratory was sited and linked to mains electricity and water supplies. Environmental microbiological analysis of water samples took place in this facility. Packed lunches were distributed to volunteers from a refrigerated van using a SBC recommended supplier. Volunteers' children were provided amusement with a bouncy castle close to the site. Safety cover was provided by the SBC lifeguards and St. John's Ambulance Brigade.

The areas of beach designated to the bathing and control groups were marked out by SBC staff to CREH guidelines using ropes and fencing stakes. A 60 m stretch of beach was allocated to the bathing group. This was divided into three 20 m sections, the four division markers defining the locations for microbiological sampling.

3.1.3 Cohort Organisation and Follow-up

The volunteers were received at the study site from 12.00 noon BST onwards. Each participant received copies of blue and red lists to enable them to identify their supervisor number within the beach area. Marshals were available to give guidance to the volunteers. Volunteers then received their exposure day interview (Yellow questionnaire, Appendix II). Afterwards bathers took a closely supervised dip in the sea during which they were asked to (I) remain in the water for 10 minutes and (II) immerse their heads completely at least three times. Diary sheets (Appendix II) were used to record the exact location and activities of the bathing group. On leaving the water, each bather was asked if they had ingested water and then were free to obtain their packed lunch. Members of the non-bathing control group collected their packed lunch after their interview and were asked to remain in the designated area of beach for a minimum of one hour. In total, 372 volunteers attended at the beach.

The follow-up questionnaire and medical interviews were held on the Friday and Saturday following the study day, i.e. 10th and 11th of July at SBC Civic Offices. A total of 323 subjects completed the third interview (Pink questionnaire, Appendix II) and gave throat and ear swabs. Additional follow-up information was obtained by personal telephone interview with 21 volunteers who were unable to attend the interview sessions. A further 21 volunteers returned questionnaires by post by prior arrangement with the project team.

Final questionnaires for self completion and return by volunteers were posted to arrive on Saturday 25th July (Blue questionnaire, Appendix II). This package also included the second faecal sample pot and instructions. The CREH office received 350 completed postal questionnaires (96% of volunteers who were followed-up at one week).

3.1.4 Questionnaire Design and Analysis

A four part questionnaire set (Appendix II) was used to obtain information on health as well as social and environmental factors describing the exposed and control groups prior to and after the exposure day. The questionnaire content had been matched with that of questionnaires used in prospective beach surveys taking place at other UK beaches during the 1992 bathing season as appropriate.

General health questions on all questionnaires (Appendix II) dealt with a wide range of individual symptoms grouped into the following broad categories: (I) 'flu and cold symptoms, (II) chest and respiratory symptoms, (III) gastrointestinal (GI) symptoms and (IV) skin symptoms. The questionnaire allowed the detailed recording of the onset and duration of recent illness. The pre-exposure interview (Green questionnaire, Appendix II) also collected information on chronic illness, use of prescription drugs, and factors such as alcohol consumption and smoking habits.

The pre-exposure questionnaire also enquired into social factors such as age, gender, social class, and size of household. The pre and post exposure interviews also focused on environmental factors including a range of exposures to marine and freshwater likely to be encountered by participants either through recreation or vocation. The exposure day and first follow-up interviews covered the volunteers' dietary history. The food types examined in this section Included; meat products (cold meats, pâté, meat pies and pasties, hot dogs and hamburgers), raw milk, raw egg products (mayonnaise), take away foods, bought sandwiches, and seafood (cockles, mussels, whelks etc.). Such foodstuffs are thought likely to increase GI illness in a volunteer group.

With the exception of the final questionnaire (3 weeks post exposure), data were collected by personal interview with each volunteer where possible. The randomly defined bathing/control status of the volunteers was not known by either the interviewers or volunteers until the exposure day. The bathing and control groups both received identical questionnaire sets.

The format of the questionnaires was virtually identical to that used in the two previous phase II studies (i.e. Moreton and Southsea). Interviewers were required to tick precoded option boxes and the right margin contained a sequence of coding boxes. The results from coded questionnaires were entered to a computer by overwriting a fixed format template. The SPSSx package (SPSS, 1989) was used for analysis. Relative risk (RR) and associated 95% confidence intervals (95% CI) were calculated using Epi Info Version 5 (Dean *et al.*, 1990). This package was also used for stratified analysis.

3.2 Methods - Microbial Investigations and Statistical Techniques

3.2.1 Microbiological Quality During the 1992 Bathing Season

Harmonised monitoring at Southend-on-Sea , Thorpe Bay, is undertaken jointly by the NRA (Thames Region) and the SBC Community Services Department. Figure 3.2 shows the results for total and faecal coliform organism and faecal streptococci concentrations during the 1992 bathing season (05.05.92 - 27.09.92). A statistical summary of these data is presented in Table 3.1 and the compliance of the results with the EC Directive (76/160/EEC) shown in Table 3.2. The results show that the samples pass the mandatory *Imperative* standards but fail the recommended *Guide* levels for these parameters.

Of 25 samples analysed for *Salmonella* spp. 4 (16.0 %) were positive. Enterovirus was detected in 10 of 18 samples (55.6 %). Values ranged from 1 to 3 plaque

forming units (pfu) 10 l⁻¹ (Figure 3.3). These results indicate failure to comply with the EC *Imperative* standard for these determinands.

3.2.2 Bacterial Water Quality on 04.07.92

The sea water in the foreshore area designated for the bather group was monitored at half hourly intervals between 13.30 and 16.30 BST during the afternoon of 04.07.92. Samples were taken at four locations 20 m apart along 60 m of shoreline. For each time and location, samples were taken at three positions in the near shore zone; surf, mid (30 cm) and chest depths. The following indicator organisms were enumerated (count 100 ml⁻¹); total and faecal coliform organisms, faecal streptococci, *Pseudomonas aeruginosa* and total staphylococci in samples (Appendix III). Enumeration of faecal coliform organisms and faecal streptococci were performed in triplicate. A set of duplicate samples were taken for quality control purposes. Additional analyses for *Cryptosporidium* spp. and *Salmonella* spp. were made on bulk samples (Appendix III). Sampling and microbiological determinations were carried out on site by staff from Altwell / Acer Environmental under the supervision of Mr Alan Godfree.

3.2.3 Viral Water Quality on 04.07.92

Three samples were taken at three of the foreshore locations (20 m, 40 m, 60 m) at half hourly intervals from 13.30 BST during the exposure period (15 samples total). These were analysed for enterovirus (pfu $10\, \Gamma^1$) and rotavirus (fluorescing foci (ff) $10\, \Gamma^1$). Sampling and analyses were supervised by Dr Helen Merrett (CREH Senior Research Fellow, formerly of Acer Environmental now of Wallace Evans Ltd), as outlined in Appendix III.

3.2.4 Clinical Samples and Examinations by Physicians

Both pre- and post-exposure interviews included an ear and throat examination by a physician. Physicians recorded details of any ear or throat infection, any redness of the throat or any discharge from the ear.

At the one week post-exposure interview, throat and ear swabs were taken for bacteriological analysis. A swab for virological analysis was also taken from each volunteer's throat. Clinical samples were transported by courier in insulated cold boxes to reach the laboratory within twelve hours. Volunteers were requested to supply samples of their faeces at one and three weeks post exposure. These were posted directly to the laboratory. Dr David Hutchinson and Dr Peter Morgan-Capner of Preston Public Health Laboratory co-ordinated sample analysis.

Ear and throat swabs were analysed for haemolytic streptococci, faecal streptococci, coliforms, *Escherichia coli* and *Staphylococcus aureus*. Ear swabs were also cultured for *Pseudomonas aeruginosa*. Viral swabs from throats were analysed for enterovirus and rotavirus.

The one week post exposure stool samples were analysed for the following organisms: Salmonella spp., Shigella spp., Campylobacter spp. and Escherichia coli O157. Samples from those volunteers reporting gastrointestinal symptoms were also analysed for Cryptosporidia spp., and for parasites, cysts and ova. A visual index of faecal sample consistency was also provided by the laboratory; whether stools were solid, semi-solid or liquid.

Three week post-exposure faeces from volunteers reporting gastrointestinal samples on the one week post-exposure questionnaire were analysed for viruses. Virus

detection was by Electron microscopy. Appendix III details analytical procedures used for human samples.

3.2.5 Packed Lunch Analysis

Randomly selected samples of food items from packed lunches supplied to the volunteers were taken for microbiological analysis. These included three chocolate biscuits and five cheese sandwiches. Samples were cultured for; coliform organisms, *Escherichia coli*, *Salmonella spp.* and faecal streptococci. Analyses were performed by the Public Health Laboratory at Chelmsford.

3.2.6 Statistical Methods

Contingency table analysis was used to examine the statistical significance of symptom reporting and swab results in the bather (exposed) and non-bather (control) groups. The relative risk (RR) of illness amongst the exposed compared to the control group and 95% confidence intervals (95% CI) were calculated using the Epi Info version 5 statistical package (Dean *et al.*, 1990; Greenland and Robins, 1985). All values of significance (p) reported are from Yates' corrected χ^2 test or Fisher's exact test, where an expected cell count was below five. Where the latter test was used the p values reported are twice the one tailed value; this approximates the Yates' corrected χ^2 value most closely (Dupont, 1986).

An initial examination of possible confounding effects of dietary habits was made using a stratified contingency table analysis. This allows the relative risk of illness from exposure to sea bathing to be assessed controlling for the effects of another exposure such as diet. The significance of stratified tables was determined from Mantel-Haenszel summary χ^2 values, weighted RR values and Greeland / Robins 95% CI listed by the Epi Info package (Dean *et al.*, 1990).

Any comparisons between mean values were made as follows. For comparing two mean values the appropriate Student's t-test was applied. For comparisons of more than two means the Student-Newman-Keuls (SNK) multiple range test was used. An α -level of 0.05 was used to define statistical significance.

3.3 Results

3.3.1 Site Conditions on 04.07.92

On the study afternoon the wind was a light to gentle breeze from the southeast. The sky was generally overcast (7 - 8 oktas) and the only sunshine (weak casting no shadows) was recorded at 14.00 and 14.30 BST. Drizzle occurred around 16.00 BST, otherwise the afternoon remained dry. The sea was calm and rippled throughout the study period and had a temperature of 19°C. No evidence of sewage solids, surface activated substances (foam), phenolic odour, mineral oil films or abnormal colour were observed in the bathing zone. However, a small fishing boat did enter the bathing area, coming on to the shore, during the bathing period. This is likely to have caused some localised disturbance of beach material, producing variations in turbidity within the bathing area. Bather density during the afternoon is shown in Figure 3.4.

3.3.2 Environmental Samples

Table 3.3a shows summary statistics for the indicator organism concentrations from 84 samples taken during the study period. Missing values, particularly for total staphylococci, were due to equipment failure. The results are summarised by sampling depth in Tables 3.3 c to d. *Cryptosporidia* spp. and *Salmonella* spp. were not detected in bulk samples. The change in geometric mean concentration of indicator organisms

is shown in Figures 3.5 to 3.9. Similar temporal patterns in concentration are evident for each sampling depth. Indicator organism concentration tends to increase from the chest depth Inshore to the surf zone.

The sets of duplicate samples taken for quality control were analysed using No significant differences, at $\alpha = 0.05$, were detected between the geometric mean values for all parameters except faecal streptococci (Appendix III, The microbiologists responsible for the analysis noted distinct large and small faecal streptococci colonies on some plates. Both types were confirmed as faecal Excluding the small colonies from the paired analysis still indicated a streptococci. significant difference in the two sample sets. However, the geometric mean faecal streptococci counts of triplicate enumerations for all samples taken on the study day were not significantly different from each other, using the SNK procedure. This was true when small colonies were included or excluded. In effect the paired quality control samples measured variability due to both (1) sampling and (11) laboratory filtration. Hence, the results may represent real differences in bacterial concentration caused by turbidity currents and associated faecal streptococci 'clumps' The triplicate filtration samples were taken from the same well mixed sample and, thus, may provide a better measure of 'laboratory' quality control, independent of environmental sampling The fact that the triplicate analyses showed no significant differences in geometric mean concentration suggests that the paired samples were indeed measuring environmental differences between sampling bottles.

Table 3.4 details the compliance of samples from the 30 cm depth with Directive 76/160/EEC. Samples showed compliance with the *Imperative* levels for total and faecal coliform organisms. Samples passed the *Guide* criteria for total coliforms and faecal streptococci. However, the samples failed to comply with the *Guide* levels for faecal coliforms on the study afternoon. The rate of non-compliance with this component of the Directive was higher than that recorded for the whole bathing season (Table 3.2).

Enterovirus and rotavirus were not-detected in-any of the 15 samples taken forvirological analysis. This indicates compliance with the EC Directive for enterovirus on the afternoon of the study.

3.3.3 Microbial Results from Human and Food Samples

3.3.3.1 Ear and Throat Swabs

Figure 3.10 shows the RR and 95% CI values of swab results from the comparison of the exposed and control groups. The analysis includes combinations of parameters present on either or both swabs. Additional details of counts, significance (p) and attack rates are given in Appendix IV. No significant differences were found between the bather and non-bather groups. Herpes simplex virus was isolated from two throat swabs, one bather and one non-bather.

3.3.3.2 Faecal Samples

Faecal samples were received from 350 volunteers at one week post-exposure. No significant growth of any of the bacteria analysed, *Salmonella* spp., *Shigella* spp. and *Escherichia coli* O157, was detected.

No cysts, ova or parasites were evident in any of the 83 samples analysed from the three week post-exposure GI positive group. Likewise, no virus particles were detected in 70 faecal samples submitted by volunteers reporting any GI symptom at one week post-exposure.

The consistency of faecal samples, on arrival at the laboratory, is detailed in Table 3.5. No significant differences were found between the exposed and control groups.

3.3.3.3 Packed Lunch Analysis

All samples of chocolate biscuit and sandwich had faecal streptococci present (20-200 $\,\mathrm{g}^{-1}$). The source of faecal streptococci in food is often not a result of direct faecal contamination and *Enterococcus faecalis* was detected in a similar analysis at Southsea the previous year (Jones *et al.*, 1991b). None of the other organisms analysed were detected in food samples.

3.3.3.4 Questionnaire Results

Frequency analysis for social and demographic factors, such as the gender and age structure of the exposed and control groups plus general health, chronic illness, prescription drug use, alcohol, smoking and recreational use of water are detailed in Appendix V. The groups were found to be broadly similar. The only significant differences were found in dinghy sailing and sub-aqua diving (Appendix V (D)).

Figures 3.10 to 3.14 show the calculated RR and 95% CI values for 26 symptoms and eight symptom groups at the pre-exposure, exposure and post-exposure stages of the project. Cell counts, significance and attack rates are listed in Appendix IV.

Prior to the exposure day significantly more bathers reported skin sores (Appendix IV (B)) or "any bathing" related symptom (any symptom excluding symptoms defined as "other" such as lassitude, dizziness etc.) (Figure 3.11). At this stage, the groups had not had their status defined. On the exposure day the bather group reported significantly more "loose motions" and "diarrhoea" (Figure 3.12). "gastrointestinal" symptom group showed no significant difference, however. week post-exposure significantly higher rates of reporting by the bather group were found for the single symptom "ear infection" and for the following symptom groups; "ear / eye infection", "gastrointestinal", "skin", "any symptom" and "any bathing symptom" (Figure 3.13). Reporting of the "ear infection" symptom was still significantly elevated in the exposed group at three weeks post-exposure (Figure 3.14). Bathers also reported significantly more "sore throat" and "dizziness" symptoms at this stage of the study (Figure Significantly higher reporting of symptoms from the following categories was 3.14). evident amongst the bathers at three weeks post-exposure; "ear/eye infection", "other", "any symptom" and "any bathing symptom" (Figure 3.14).

The effects of swallowing seawater on the exposure day and the reporting of post-exposure GI symptoms was investigated as follows. Approximately half of the exposed group reported ingesting seawater during their dip in the sea. Reporting of GI symptoms by this group was compared to that in the control group at one and three weeks post-exposure (Figures 3.15 and 3.16). This analysis revealed significantly elevated reporting of "appetite loss", "diarrhoea" and any "gastrointestinal" symptom by the exposed group. The results of a similar comparison of those bathers who did not ingest seawater and the control group revealed no significant differences in GI symptom reporting at either post-exposure interval (Figure 3.17 and 3.18). Further detail is given in Appendix IV (C).

Differences in the dietary habits of the bather and control groups were examined using information collected on the exposure day and at one week post-exposure. Significantly more volunteers in the bathing group were found to consume the following food types; mayonnaise on the exposure day, bought sandwiches at one week post-exposure and seafood at both times (Appendix IV (D)). Stratified contingency tables were used to examine gastrointestinal symptoms controlling for the

effects of these four food categories. The results are shown in Figures 3.19 to 3.22 and details given in Appendix IV (D). Taking the exposure day results, controlling for consumption of mayonnaise had no effect on significant symptom reporting, "loose motions" and "diarrhoea" remain significantly higher in the bathing group (Figure 3.19). However, the differences in reporting of these two symptoms became insignificant when consumption of seafood was controlled for (Figure 3.20). This suggests that the difference in GI symptom reporting on the exposure day is related to dietary habit (eating seafood). The one week post-exposure analysis showed that significant reporting of any "gastrointestinal" symptom was not affected by controlling for dietary factors (Figures 3.21 and 3.22), and may thus be an effect of exposure to sea bathing. The effects of confounding factors are further explored in Section 4 of this report.

Indices of serious illness in the follow-up period included (I) GP consultation, (II) illness interfering with normal daily activities and (III) hospital consultation. Positive occurrences were low in all categories and bather vv non-bather differences were not statistically significant (Appendix IV (E)).

Figure 3.23 shows the result of contingency table analysis for medical diagnoses of throat and ear conditions. No significant differences were evident between the exposed and control groups (Appendix IV (F)).

PART II

Analysis of the Four Controlled Cohort Studies

Initial categorical analyses presented in Part II of this report were completed at University of Wales and SUNY. The logistic regression analysis presented here was completed at SUNY by Professor J Flelsher using the BMDP package. Further logistical regression analyses have been completed by Dr R Salmon of the PHLS CDSC Welsh Unit using Epi Info, Version 5 and MULTLR. These parallel analyses employed different variable selection routines and model building criteria. However, they produced very similar dose response-relationships linking reported GI illness and concentrations of faecal streptococci concentrations in the bathing water.

4. Analysis of the Four Controlled Cohort Studies

This study was planned as a four year investigation into the possible health effects of sea bathing using a randomised trial of healthy volunteers. The reporting of the 1992 investigation at Southend-on-Sea, in the preceding section, follows the format of the previous three studies and draws a direct comparison between the exposed (bather) and control (non-bather) groups at this particular site. Data from the four studies were combined into a single data file containing information on every volunteer, their socio-demographic characteristics, the microbial water quality to which bather volunteers were exposed, potential non-water-related confounding factors such as food intake and finally the health status of the individual.

Completion of the four site investigations allowed the potential pooling of data from each study, given appropriate steps to demonstrate the validity of this process. The objective of the analysis of the pooled data outlined in this section of the report extends far beyond the limited bather vv non-bather comparisons reported for individual sites (see section 4.2). The purpose of the statistical analysis presented below is to; (I) investigate and demonstrate the appropriateness of pooling the data from the four studies, (II) examine the data for any dose-response relationships present, where "dose" (x) is a measured index of water quality and "response" (y) is a disease outcome:

$$y = f x ag{4.1}$$

and (III) quantify the effects of possible confounding factors such as food intake.

This analysis was completed using a combination of categorical techniques and logistic regression analysis. The approach taken in this work is outlined in detail below. Any negative findings have been reported to allow a critical evaluation of the methods employed. The emphasis in this section of the report has centred on the health outcome of most immediate policy relevance, namely, gastroenteritis. Where possible, outcome measures of disease (i.e. symptom groupings) and ordinal variable break points (i. e. microbial water quality measures) have been chosen by reference to the sparse available literature reporting epidemiological studies which have been used to develop water quality standards (Cabelli et al., 1982; USEPA, 1986).

4.1 Comparisons of Environmental Microbiology

Figure 4.1 summarises the geometric means and ranges of indicator concentrations from samples taken in the in-shore bathing zone, including all surf, 30 cm and chest depth samples. Significant differences in geometric mean indicator concentrations between the studies are detailed in Tables 4.1 to 4.3 by sampling depth. The only parameter for which no significant difference was found between any studies was faecal streptococci concentration at 30 cm and chest depths.

Tables 4.1 to 4.3 also rank the studies by geometric mean. This allows relative comparisons to be made. For instance the lowest geometric mean total and faecal coliform concentrations were found at Langland Bay at all depths. However, Langland Bay also exhibited the highest geometric mean faecal streptococci concentrations at 30 cm and chest depths.

4.2 Comparison of Crude Results

The significant results of crude bather vv non-bather comparisons from all four sites is presented in Table 4.4. This table indicates several similarities between the individual site investigations. All phase II studies (Moreton, Southsea, Southend-on-Sea) showed significant bather vv non-bather differentials for the GI symptom group in the first follow-up period. The Moreton and Southsea studies also showed significance for this

symptom group at the postal questionnaire stage. The pilot study at Langland Bay also showed evidence of significantly elevated gastrointestinal illness amongst bathers, as diarrhoea, In the three week post exposure period. The four studies demonstrate some degree of communality with respect to ear infections, sore throats and skin symptoms.

4.3 Logistic Regression Modelling of Gastrointestinal Illness

4.3.1 Study Population

At Langland Bay, 266 participants completed the initial interview and medical examination. These 266 participants were randomised into 133 bathers and 133 non-bathers. Nine members of the bather cohort and 11 members of the non-bather cohort reported having a chronic condition that will predispose to symptoms of gastroenteritis (e.g., colitis, irritable bowel syndrome, etc.) or reported symptoms of gastroenteritis on the trial day, and were excluded from the study. An additional 13 bathers lacked exposure data and were also excluded from subsequent analysis. Therefore, the overall follow-up rate at Langland Bay was 87.6%...

At Moreton, 303 study participants completed the initial interview and medical examination. The 303 study participants were then randomised into 138 bathers and 165 non-bathers. Four members of the bather cohort and 11 members of the non-bathers reported having a chronic condition that will predispose to symptoms of gastroenteritis, or reported symptoms of gastroenteritis on the trial day, and were excluded from subsequent analysis. In addition, 36 bathers lacked data on the time and place that they entered the water and were also excluded from the study. The overall rate of follow-up at Moreton was 83.2%...

Three hundred and sixty-four study participants completed the initial interview and medical examination at Southsea. The 364 participants were then randomised into 178 bathers and 186 non-bathers. Thirteen bathers and 22 non-bathers reported either chronic gastrointestinal illness or symptoms of gastroenteritis and were excluded from subsequent analysis. An additional five bathers lacked exposure data and were also excluded. The overall rate of follow-up at Southsea was 89.0%...

At Southend-on-Sea, 413 participants completed the initial interview and medical examination. Of these 372 attended the beach. Of those 32 did not fill out subsequent questionnaires, and 37 were excluded due to gastrointestinal illness on the exposure day (17 bathers and 20 non-bathers). This left a randomised cohort of 138 bathers and 165 non-bathers, yielding an overall follow-up rate of 73.4%. The follow-up rate for all four studies combined was 82.6%...

The mean age for the entire bather cohort was 35.5 years vv. 34.9 years for the non-bather cohort (p = 0.52). When broken down by study location, only Southsea showed bathers to be slightly older than non-bathers (Table 4.5).

Prior to analysis, gastrointestinal (GI) symptoms reported at follow-up were divided into two categories - subjective and objective. The subjective category of symptoms included all reported cases of diarrhoea, indigestion, vomiting, or nausea on either the one-week follow-up interview or the postal questionnaire. Objective symptoms of gastroenteritis included all cases of vomiting or diarrhoea, and all reported cases of subjective symptoms accompanied by a fever. The reporting of objective symptoms of gastroenteritis are less likely to be affected by inter-individual variation in perception of specific symptoms and, thus, provide a more reliable estimate of the occurrence of gastroenteritis among study subjects. The objective symptom group is less subject to recall bias among volunteers, given the nature of the symptoms and the relatively short period between exposure and follow-up. In addition, this categorisation of symptoms is similar to those used in the large prospective study conducted by the USEPA (Cabelli et

al. 1982) and therefore maximises the potential for comparison between the UK and US investigations.

The crude rates of subjective and objective GI symptoms are shown in Table 4.6. Inspection of Table 4.6 shows bathers to have higher rates of both categories of GI symptoms at all four study locations, which become statistically significantly higher when the data from all four sites are combined. Subsequent analysis was designed to show if gastrointestinal illness rates were affected by (I) indicator organism density (IOD), and (II) possible confounding non-water-related risk factors for gastroenteritis.

4.3.2 Indicator Organism Density and Illness Among Bathers

The indicator organisms measured were total coliform, faecal coliform, faecal streptococci, *Pseudomonas aeruginosa*, and total staphylococci. Measurements of each indicator organism were taken at three different depths: (I) the surf zone; (II) in waters approximately 30 cm deep; and (III) at "chest depth" (i.e. 1.3-1.4 m) where bather exposure actually took place. The initial analytical strategy was to compare rates of both subjective and objective GI symptoms among bathers at various levels of IOD with rates among non-bathers. The bather group was, initially, divided into categories of indicator organism exposure based on current or previous standards or, where standards could not be found, on the median IOD observed at all four locations. This was done prior to analysis to ensure objectivity in the choice of cut-points.

Total coliform exposure was divided into two categories: less than 2400 vv greater than 2400 organisms/100 ml. This grouping was based on the fact that these values were previously used as standards within the United States (Stevenson, 1953; New York City Department of Health, 1980). Faecal coliform exposure was divided as follows: exposure to less than 200 vv exposure to greater than 200 organisms/100 ml. These values were previously used as USEPA criteria for marine bathing waters (USEPA, 1976; USEPA, 1986). Faecal streptococci exposure was divided as follows: 0-34, 35-69, and 70+ organisms per 100 ml of sample. These cut-points reflect the current USEPA criterion for marine recreational waters (i.e. 35 per 100 ml) and a critical re-evaluation of this standard, based upon a re-analysis of the data from which it was derived (Fleisher, 1991). Since no quantitative information could be found in the literature regarding appropriate cut-points for Pseudomonas aeruginosa or total staphylococcl, the median levels observed at all study locations were used as cut-points of exposure. These levels correspond to 173 total staphylococci organisms per 100 ml and zero Pseudomonas aeruginosa organisms per 100 ml of sample. Separate analyses of contingency tables were undertaken at the State University of New York (SUNY) and at the University of Wales (UW), using different statistical packages (SAS and SPSS), to double check results.

Rates of GI illness among bathers whose exposures fell within the groupings described above were compared to rates of GI illness among non-bathers. This was done separately for (I) each indicator organism used, (II) each of the sampling depths used and (ill) both objective and subjective GI symptoms. The results for subjective and objective GI symptoms are shown in Tables 4.7-4.11. Inspection of Tables 4.7-4.11 shows that among the 25 comparisons yielding a statistically significant trend, 18 have illness rates such that bathers in the highest exposure group have rates of illness lower than bathers in the next lower exposure group. The p value for trend is based on the Mantel-Haenszel χ^2 statistic, which tests the alternative hypothesis that there is a linear association between the row variable and the column variable (SAS Institute, 1985). In assessing the existence of a dose-response relationship, it becomes important to ensure that the trend statistic is not being unduly influenced by a large difference between the reference group (non-bathers) and the lowest exposure group, while the risks among the different exposure levels remain constant. In this situation, the trend statistic can yield a statistically significant p value even though the only significant increase in risk occurs between the reference group and the group with the lowest exposure (Maclure and Greenland, 1992). A suggested method to ensure that this phenomenon is not occurring is to exclude the reference group and assess for trend among the remaining exposure groups (Breslow and Day, 1980). Further inspection of Tables 4.7-4.11 indicates that faecal streptococci sampled at "chest depth" is the only indicator organism to show an increasing statistically significant trend in illness rates with and without the inclusion of the reference group for objective GI symptoms (p < 0.001) and a nearly significant increasing trend in illness rates for subjective GI symptoms when the reference group is excluded from the analysis (p = 0.056 without reference group and p < 0.001 with the inclusion of the reference group). The results of contingency table analysis were identical from SUNY and UW.

These results strongly suggest that the faecal streptococci, when sampled close to the time and place where the actual bather exposure took place, were the only indicator organisms to be associated with an increased risk of gastroenteritis among bathers in marine waters contaminated with domestic sewage at the sites studied.

4.3.3 Non-Water-Related Risk Factors for Gastroenteritis

Table 4.12 lists the non-bathing-water-related risk factors tested as potential confounders of the relationship between gastroenteritis and increasing indicator organism density. χ^2 analysis was used to identify statistically significant risk factors among those listed in Table 4.12. Separate analyses were conducted comparing bathers vv non-bathers, and ill bathers (using both subjective and objective GI symptoms) vv bathers not reporting such symptoms. Where expected cell frequencies were less than five Fisher's Exact Test (2-tailed) was used in lieu of the Pearson χ^2 statistic. Age was grouped by 10-year intervals prior to analysis. The results of these analyses are shown in Tables 4.13-4.15.

4.3.4 Bather vv Non-Bather Contrasts

Due to the recent demonstration of site-specific associations between gastroenteritis and enterococci density (Fleisher, 1991), the data from each of the four study locations were initially analysed separately to see if the pooling of all data was appropriate. For the reasons discussed earlier, faecal streptococci densities derived from samples taken at "chest depth" were used as the indicator of exposure among bathers in all subsequent analyses.

The study cohort at each study location was divided into the following categories: (I) Non-bathers; (II) Bathers exposed to less than 35 faecal streptococci per 100 ml; (III) Bathers exposed to between 35-69 faecal streptococci per 100ml; and (IV) Bathers exposed to 70 or more faecal streptococci per 100 ml. The Mantel-Haenszel χ^2 statistic for linear trend was used to test for dose-response relationships over the four categories of exposure within each study location. Table 4.16 shows the results of these analyses. Examination of Table 4.16 reveals evidence of an increasing trend in both subjective and objective symptoms over increasing faecal streptococci density at all four study locations with the possible exception of objective GI symptoms at Southsea. A χ^2 analysis was then conducted to test whether rates of GI illness differed within each category of exposure between study locations. For subjective GI symptoms, only the 0-34 category showed a significant difference between rates of illness between study locations (p = 0.007). No statistically significant differences were seen for objective GI symptoms for any exposure category between study sites. It was therefore decided to pool the data from the four study locations in all subsequent analyses.

Multiple logistic regression analysis was used to assess the effect of the non-water-related risk factors shown in Table 4.13 on the four categories of exposure defined above. The results are shown in Table 4.17.

Table 4.17 shows the adjusted odds ratios to be slightly higher that the crude odds ratios for both subjective and objective symptoms. However, Table 4.17 exhibits little evidence of any appreciable amount of confounding.

To further delineate the faecal streptococci density at which bathers' rates of illness exceed those of non-bathers, the pooled data set was used to assess the occurrence of GI symptoms among non-bathers vv bathers at 20-unit intervals of faecal streptococci exposure. Table 4.18 shows the results of this analysis. With respect to subjective GI symptoms, Table 4.18 shows a statistically significant increase in risk among bathers exposed to 0-19 faecal streptococci, and among bathers exposed to 40 or more faecal streptococci relative to non-bathers. With respect to objective GI symptoms, Table 4.18 clearly shows that risk of illness among bathers does not exceed risk to non-bathers until faecal streptococci densities are above 39. Since the objective GI symptom category is less subject to bias caused by inter-individual perception of symptoms and differential recall among ill vv non-ill bathers, the relationship between objective GI symptoms and increasing faecal streptococci density should be given the greater weight.

4.3.5 Contrasts of Ill Bathers vv Well Bathers

Multiple logistic regression analysis of the pooled data set was used to model the relationship between increasing faecal streptococci densities and GI illness among bathers while controlling for non-water-related risk factors. Faecal streptococci density was modelled in 20-unit intervals, with densities less than 40 per 100 ml used as the reference category of exposure. Three indicator variables were constructed corresponding to exposure to 40-59, 60-79, and 80+ faecal streptococci per 100 ml. In order to make the logistic models more parsimonious, a composite variable was constructed that included any food items shown to be consumed significantly more often by ill w non-ill bathers plus any symptoms of illness that might predispose to gastroenteritis that were shown on univariate analysis to occur significantly more often among ill vv non-ill bathers. For subjective GI symptoms, the co-variates included in this composite variable were consumption of hamburgers, cold meat pies, or purchased sandwiches at Moreton, bathers suffering from unusual fatigue of stress lasting more than 24 hours within three weeks of the initial interview at Moreton and Southsea respectively. For objective GI symptoms, the composite variable included consumption of hamburgers or take-away foods at Moreton, consumption of purchased sandwiches at Langland Bay, bathers without any diagnosed chronic illness that reported a predisposition to diarrhoea at Langland Bay, or any bather suffering from diarrhoea or unusual fatigue lasting for more than 24 hours within three weeks of the initial interview. In addition, three indicator variables were used to assess any possible site-specific differences in risk in all logistic models. Except when testing for interaction among main effects or assessing the presence of confounding, a backward selection procedure was used. All logistic regression modelling was conducted using the BMDP package of statistical software (Dixon, 1988).

Table 4.19 shows the results of the multiple logistic regression analysis of subjective GI symptoms. Inspection of Table 4.19 shows study location, non-bathing-water-related risk factors for gastroenteritis, and gender to be statistically significant predictors of subjective GI symptoms. When modelled as a categorical variable, faecal streptococci density does not achieve statistical significance. When faecal streptococci at chest depth is modelled as a continuous variable, however, statistical significance is achieved (p = 0.0320). When modelled in this manner, the odds ratio for each 20-unit increase in faecal streptococci density equalled 1.40 (95% CI 1.03-1.90). The estimated odds ratios for the remaining variables shown in Table 4.19 remained unchanged. There was no statistically significant interaction between faecal streptococci density and study location, non-water-related risk factors, or gender. With regard to confounding, the odds ratio for subjective GI symptoms modelled as a continuous variable in terms of 20-unit intervals of faecal streptococci exposure equalled 1.21 prior to adjustment, but equalled

1.40 after adjusting for the effects of study location, non-water-related risk factors for subjective GI symptoms, and gender. This indicates a moderate degree of confounding as shown in Table 4.20. Indeed Table 4.20 shows that failure to adjust for the effects of study location, non-water-related risk factors, and gender would have resulted in an underestimate of the risk due to bathing by a minimum of 13.6% and a maximum of 35.7%. This finding has obvious implications in the interpretation of previously published studies and in the design of future epidemiological studies.

Table 4.21 shows the results of the logistic modelling using objective GI symptoms as the outcome variable. Table 4.21 demonstrates that faecal streptococci density, non-water-related risk factors, age, the occurrence of GI symptoms among family members that **preceded** the occurrence of gastroenteritis in the bather, and gender are all predictors of objective GI symptoms. Again, there was no statistically significant interaction between faecal streptococci density and the other predictors of objective GI symptoms shown in Table 4.21. It is interesting to note that a decreasing risk of gastroenteritis was observed to occur with increasing age, and that this relationship is independent of the effect of increasing faecal streptococci densities. This is in general agreement with the findings of a recent beach survey study (Balarajan *et al.*, 1991), which reported young adults to be at highest risk. The study seemed to imply that this increase in risk was related to increasing indicator organism density. Our finding of no significant interaction between age and faecal streptococci density renders age an *independent* predictor of gastroenteritis that is not related to bathing water quality.

The finding that females are 1.81 times more likely to report objective symptoms of gastroenteritis relative to males is surprising in that no biological basis for this seems plausible. It has been well established, however, that females seek medical care more often than males, so that this finding probably reflects an under-reporting of symptoms by males rather than true excess risk among females. The finding that bathers with family members acquiring GI symptoms that **preceded** their own were 4.44 times more likely to report objective GI symptoms than bathers whose family members did not have GI symptoms underscores the importance of controlling for non-bathing-water-related person-to-person transmission of gastroenteritis in bathing water studies. To our knowledge, the UK sea bathing investigations are the first to control for this risk factor.

In order to assess whether the risk factors shown in Table 4.21 confounded the relationship between increasing faecal streptococci levels and objective GI symptoms, the four categories of faecal streptococci exposure shown in Table 4.21 were modelled as a continuous variable. When modelled in this manner, the odds ratio for each 20-unit increase in faecal streptococci exposure equalled 1.58 (95% CI 1.22-2.06). Again, the odds ratios for the non-bathing-water-related risk factors remained unchanged (Table 4.21). The crude odds ratio for each 20-unit increase in faecal streptococci exposure equalled 1.62. This estimate, when compared to the adjusted odds ratio of 1.58 per 20-unit increase in faecal streptococci density, indicates that there was no appreciable confounding by the other risk factors for objective GI symptoms shown in Table 4.21.

4.3.6 The Effect of Duration of Exposure Among Bathers

In order to assess whether the time a bather spent in the water affected risk of gastroenteritis, the time each bather entered and left the water were recorded. A three-way analysis of variance procedure was computed to assess (I) the effect of duration of exposure on illness status, (II) the potential differences in duration among different study locations, and (III) the potential differences in duration of exposure to different levels of faecal streptococci density. This analysis was conducted for objective GI symptoms and is shown in Table 4.22. Inspection of Table 4.22 shows that the average time bathers spent in the water did not differ by illness status or differing faecal streptococci exposure, but did differ among study sites. Table 4.23 shows the results of the Student-Newman-Keuls Test on the three main effects of the analysis of variance procedure shown in Table 4.22. Inspection of Table 4.23 shows that bathers at the Southsea and Southend-on-Sea

study locations spent an average of 3-5 minutes less time in the water than bathers at the other two study locations. The fact that Table 4.23 indicates no relationship between the time bathers spent in the water and the occurrence of objective GI symptoms or with exposure to differing levels of faecal streptococci density, argues that even short exposures to bathing waters contaminated with domestic sewage will result in a clear and measurable health outcome. This is reinforced by the fact that the average time bathers spent in the water over all four study locations was 14.5 (+/-6.90) minutes. (The same analysis of variance procedure was computed for subjective GI symptoms, and yielded similar results).

4.3.7 The Effect of Swallowing Water on Bathing-Associated Illness

At both the Southsea and Southend-on-Sea studies bathers were asked if they had "swallowed any water" on leaving the water. At Southsea 82.6% of bathers reporting subjective GI symptoms reported swallowing water, while 81.2% of bathers not reporting subjective GI symptoms reported swallowing water (p = 0.84). For objective GI symptoms, the corresponding proportions were 70.0% vv 83.3% (p = 0.21).

At Southend-on-Sea, 72.0% of bathers reporting subjective symptoms reported swallowing water vv 44.2% of bathers not reporting subjective GI symptoms (p = 0.012). The corresponding proportions for objective GI symptoms were 77.8% vv 45.0% (p = 0.009).

The discrepancy in the effect of swallowing bathing water on bathing-associated gastroenteritis might be explained, in part, by the difference in the proportion of bathers exposed to 40 or more faecal streptococci organisms per 100 ml at these two study locations. At Southsea, only 11.2% of bathers were exposed to waters containing 40-59 faecal streptococci per 100 ml, while no bather was exposed to more than 59 organisms per 100 ml. At Southend-on-Sea, however, 13.8% of bathers were exposed to 40-59 faecal streptococci per 100 ml, while an additional 8.7% were exposed to faecal streptococci densities between 60-79 per 100 ml. Since prior analysis-shows increased risk of bathing-associated illness to occur at about 40 faecal streptococci organisms per 100 ml, it is possible that the difference in the proportion of bathers at the two study locations, who were exposed to densities over 40 per 100 ml, explains the observation that swallowing water is associated with gastroenteritis at Southend-on-Sea, but not at Southsea.

4.3.8 Defining the Threshold of Risk

Prior analysis of the association between increasing faecal streptococci densities and gastroenteritis among bathers presented in this report utilised broad groupings of faecal streptococci exposure. This was done to both reflect the limited precision inherent in current methods of indicator organism enumeration (Fleisher, 1980, 1985, 1990), and to illustrate the high degree of concordance between the logistic regression models and the results of cross-tabulation analyses. It is, however, necessary in the formulation of water quality criteria to define the threshold of risk in more precise terms than units of 20 faecal streptococci.

The previous analyses presented in this report strongly suggest that there is no increase in the risk of either subjective or objective symptoms of gastroenteritis until approximately 40 faecal streptococci organism per 100 ml of sample. In order to define the threshold of risk more precisely, logistic regression modelling of III vv non-iII bathers was used as the outcome variable while faecal streptococci density modelled as a continuous variable was used as the only main effect. This analysis was restricted to objective symptoms due to the fact that this category of symptoms is the more reliable category and that previous logistic regression modelling utilising units of 20 faecal streptococci per 100 ml showed that there was no confounding of the relationship between faecal streptococci density and objective GI symptoms by the non-bathing-

water-related risk factors incorporated in the model. Since all prior models indicated no excess risk among bathers exposed to 20-39 faecal streptococci per 100 ml, the median faecal streptococci density to which bathers were exposed within this exposure grouping was chosen as a cut-point on which to conduct separate logistic regression analysis, i.e., separate logistic models were computed for bathers exposed to less than 32 faecal streptococci per 100 ml and for bathers exposed to 32 or more faecal streptococci per 100 ml. Since faecal streptococci density was modelled as a continuous variable in these analyses, a square root transformation was necessary to ensure linearity in the logit.

The logistic model for bathers exposed to less than 32 faecal streptococci was not significant (likelihood ratio χ^2 = 0.67, p = 0.41). The logistic regression model for bathers exposed to 32 or more faecal streptococci organisms resulted in the following model:

$$v = a \sqrt{(x - 32)} + c$$
 4.2

where:

y = Ln odds of illness (Objective GI symptoms in this case)

x = Faecal streptococci density (per 100 ml)

 σ = slope coefficient (0.20102)

c = a constant (-2.3561).

The likelihood ratio χ^2 for the above model was 6.33, p=0.012. The model was then used to calculate the probability (p) of objective GI symptoms with increasing faecal streptococci exposure:

$$p = (1/(1+(\exp - m)))$$
 4.3

where:

$$m = a \sqrt{(x - 32)} + c$$
 (from equation 4.2).

It is interesting to note that the model predicts the probability of illness as 0.0866 at 32 faecal streptococci per 100 ml and a probability of 0.1039 at 33 faecal streptococci per 100 ml. When compared with the observed rate of objective symptoms among non-bathers (0.0975) the model indeed provides strong evidence that 33 faecal streptococci organisms per 100 ml is the threshold of increased risk. Figure 4.2 is a plot of the excess probability of acquiring an objective GI symptom (relative to the probability at 32 faecal streptococci) by increasing faecal streptococci density following the function:

$$XSp = (1/(1+(exp-m))) - p_{32}$$
 4.4

where:

 $m = a \sqrt{(x - 32)} + c$ (from equation 4.2) $p_{32} = \text{probability at 32 faecal streptococci per 100 ml (0.0866)}.$ The odds ratio (OR) provides a measure of relative risk to bathers exposed to 32 or more faecal streptococci:

$$OR = \exp m$$

where:

 $m = a\sqrt{(x - 32)}$ (see equation 4.2) x = Faecal streptococci density (per 100 ml).

This function is illustrated in Figure 4.3.

Table 4.24 and Figure 4.4 compare the excess probability of illness due to increasing faecal streptococci exposure among bathers with the probability of illness due to the non-water-related risk factors shown in Table 4.21. Table 4.24 shows risk to rise rapidly with increasing faecal streptococci density. It is also true, however, that the probability of illness due to exposure to bathing waters contaminated with domestic sewage (as measured by faecal streptococci density) must be viewed in the context of the competing risks represented in Table 4.24 when deciding upon appropriate bathing water criteria. Thus, there is a statistically significant risk of acquiring GI illness when faecal streptococci concentration exceeds 33 per 100 mi at chest depth. However this risk is considerably lower than that attributable to non-water-related risk factors such as GI illness in the household (Figure 4.4).

The multiple logistic regression analysis presented above was undertaken at SUNY. Further logistic regression analysis was undertaken at the CDSC Welsh unit using different software (Epi Info version 5 and MULTLR) as well as different variable selection routines and model building criteria. The results of these independent analyses were very similar in terms of the slope of the calculated dose-response relationships and the predicted probability of excess illness at a given faecal streptococci level. The relationships linking faecal streptococci exposure to gastroenteritis in bathers are robust. They could be verified by studying whether observed illness in bathers is at the levels which faecal streptococci measurements would predict prospectively.

4.4 Conclusions - Setting Standards for UK Bathing Waters

The interpretation of these results in terms of appropriate standards is important. It would be possible, for example, to suggest that the aim of any standard should be to restrict "risk" to that level which is normally experienced by members of the public (Kay, 1992; Jones and Kay, 1992; Fleisher et al., 1993). It could be argued that this is equivalent to the risk attributable to the non-water-related risk factors identified here which correspond to an exposure of approximately 73 faecal streptococci per 100 ml at chest depth (Figure 4.4). An equally plausible argument could be constructed to suggest that the standards might seek to ensure that no statistically significant excess risk of disease could be identified in the exposed (bather) compared to the control (non-bather) groups. This would imply a starting point for a "standard" of 33 faecal streptococci per 100 ml at chest depth as outlined above.

The results of this research provide objective information on which to base standards, or water quality objectives, for marine recreational waters as used by the average family group (NRA, 1991; Grantham, 1992; 1993). The limitation of risk, however, will always depend upon a clear understanding of the statistical distribution of "environmental" faecal streptococci concentrations at any site. The definition of a standard system will therefore require information in four main areas:

- (I) dose-response relationships
- (II) environmental variability in the chosen indicators at a range of relevant sites
- (III) a statistical definition of standard (i.e. geometric mean values, percentile and/or upper limit values for compliance)
- (IV) definition of the risk to which the population is exposed at each site under PASS and FAIL conditions (i.e. using data from (I), (II) and (III) above)

The last three stages outlined are beyond the scope of this report. However the authors believe that the analysis presented above provides sufficient epidemiological data for the competent authorities to design appropriate water quality objectives for "normal" water contact activities undertaken by adults in UK marine recreational waters. Further epidemiological data would be required before these objectives are extended to (1) special interest groups in the marine environment and/or (11) fresh recreational waters (Fewtrell et al., 1992; 1993).

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Acknowledgements

The authors' are grateful for the professional assistance and overall project supervision provided by Dr E. B. Pike of the Water Research Centre. A team of dedicated scientists, environmental health professionals and volunteer supervisors ensured the success of the final controlled cohort study at Southend-on-Sea. These included; Mr J. Beeby, Ms L. Boulton, Ms S. Cameron, Mr S. Casey, Prof. R. Cartwright, Mr J. Cranshaw, Mrs H. Davies-Casey, Ms H. Dawson, Ms H. Dawber, Dr A. Delahunty, Mr A. Eaton, Mrs L. Fewtrell, Mrs P. Hopkins, Ms A. Hughes, Mr E. Laurier, Mr A. Longford, Mr D. Lowthion, Dr H. Merrett, Ms R. Morano, Mr P. Naylor, Dr J. Norris-Hill, Mr K. Osborn, Mrs C. Pownall, Mr I. Sinclair, Ms S. Sumner, Mr C. Thomas, Mr A. West, Mrs C. Whittingham, Mr J. Whittingham, Dr P. Whittle, Ms J. Yeo.

We are indebted to the officers of Southend-on-Sea Borough Council for their extensive logistical and professional support for this project. We extend warm thanks to Mr L. Barker (Director of Community Services), Mrs S. Cooper (Community Services), Mr D. Connor (Community Services), Mr D. Reader (Engineering), Mr M. Pressling (Marketing Director), Mr I. Davies (Leisure Services), Mr A. Herbert (Environmental Health and St. John's Ambulance Brigade). Professional safety cover was provided by the St. John's Ambulance Brigade and the Southend-on-Sea Life Guards. Advice on medical and statistical aspects of the project were provided by the staff of PHLS Preston and the Communicable Disease Surveillance Centre (Welsh Unit), in particular Mr M. Hindle, Dr D. Hutchinson, Dr P. Morgan Capner, Dr R. Smith and Dr D. Thomas. We also thank those staff of St. David's University College who provided logistical support beyond the call of duty, in particular Mr K. Evans, Mr A. Jones, Mr E. Jones, Mr G. Jones, Mr V. Lote and Mr A. J. Rogers.

We thank the volunteers from the Southend-on-Sea area who took part in this final investigation, they gave freely of their time to demonstrate, yet again, a public willingness to assist in environmental research.

Finally we would like to offer our sincere thanks to the many hundreds of people involved in the four controlled cohort investigations over the past four years. The commitment of the volunteers and research teams alike have provided a great way forward in objective environmental decision making.

Tables



Table 3.1 Summary statistics for microbiological determinands, Southend-on-Sea , Thorpe Bay, 1992 bathing season

Variable	Arith. Mean	Std. Dev.	Min.	Max.	Geo. Mean	Log ₁₀ Std. Dev.	N
Total coliform	681.935	969.073	9	4300	280.190	0.640	62
Faecal coliform	348.306	580.105	0	2900	127.529	0.658	62
Faecal streptococci	220.800	554.155	9	2500	47.641	0.721	20

Units: count 100 mt⁻¹.

Table 3.2 Compliance with EC bathing waters directives Southend-on-Sea , Thorpe Bay, 1992 bathing season

Indicator	Imperative	Guide	N
	No. samples not exceeding: 2000 100 mt ⁻¹ (95% to comply)	No.samples not exceeding: 100 100 mF ¹ (80% to comply)	
Faecal coliform	60 (96.8%)	29 (46.8%)	62
	No. samples not exceeding: 10,000 100 ml ⁻¹ (95% to comply)	No.samples not exceeding: 500 100 ml ⁻¹ (80% to comply)	
Total coliform	62 (100%)	40 (64.5%)	62
	-	No.samples not exceeding: 100 100 ml ⁻¹ (90% to comply)	
Faecal streptococci		13 (65.0%)	20

Table 3.3 Summary statistics for microblological determinations (count 100 ml⁻¹) on samples taken at Southend, Thorpe Bay, 04.07.91.

Variable	Arith. Mean	Std. Dev.	Min.	Max.	Geo. Mean	Log10 \$td. Dev.	N
Total coliform	457.232	738.030	45	4126	280.190	0.363	82
Faecal coliform	209.869	208.168	2	1212	133.586	0.488	84
Faecal streptococci	156.655	838.199	0	7697	39.644	0.514	84
Pseudomonas aer.	31.607	124.551	1	720	2.177	0.615	84
Total staphylococci	306.229	328.770	ì	1000	133.586	0.685	48

(b) Surf samples

Variable	Arith. Mean	\$td. Dev.	Min.	Max.	Geo. Mean	Log ₁₀ Std. Dev.	N
Total coliform Faecal coliform Faecal streptococci Pseudomonas aer. Total staphylococci	818.037 267.071 356.429 46.536 414.688	1180.861 238.130 1442.791 152.777 373.923	72 36 3 1 8	4126 1118 7697 720 1000	418.759 189.546 57.614 3.074 222.872	0.466 0.375 0.617 0.734 0.603	27 28 28 28 28 16

(c) 30cm samples

Variable	Arith. Mean	Std. Dev.	Min.	Max.	Geo. Mean	Log ₁₀ Std. Dev.	N
Total coliform	337.889	273.886	54	1351	267.534	0.298	27
Faecal coliform	203.250	223.536	2	1212	129.317	0.495	28
Faecal streptococci	58.250	66.455	7	332	37.994	0.397	28
Pseudomonas aer.	43.750	151.171	1	720	2.428	0.698	28
Total staphylococci	339.438	326.130	9	920	169.608	0.623	16

(d) Chest samples

Variable	Arith. Mean	Std. Dev.	Min.	Max.	Geo. Mean	Log ₁₀ Std. Dev.	N
Total coliform Faecal coliform Faecal streptococci Pseudomonas aer. Total staphylococci	224.393 159.286 55.286 4.536 164.563	135.635 142.985 107.812 18.709 239.520	45 2 0 1	811 661 573 100 940	198.067 97.175 28.309 1.301 62.826	0.216 0.551 0.474 0.322 0.737	28 28 28 28 28 16

Units: count 100 ml⁻¹.

Compliance with EC bathing waters directives, Southend, Thorpe Bay 04.07.92Table 3.4

Indicator	Imperative	Guide	N
	No. samples not exceeding: 2000 100 ml ⁻¹ (95% to comply)	No.samples not exceeding: 100 100 ml ⁻¹ (80% to comply)	
Faecal coliform	28 (100%)	7 (25.0%)	27
	No. samples not exceeding: 10,000 100 ml ⁻¹ (95% to comply)	No.samples not exceeding: 500 100 mi ⁻¹ (80% to comply)	
Total coliform	27 (100%)	24 (88.9%)	27
	¢.€-	No.samples not exceeding: 100 100 ml ⁻¹ (90% to comply)	
Faecal streptococci		26 (92.9%)	28

Table 3.5 Faecal sample consistency

Status	Bathers	Non Bathers	
Solld	97	109	_
Semi solid	49	6 9	
Liquid	10	8	<i>p</i> =0.429

9

Table 4.1 Results of Student-Newman-Keuls multiple range tests, by site, Surf depth samples						
Total coliforms						
Geo. mean (count 100ml ⁻¹)	Site	Langland	Southsea	Moreton	Southend	
82.907	Langland					
207.497	Southsea	•				
326.039	Moreton	•	•			
419.759	Southend	•	•			
Faecal coliforms		-				
Geo. mean	Site	Langland	Moreton	Southend	Southsea	
(count 100ml ⁻¹)		J				
46.621	Langland	· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·			
161.443	Moreton	•				
189.546	Southend	•				
198.894	\$outhsea	•				
Faecal streptoco Geo. mean (count 100ml ⁻¹)	cci Site	Moreton	Langland	Southsea	Southend	
27.747	Moreton					
45.666	Langland	•				
51.360	Southsea	•				
57.627	Southend	•				
Pseudomonas ae	eruginosa	. ,				
Geo. mean (count 100ml ⁻¹)	Site	Langland	Southend	Moreton	Southsea	
0.622	Langland					
3.075	Southend	•				
9.718	Moreton	•	•			
10.830	Southsea	•	•			
Total staphyloco	cci					
Geo. mean	Site	Moreton	Southend	Southsea		
(count 100ml ⁻¹)						
135.207	Moreton					
135.207 222.872 791.866	Moreton Southend Southsea					

^{*} denotes significant difference in geometric mean concentration (count 100 mir 1) between sites at α = 0.05.

Table 4.2	Results of Student-Newman-Keuls multiple range tests, by site, 30 cm depth samples						
Total coliforms							
Geo. mean (count 100ml ⁻¹)	Site	Langland	Southsea	Southend	Moreton		
49.026	Langland	•					
139.378 308.030	Southsea Southend	•	•				
314.283	Moreton	•	•				
Faecal coliforms							
Geo. mean	Site	Langland	Southend	\$outhsea	Moreton		
(count 100ml ⁻¹)		•					
39.281	Langland			· -			
129.317	Southend	•					
141.922	Southsea	•					
160.287	Moreton						
Faecal streptoco	cci						
Geo. mean	Site	Moreton	Southsea	Southend	Langland		
(count 100ml ⁻¹)							
29.304	Moreton						
36.420	Southsea						
37.994	Southend		No significan	t differences			
43.844	Langland		-	34.19.1.19.1			
Pseudomonas ae	əruginosa						
Geo. mean	Site	Moreton	Southsea	Southend	Langland		
(count 100mi ⁻¹)							
0.216	Langland	_					
2.427	Southend	•					
5.937	Southsea	•	•				
6.130	Moreton		<u> </u>				
Total staphyloco	ccł						
Geo. mean	Site	Moreton	Southend	Southsea			
(count 100ml ⁻¹)							
123.022	Moreton						
123.022 169.530 452.628	Moreton Southend Southsea		_				

^{*} denotes significant difference in geometric mean concentration (count 100 ml⁻¹) between sites at α = 0.05.

Table 4.3 Results of Student-Newman-Keuls multiple range tests, by site, Chest depth samples Total coliforms Geo. mean Site Langland Southend Southsea **Moreton** (count 100ml⁻¹) 34.711 Langland 71.644 Southsea 168.434 **Moreton** 198.113 Southend Faecal coliforms Site Geo. mean Langland Southsea Southend **Moreton** (count 100ml⁻¹) 14.014 Langland 74.214 Southsea 97.243 Southend 148.589 Moreton Faecai streptococci Geo. mean Site Southsea Moreton Southend Langland (count 100ml⁻¹) 21.751 Southsea 23.714 Moreton 28.309 Southend No significant differences 31.769 Langland Pseudomonas aeruginosa Geo. mean Site Langland Southend Moreton Southsea (count 100ml⁻¹) 0.212 Langland 1.301 Southend 1.531 **Moreton** 3.221 Southsea Total staphylococci Geo. mean Site Southend Southsea Moreton (count 100ml⁻¹) 62.885 Southend 147.662 Moreton 428.536 **Southsea**

^{*} denotes significant difference in geometric mean concentration (count 100 ml⁻¹) between sites at $\alpha = 0.05$.

Table 4.4 Summary of significant results (RR, 95%CI) of crude Bather vv Non-bather comparisons from the post exposure stages of the healthy volunteer studies

	1 st post exp	oosure		4		2 nd post ex	posure	
Langland	Moreton	Southsea	Southend		Langland	Moreton	Southsea	Southend
a. Single symp	otoms							
O	0	1	Parlada akam	•	Disado o se		Newson	Cara threat
Sore throat 2.08(1.01-4.27)	Sore throat 3.01(1.50-6.05)	Loose motions 1.56(1.01-2.40)	Ear infection 5.10(1.48-17.60)		Diarrhoea 3.22(1.22-8.55)		Nausea 3.7(1.65-8.32)	Sore throat 1.61(1.08-2.41)
Eye infection	Dry cough	Nausea		4				Ear infection
8.25(1.09-65.02)				1				3.94(1.49-10.44)
Ear Infection*	Ear infection							
(p=0.03)	5.38(1.18-24.49)							
	Stomach pain							
	2.77(1.09-7.02)							
	Loose motions							
	2.3(1.15-4.60)							
b. Symptom gre	oups†							
		•				IP9 4 4 1	0.1	•
	'Flu/cold 2.26(1.4-3.65)	GI 1.76(1.31-2.38)	Ear/eye 2,86(1.22-6.73)			'Flu/cold 1.40(1.08-2.75)	GI 1.51(1.11-2.06)	Ear/eye 2.78(1.37-5.64)
	2.20(1.4 0.00)	1.70(1.01 2.00)	2.00(1.22 0.70)	1		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	7,6 7(11.7 2,66)	2,, 0(1,0, 0,0,)
	Chest		GI	,		GI	Skin	
	1.83(1.04-3.23)		1.81(1.22-2.71)	T		1.57(1.01-2.44)	1.97(1.02-3.84)	
	GI		Skin	1				7
	1.70(1.06-2.72)		2.50(1.16-5.38)	•				

cell count zero, RR not calculable
 t Symptom groups not applicable for the Langland study

Table 4.5 Mean age of bathers and non-bathers by study location

Study Location	Status	Mean age	P
Langland Bay	Bather Non-bather	3 6.5 3 5.8	0.70
Moreton	Bather Non-bather	33.4 36.5	0.10
Southsea	Bather Non-bather	33.6 29.8	0.003†
Southend on Sea	Bather Non-bather	38.4 38.1	0.83

[†] significant at α =0.05.

Table 4.6 Site specific rates of gastroenteritis

Stite	Bathers	hers Non-bathers	
a. Subjective sympton	ns ‰		
Langland Bay	234	156	0.13
Moreton	153	117	0.41
Southsea	294	177	0.01 3 †
Southend on Sea	181	139	0.32
All sites combined	223	147	0.001†
b. Objective symptom	าร ‰		
Langland Bay	207	131	0.12
Moreton	133	71	0.11
Southsea	131	116	0.67
Southend on Sea	130	79	0.14
All sites combined	148	97	0.010†

[†] significant at α =0.05.

Table 4.7 Subjective and objective GI symptoms among non-bathers, bathers exposed to less than 2400 total coliforms per 100 ml and bathers exposed to greater than 2400 total coliforms per 100 ml

		Subjective GI		Objective GI	
Status	N	Rate ‰	p (Trend)	Rate ‰	p (Trend)
a. Surf zone					
Non-bathers	605	147	0.004†	97	0.016†
Bathers 0-2399	472	229		150	
Bathers 2400+	31	161		129	
		Bathers on	y p = 0.38	Bathers or	p = 0.75
b. 30 cm					
Non-bathers	605	147	0.004†	97	0.030†
Bathers 0-2399	503	225		149	
Bathers 2400+	1	0		00	
		Bathers only $p = 0.59$		Bathers or	p = 0.77
c. Chest depth			<u> </u>		
Non-bathers	605	147	0.001†	9.7	0.010†
Bathers 0-2399	506	223	·	14.8	·
Bathers 2400+	0	0		0	
		·····			

[†] significant at α =0.05.

Table 4.8 Subjective and objective GI symptoms among non-bathers, bathers exposed to less than 200 faecal coliforms per 100 ml and bathers exposed to greater than 200 faecal coliforms per 100 ml

505 277 230	147 242 200	p (Trend) 0.014†	Rate %	p (Trend)
277	242	0.014†	97	
277	242	0.014†	97	
			, ,	0.24
230	200		184	
	200		104	
	Bathers only $p = 0.26$		Bathers only $p = 0.0$	
_				
505	147	0.004†	97	0.029†
324	225		151	
183	219		142	
	Bathers only $p = 0.86$		Bathers only $p = 0.78$	
505	147	0.029†	97	0.041†
414	239		152	
88	148		125	
	Bathers on	ly p = 0.062	Bathers	only $p = 0.51$
	114	505 147 114 239 88 148	505 147 0.029† 114 239	505 147 0.029† 97 414 239 152 88 148 125

[†] significant at α =0.05.

Table 4.9 Subjective and objective GI symptoms among non-bathers, bathers exposed to less than 34, 35-69 and greater than 70 faecal steptococci per 100 ml

		Subjec	tive GI	Objecti	ve Gl
Status	N	Rate ‰	p (Trend)	Rate ‰	p (Trend)
a. Surf zone					
Non-bathers	605	147	0.002†	97	0.009†
Bathers 0-34	143	209		126	
Bathers 35-69	170	223	10.14	159	
Bathers 70+	194	232		155	
		Bathers onl	p = 0.63	Bathers	only $p = 0.49$
b. 30 cm					
Non-bathers	605	147	0.002†	97	0.015†
Bathers 0-34	160	175		119	
Bathers 35-69	245	278		180	
Bathers 70+	102	167		118	
		Bathers on	ly $p = 0.77$	Bathers	only $p = 0.74$
c. Chest depth					
Non-bathers	605	147	<0.001†	97	<0.001†
Bathers 0-34	307	205		111	
Bathers 35-69	149	215		161	
Bathers 70+	51	353		333	
		Bathers on	ly $p = 0.056$	Bathers or	p = < 0.001 + 0.001

t significant at α =0.05.

Table 4.10 Subjective and objective GI symptoms among non-bathers, bothers exposed to less than 173 Total staphylococci per 100 ml and bathers exposed to greater than 173 Total staphylococci per 100 ml*

		Subjective GI		Objective GI		
Status	N	Rate ‰	p (Trend)	Rate ‰	p (Trend)	
a. Surf zone						
Non-bathers	483	145	0.001†	89	0.036†	
Bathers 0-172	107	168		131		
Bathers 173+	230	252		139		
		Bathers only $p = 0.087$		Bathers only $p = 0.84$		
b. 30 cm			· · · · · · · · · · · · · · · · · · ·	_		
Non-bathers	483	145	0.001†	89	0.044†	
Bathers 0-172	102	176		137		
Bathers 173+	235	247		136		
		Bathers only $p = 0.16$		Bathers only $p = 0.98$		
c. Chest depth						
Non-bathers	483	14.5	0.001	8.9	0.086	
Bathers 0-172	107	18.7		15.9		
Bathers 173+	230	24.3		12.6		
		Bathers on	ly $p = 0.25$	Bathers on	ly p = 0.41	

Langland Bay study location excluded from this analysis Total staphylococci not measured at Langland Bay.

[†] significant at α =0.05.

Table 4.11 Subjective and objective GI symptoms among non-bathers, bathers exposed to zero *Pseudomonas aeruginosa* organisms per 100 ml and bathers exposed to one or more *Pseudomonas aeruginosa organism* per 100 ml

		Subjective GI		Objective GI		
Status	N	Rate ‰	p (Trend)	Rate ‰	p (Trend)	
a. Surf zone						
Non-bathers	605	147	0.004†	97	0.078	
Bathers @ 0	108	250		213		
Bathers 1+	399	215		130	-	
		Bathers only $p = 0.45$		Bathers only $p = 0.032$		
b. 30 cm					-	
Non-bathers	605	147	0.003†	97	0.072	
Bathers @ 0	111	243		207		
Bathers 1+	396	217		131		
		Bathers only $p = 0.56$		Bathers only $p = 0.047$ †		
c. Chest depth				<u> </u>		
Non-bathers	605	147	0.004†	97	0.078	
Bathers @ 0	108	250		213		
Bathers 1+	399	215		130		
		Bathers on	ly p = 0.45	Bathers onl	y p = 0.032†	

[†] significant at α =0.05.

Age (Grouped by 10 year intervals)

Gender

History of migraine headaches

History of stress or anxiety frequency of Diarrhoea (often, sometimes, rarely, or never)

Current use of prescription drugs

Illness within 4 weeks prior to trial day lasting more than 24 hours

Use of prescription or over-the-counter drugs within 4 weeks of the trial day

Consumption of foods containing fresh mayonnaise

(3 days prior to or 7 days after trial day)

Consumption of purchased sandwiches

(3 days prior to or 7 days after trial day)

Consumption of chicken

(3 days prior to or 7 days after trial day)

Consumption of eggs

(3 days prior to or 7 days after trial day)

Consumption of hamburgers

(3 days prior to or 7 days after trial day)

Consumption of hot dogs

(3 days prior to or 7 days after trial day)

Consumption of raw milk

(3 days prior to or 7 days after trial day)

Consumption of meat pies

(3 days prior to or 7 days after trial day)

Consumption of sea food

(3 days prior to 7 days after trial day)

Illness in household within 3 weeks after trial day

Additional bathing within 3 days prior to and 3 weeks after trial day*

Frequency of usual alcohol consumption

Taking of laxatives within 4 weeks of trial day

Taking of other stomach remedies within 4 weeks of trial day

This was included in order to control for possible confounding due to multiple exposures among bathers and exposure among non-bathers prior to or after the trial day.

Table 4.13 Non-water-related risk factors, bathers vv non-bathers

	Percentage with Risk factor				
Risk factor	Site	Bathers	Non-Bathers	P	
Predisposition to diarrhoea*	all	11.1	7.5	0.037†	
Indigestion lasting more than 24 hours within 4 weeks of initial interview	all	8.5	5.0	0.019†	
Taking of prescription or non-prescription drugs within 4 weeks of initial interview	all	44.0	50.3	0.035†	
Bathing within 3 weeks of trial date	f all	24.7	18.3	0.011†	
Gender (% males)	all	54.4	47.5	0.022†	
Food items¶:					
Mayonnaise	Langland Southend- on-Sea	21.5 25.5	8.5 12.2	0.006† 0.003†	
Raw milk	Southsea	23.3	8.6	0.0003†	
Seafood	Southend- on-Sea	30.1	15.8	0.003†	
Meat pies	Moreton	38.1	24.3	0.023†	
Purchased sandwiches	Moreton Southend- on-Sea	21.6 45.2	59.1 32.1	<0.0001† 0.024†	

Predisposition to diarrhoea = having diarrhoea at least once per month vv having diarrhoea less than twice per year.

[¶] Ail food items were consumed within the time period of within 3 days prior to exposure or within 7 days subsequent to the exposure day.

[†] significant at α =0.05.

Table 4.14 Non-water-related risk factors, ill bathers vv well bathers - Subjective GI symptoms

	Percentage with Risk factor				
Risk factor	Site	III Bathers	Well Bathers	P	
Bathing within 7 days subsequent to trial date	all	21.4	13.6	0.045†	
Unusual fatigue lasting for more than 24 hours within 3 weeks of initial interview	Moreton	20.0	2.4	0.025†	
Unusual stress or anxiety lasting for more than 24 hours within 3 weeks of					
initial interview	Southsea	6.5	0	0.023†	
Gender (% females)	all	61.1	41.1	<0.0001†	
GI symptoms in family members*	all	5.3	1.8	0.047†	
Food items¶:					
Hamburgers	Moreton	64.3	25.3	0.009†	
meat pies	Moreton	60.0	32.5	0.042†	
Purchased sandwiches	Moreton	42.9	18.1	0.022†	

GI symptoms among family members that preceded any GI symptoms that occurred among individual bathers.

[¶] All food items were consumed within the time period of within 3 days prior to exposure or within 7 days subsequent to the exposure day.

[†] significant at α =0.05.

Table 4.15 Non-water-related risk factors, ill bathers vv well bathers - Objective GI symptoms

	Percentage with Risk factor				
Risk factor	Site	III Bathers	Well Bathers	P	
Predisposition to diarrhoea*	Langland	8.7	0	0.041†	
Diarrhoea lasting for more than 24 hours within 3	÷	* * *	ġ.		
weeks of Initial interview	ail	17.3	8.1	0.012†	
GI symptoms in family members §	all	6.7	1.8	0.015†	
Gender (% females)	all	57.3	43.5	0.027†	
Unusual fatigue lasting for more than 24 hours within 3 weeks of initial interview	Moreton	13.3	6.3	0.030†	
Food items¶:					
Hamburgers Take-out foods Purchased sandwiches	Moreton Moreton Langland	66.7 76.9 39.1	25.9 41.7 19.3	0.004† 0.018† 0.046†	
Age					
Less than 25 25 -34 35 - 44 45 - 54 55 and over	all	16.3 17.8 14.3 9.1 7.4	-	p (trend) = 0.044†	

Predisposition to diarrhoea = having diarrhoea at least once per month vv having diarrhoea less than twice per year.

[§] GI symptoms among family members that preceded GI symptoms experinced by individual bathers.

[¶] All food items were consumed within the time period of within 3 days prior to exposure or within 7 days subsequent to the exposure day.

[†] significant at α =0.05.

Subjective and objective GI symptoms, non-bathers vv bathers by units of 35 faecal streptococci per 100 ml exposure

		Symptoms	‰ stucty subject	ets	
	Langland Bay	Moreton	Southsea	Southend -on-Sea	All sites combined
a. Subjective	GI symptom	\$			
Non-Bathers	156	117	177	139	147
Bathers 0-34	167	105	302	159	205
Bathers 35-69	196	151	268	263	215
Bathers 70+	355	500		250	353
p (Trend) =	0.032†	0.051	0.042†	0.14	<0.001†
b. Objective	GI symptoms	3			-
Non-Bathers	131	71	116	79	97
Bathers 0-34	125	70	143	93	111
Bathers 35-69	179	151	98	263	161
Bathers 70+	323	500		250	333
p (Trend) =	0.024†	0.002†	0.96	0.011†	<0.000†

[†] significant at α =0.05.

Table 4.17 Multiple logistic regression estimates of odds ratios of subjective and objective GI symptoms with and without adjustment for non-water-related risk factors, bathers vv non-bathers

	Crude Odds Ratio	95% CI	Adjusted Odds Ratio	95% CI
a. Subjective (GI symptoms			
Non-Bathers	1.00		1.00	
Bathers 0-34	1.53	1.05 - 2.22	1.58	1.07 - 2.33
Bathers 35-69	1.49	0.92 - 2.42	1.64	0.99 - 2.72
Bathers 70+	3.01	1.44 - 5.85	3.81	1.84 - 7.88
b. Objective 6	SI symptoms	· · · · · · · · · · · · · · · · · · ·		
Non-Bathers	1.00		1.00	
Bathers 0-34	1.16	0.72 - 6.75	1.18	0.73 - 1.91
Bathers 35-69	1.84	1.07 - 3.15	1.94	1.11 - 3.36
Bathers 70+	4.31	2.15 - 8.61	5.29	2.58 - 10.82

Table 4.18 Subjective and Objective GI symptoms, non-bathers vv bathers, by 20 unit intervals of faecal streptococci exposure per 100 ml - all sites combined

Exposure status	Subjective GI Rate ‰	Exposure status	Objective GI Rate ‰
Non-bather	147	Non-bather	86
Bather 0-19	239†	Bather 0-19	87
Bather 20-39	167	Bather 20-39	99
Bather 40-59	219†	Bather 40-59	171†
Bather 60-79	281 †	Bather 60-79	281†
Bather 80+	348†	Bather 80+	217†
p	(Trend) = 0.001		p (Trend) < 0.0001

[†] Indicates rates significantly different relative to non-bather rates (p < 0.05)

Table 4.19 Logistic regression analysis of subjective GI among bathers

Variable	Likelihood Ratio χ^2	P	Odds Ratio	.95% CI
Faecal			į.	<u></u>
Streptococci*	4.81	0.19		
0-39			1.00	
40-59			1.24	0.65 - 1.54
60-79			1.90	0.87 - 4.19
80+			3.00¶	1.02 - 8.83
			1	
Study Location	16.30	0.001		
Langland			1.00	
Moreton			0.28	0.10 - 0.80
Southsea			1.49	0.69 - 3.22
Southend			1.05	0.50 - 2.21
Non-Water §	10.79	0.001	3.51	1.62 - 7.57
Gender †	12.09	0.0005	2.21	1.40 - 3.47

Faecal streptococci density per 100 ml of sample

Table 4.20 Odds ratio for aquiring a subjective GI symptom before and after adjustment for confounding factors*

Faecal streptococci exposure (per 100 ml)	Crude Odds Ratio	Adjusted Odds Ratio	% Change
0 - 39	1.00	1.00	
40 - 59	1.21	1.40	13.6%
60 - 79	1.46	1.96	25.5%
80+	1.76	2.74	35.7%

Adjusted for the effects of study location, non-water-related risk factors for gastroenteritis and gender.

[¶] P(Trend) = 0.032

[§] Non-Water = Consumption of hamburgers, cold meat pies, or purchased sandwiches at the Moreton study location, or bathers suffering from unusual fatigue or unusual stress at the Moreton and Southsea study locations respectively

[†] Reference group - males.

Table 4.21 Logistic regression analysis of objective GI among bathers

Variable	Likelihood Ratio χ ²	P	Odds Ratio	95% CI	
Faecal	1.00	0.000			
Streptococci* 0-39 40-59 60-79 80+	11.83	0.008	1.00 1.91 2.90 - 3.17¶	1.60 - 2.28 1.43 - 5.88 1.12 - 8.97	
Non-Water§	3.54	0.06	1.72	0.98 - 2.99	
Age#	3.66	0.056	O. 8 1	0.65 - 1.01	
GI Symptoms in Family Member		0.02	4.44	1.34 - 14.64	
Gender †	5.09	0.02	1.81	1.08 - 3.04	

Faecal streptococci density per 100 ml of sample.

- § Non-Water = Consumption of hamburgers or take- out foods at Moreton; consumption of purchased sandwiches at Langland Bay; Bathers having a predisposition to diarrhoea at Langland Bay; or bathers suffering from diarrhoea or unusual fatigue which lasted for more than 24 hours within 3 weeks of the initial interview from all studies.
- # Modelled continuously in intervals of 10 years.
- † Objective GI symptoms in family members that preceded any symptoms in individual bathers.
- † Reference group males.

[¶] p (Trend) = 0.009.

Analysis of variance of time individual bathers spent in the water by illness status (objective GI symptoms), study site and exposure to faecal streptococci

Source	Degrees of freedom	Sum of Squares	Mean of squares	F	p
Illness Status*	1	33.64	33.64	0.78	0.38
Study Site	3	2204.99	734.99	17.01	0.0001
Faecal Strept Density¶	ococci 4	304.41	76.10	1.76	0.13
Error	485	20962.17	43.22		

^{*} Compares bathers with objective GI symptoms with bathers without objective GI symptoms.

Table 4.23 Student-Newman-Keuls test on main effects of analysis of variance of time spent in the water (Table 4.22)

Main effect	Mean duration of exposure (minutes)*
Objective GI symptoms	
No	14.63
Yes	13.89
Study location	
Moreton	17.70
Langland Bay	16.71
Southend-on-Sea	13.80
Southsea	12.11
Faecal streptococci	
density exposed to (per 100 ml)	<i>)</i> :
0-19	13.36
20-39	14.79
40-59	16.20
60-79	14.89
80+	15.22

[•] Means <u>not</u> connected by a line are significantly different (p < 0.05).

[¶] Faecal streptococci densities (per 100 ml) grouped by 20 units of exposure (0-19, 20-39, 40-59, 60-79, 80+).

Table 4.24 Comparison between the excess probability of objective GI symptoms among bathers exposed to 33 or more faecal streptococci and the probability of acquiring objective GI symptoms due to non-water-related risk factors (see Figure 4.4)

Faecal streptococci exposure*	Excess Probability	Non-water- related risk factor¶	GI illness in household§	Age	Probability
33	0.02	0.17	0.34	18-25	0.09
40	0.06			25-34	0.07
50	0.09			35-44	0.06
60	0.13			45-44	0.05
70	0.16			55+	0.04
80	0.19				
90	0.22				
100	0.24				

Faecal streptococci per 100 ml of sample, modelled continuously.

- ¶ Non-Water = Consumption of hamburgers or take-out foods at Moreton; consumption of purchased sandwiches at Langland Bay; Bathers having a predisposition to diarrhoea at Langland Bay; or bathers suffering from diarrhoea or unusual fatigue lasting for more than 24 hours within 3 weeks of initial interview from all studies.
- § GI illness in household that preceded an Individual bather's GI illness.

Figures



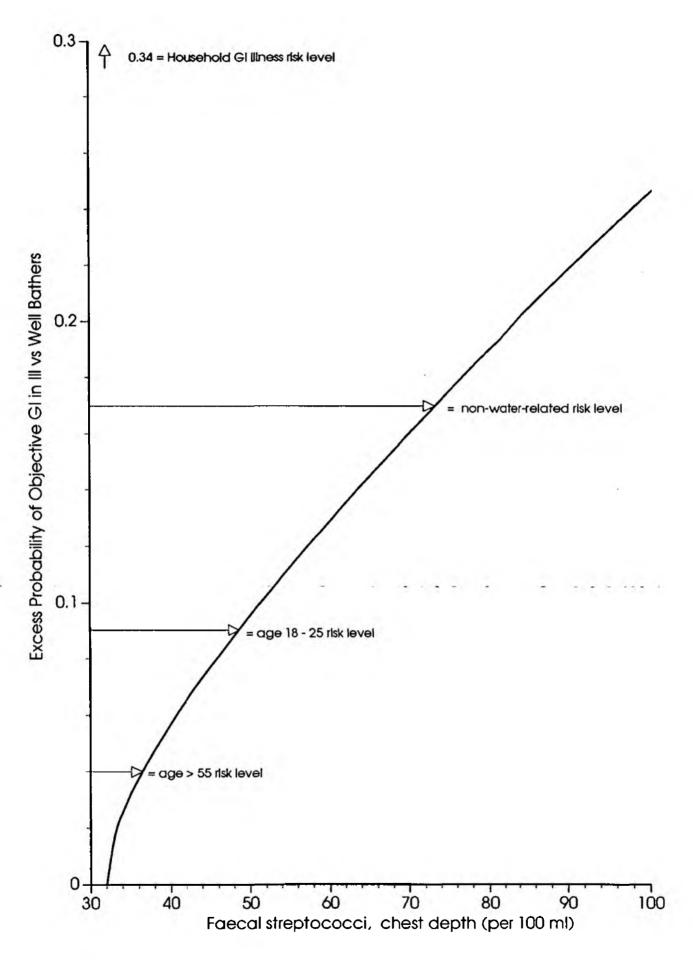


Figure 1 Comparison of riskof objective gastroenteritis from different sources with risk associated with exposure to sewage contaminated sea water indexed by faecal streptococci (per 100 ml) at chest depth

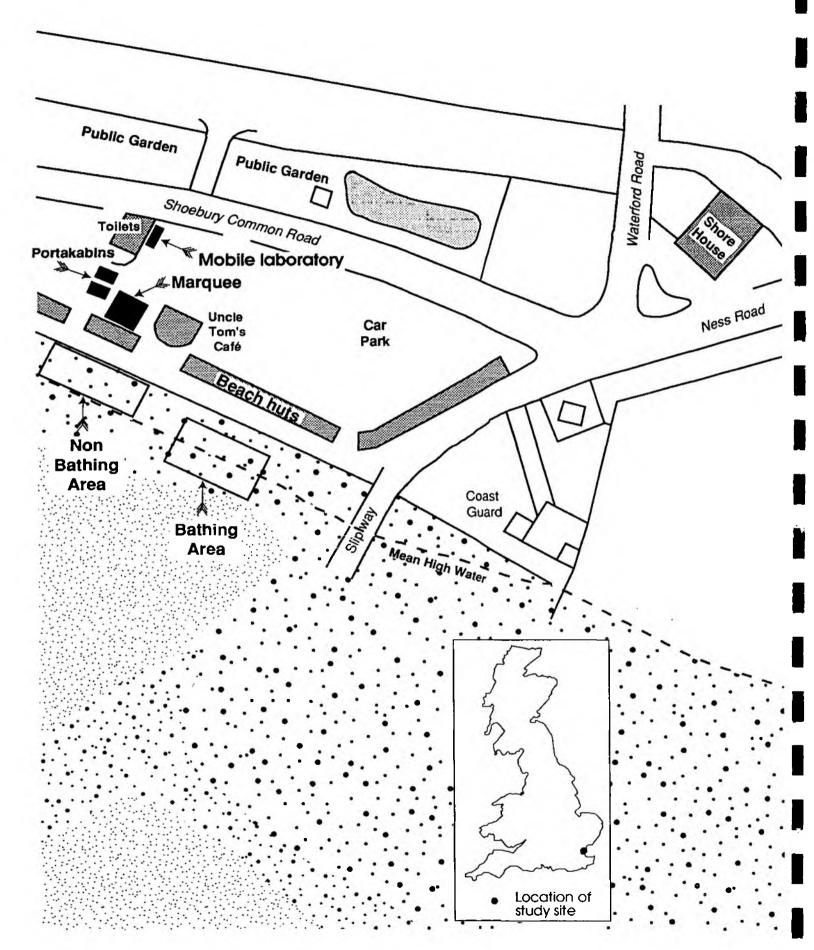


Figure 3.1 Schematic map of the study site

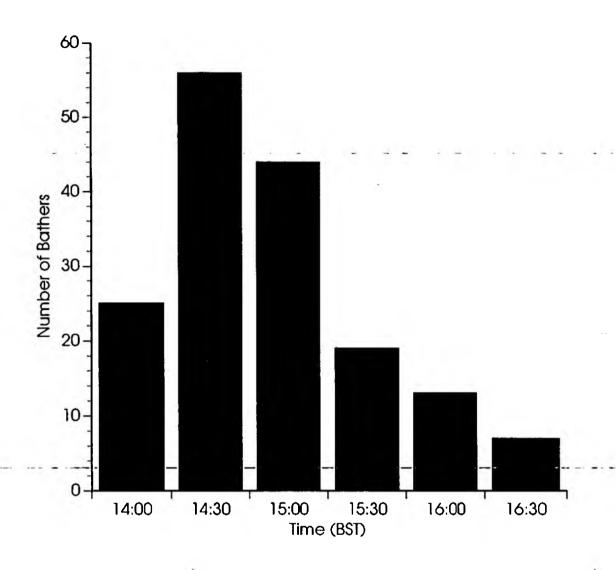


Figure 3.4 Bather density in the bathing area Southend-on-Sea, Thorpe Bay, 04.07.92

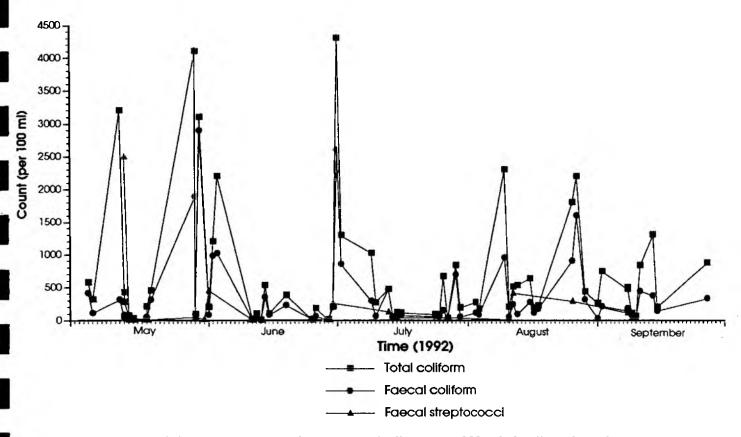


Figure 3.2 Indicator organism concentrations (per 100 ml) Southend-on-Sea, Thorpe Bay, 1992 bathing season

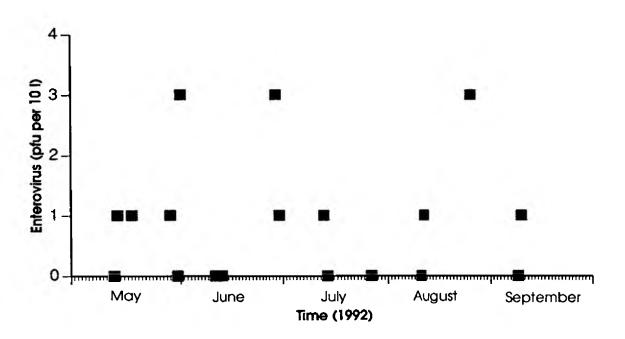


Figure 3.3 Enterovirus concentrations (pfu per 10 l) Southend-on-Sea, Thorpe Bay, 1992 bathing season

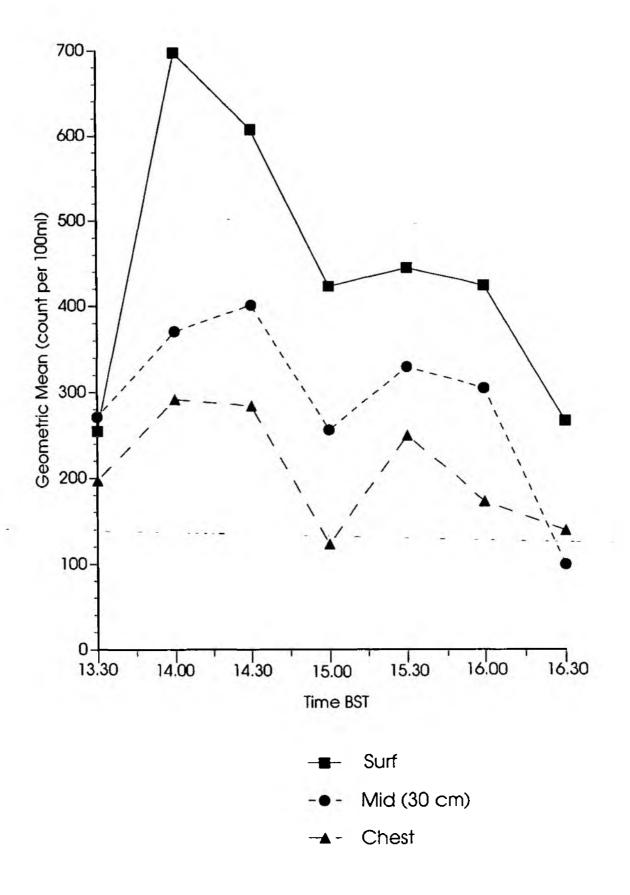


Figure 3.5 Geometric mean total coliform concentrations (count per 100 ml) Southend-on-Sea, Thorpe Bay, 04.07.92

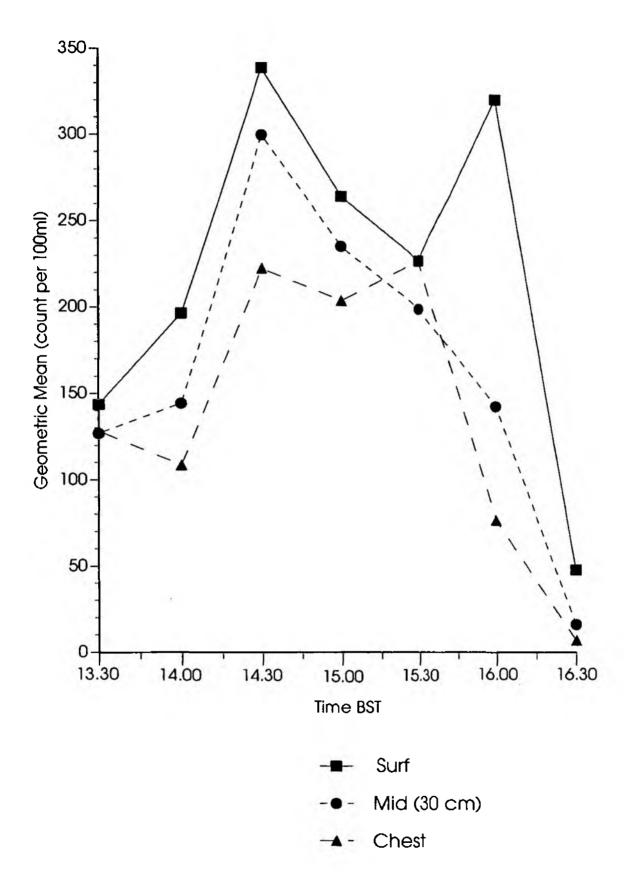


Figure 3.6 Geometric mean faecal coliform concentrations (count per 100 ml) Southend-on-Sea, Thorpe Bay, 04.07.92

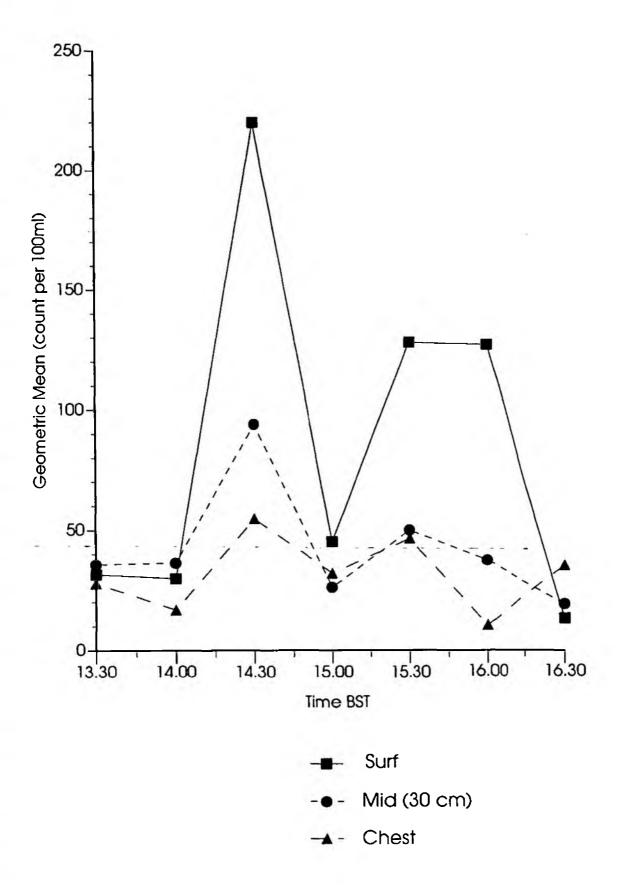


Figure 3.7 Geometric mean faecal streptococci concentrations (count per 100 ml) Southend-on-Sea, Thorpe Bay, 04.07.92

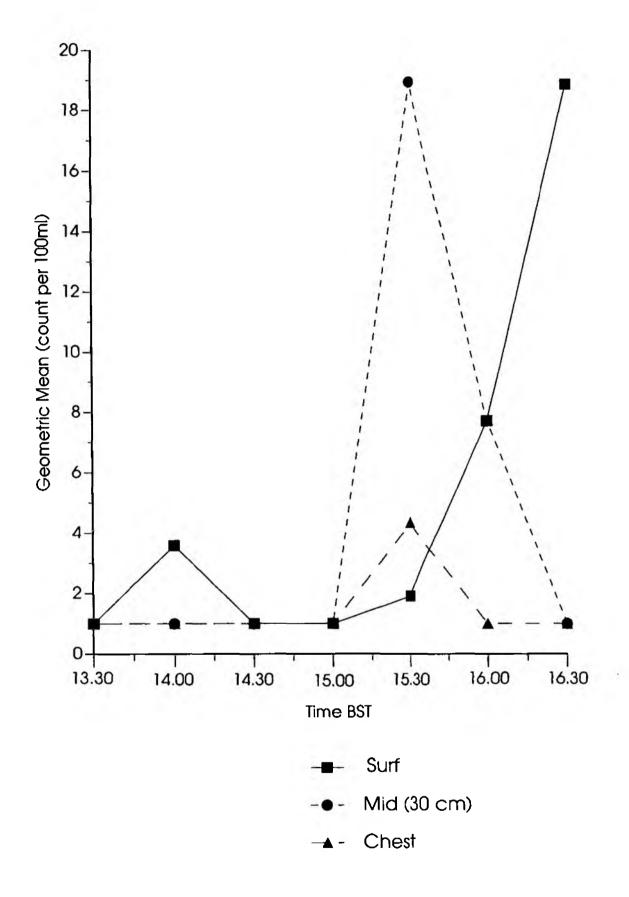


Figure 3.8 Geometric mean *Pseudomonas aeruginosa* concentrations (count per 100 ml) Southend-on-Sea, Thorpe Bay, 04.07.92

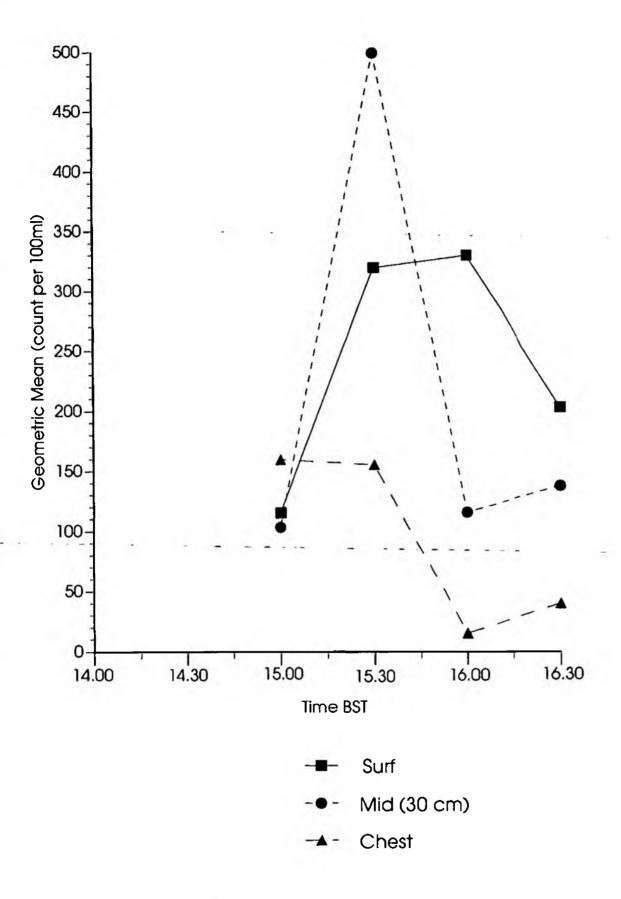
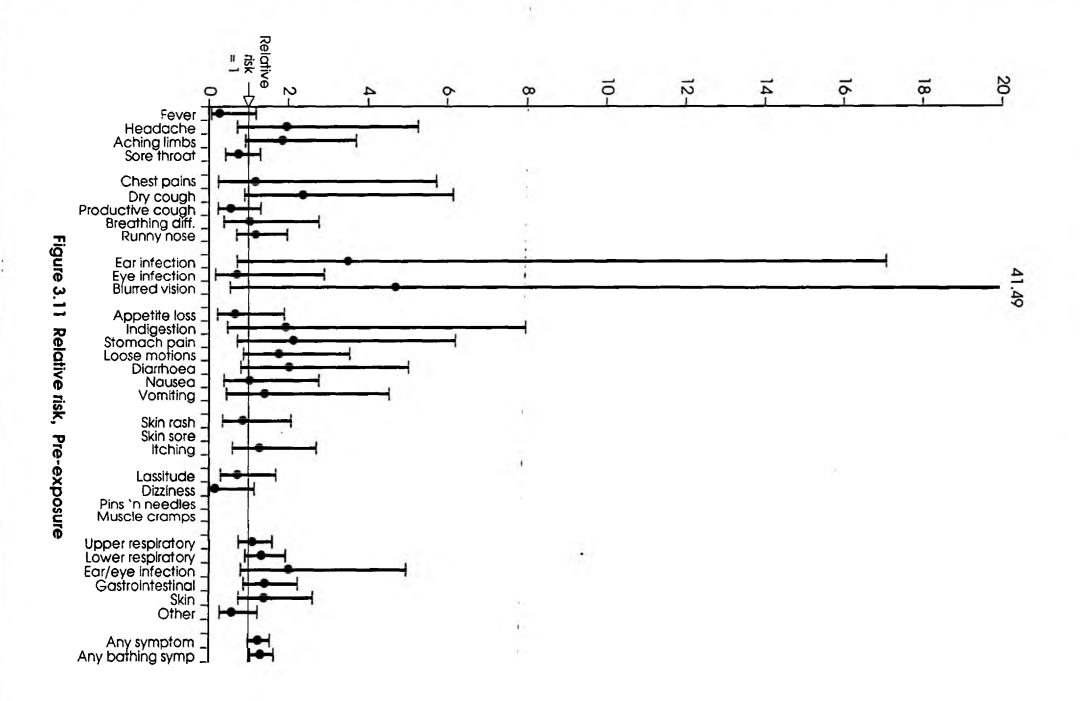
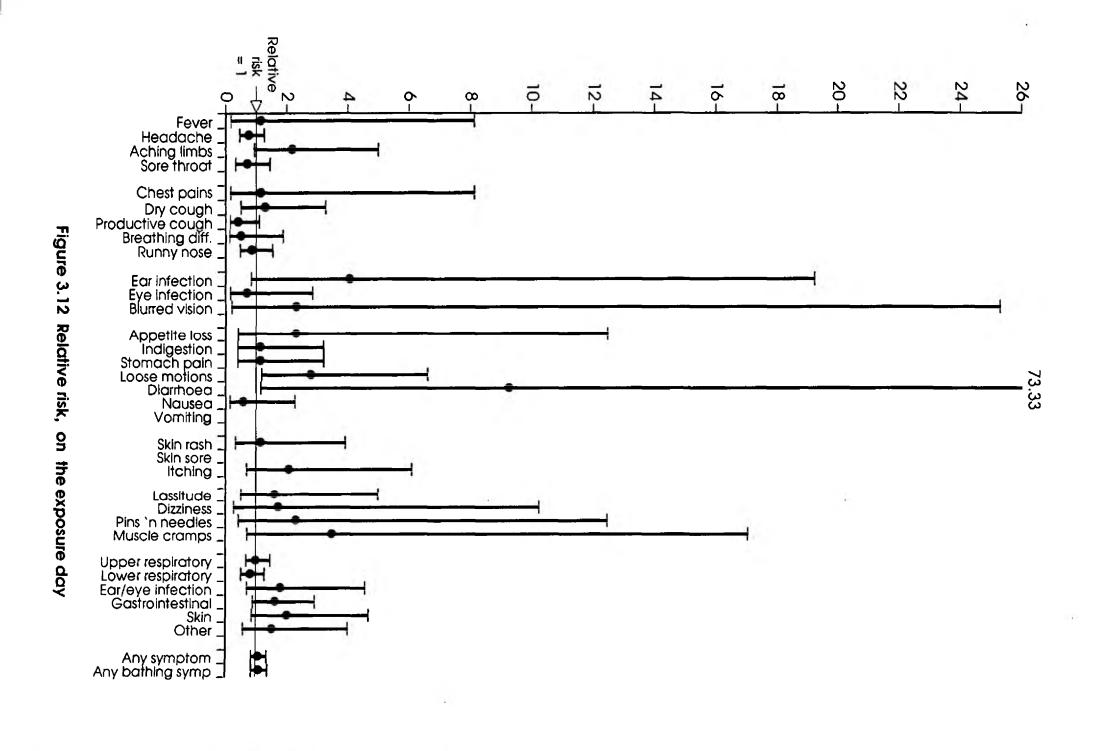
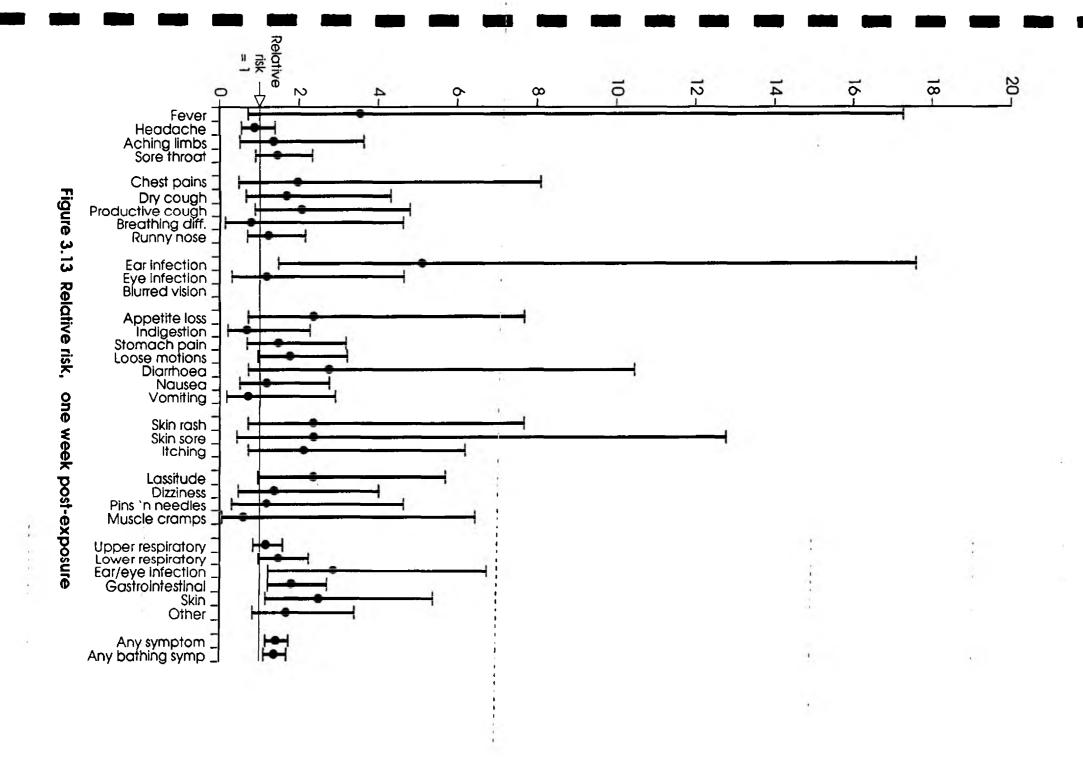


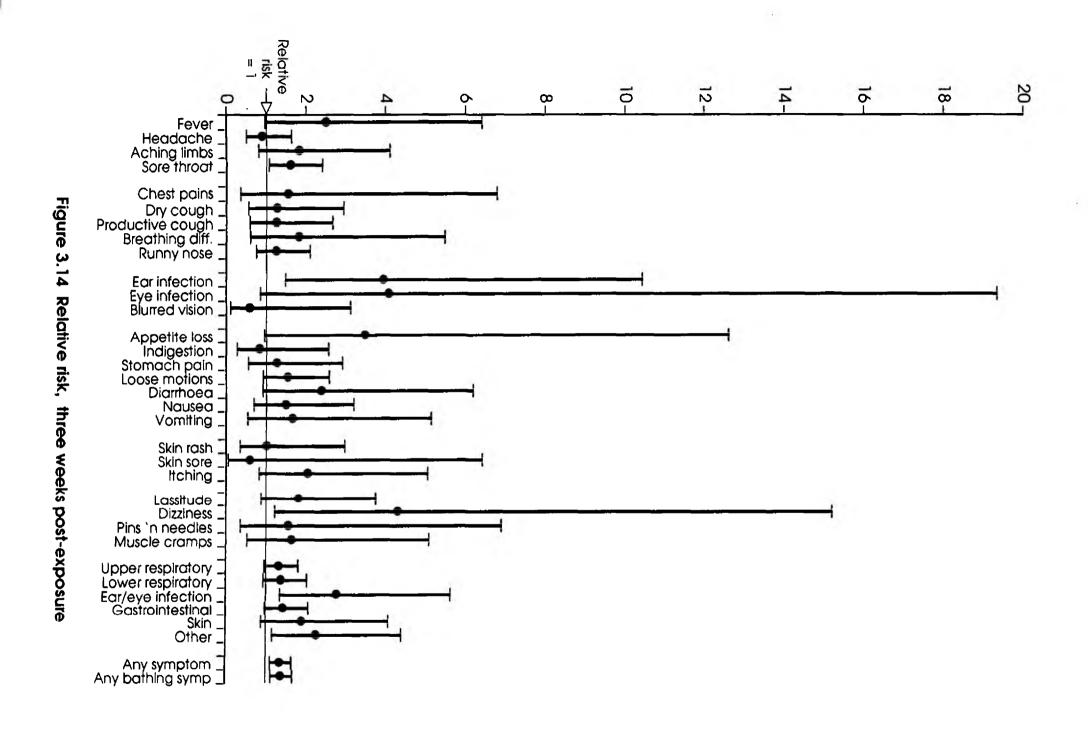
Figure 3.9 Geometric mean total staphylococci concentrations (count per 100 ml) Southend-on-Sea, Thorpe Bay, 04.07.92

Figure 3.10 Relative risk, swab results









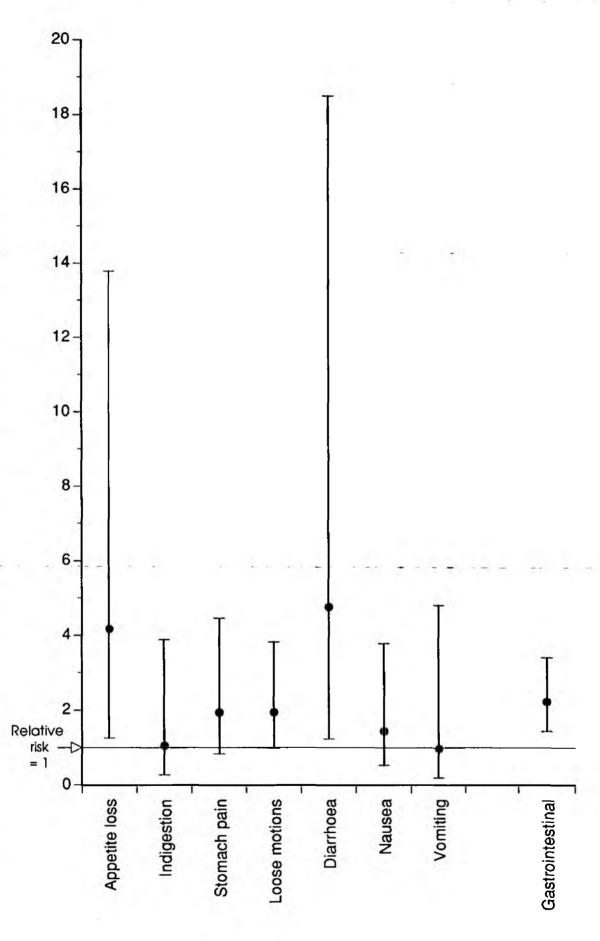


Figure 3.15 Gastrointestinal symptom relative risk, bathers who ingested seawater vv non-bathers at one week post-exposure

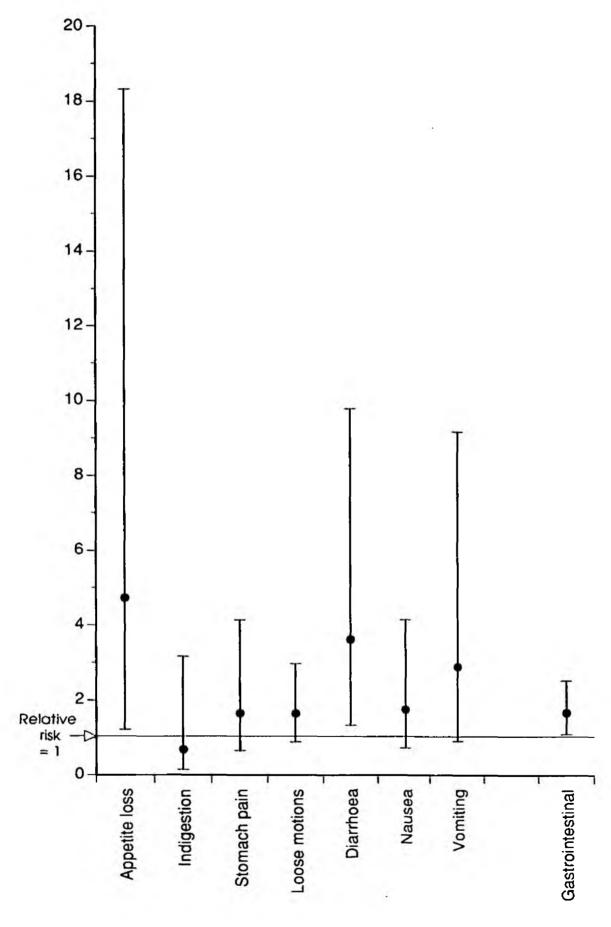


Figure 3.16 Gastrointestinal symptom relative risk, bathers who ingested seawater vv non-bathers at three weeks post-exposure

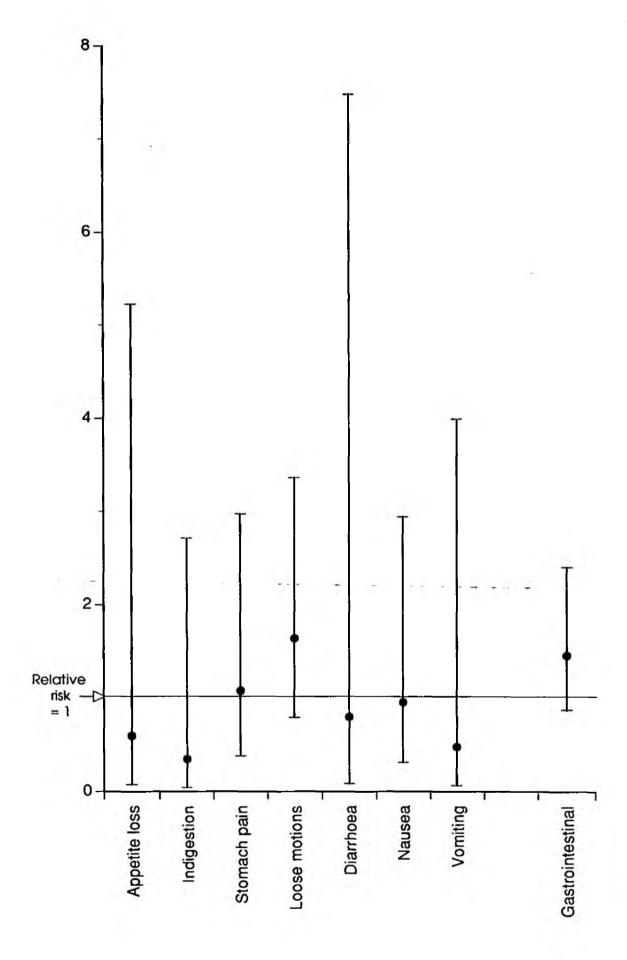


Figure 3.17 Gastrointestinal symptom relative risk, bathers who did not ingest seawater vv non-bathers at one week post-exposure

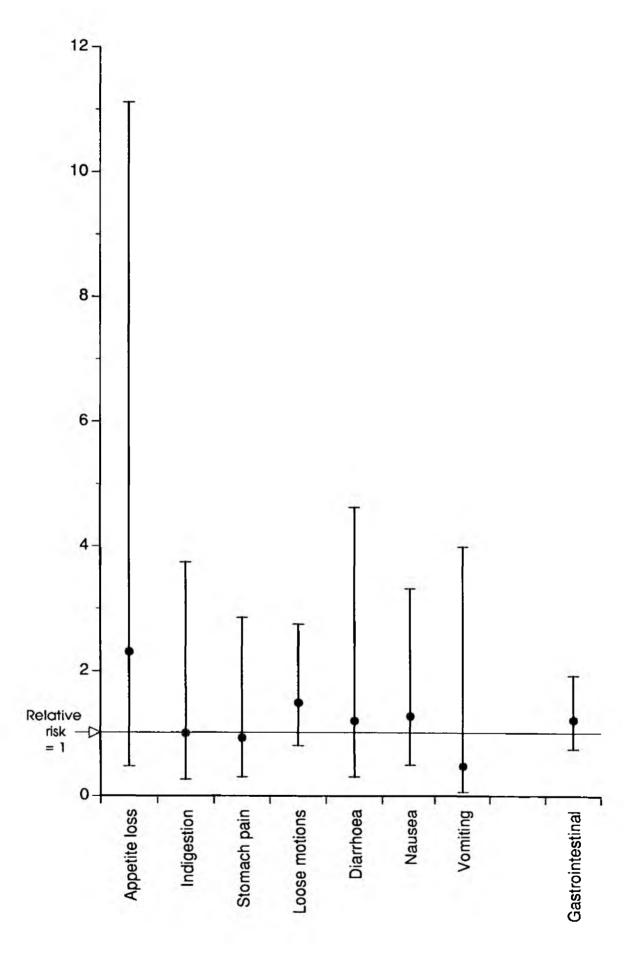


Figure 3.18 Gastrointestinal symptom relative risk, bathers who did not ingest seawater vv non-bathers at three weeks post-exposure

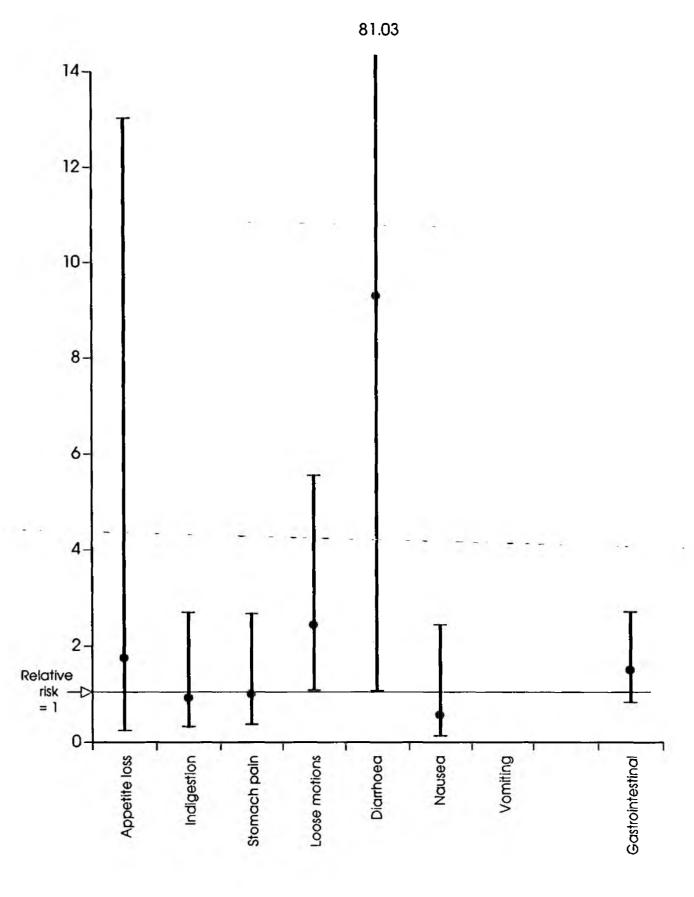


Figure 3.19 Gastrointestinal symptom relative risk controlling for mayonnaise consumption on the exposure day



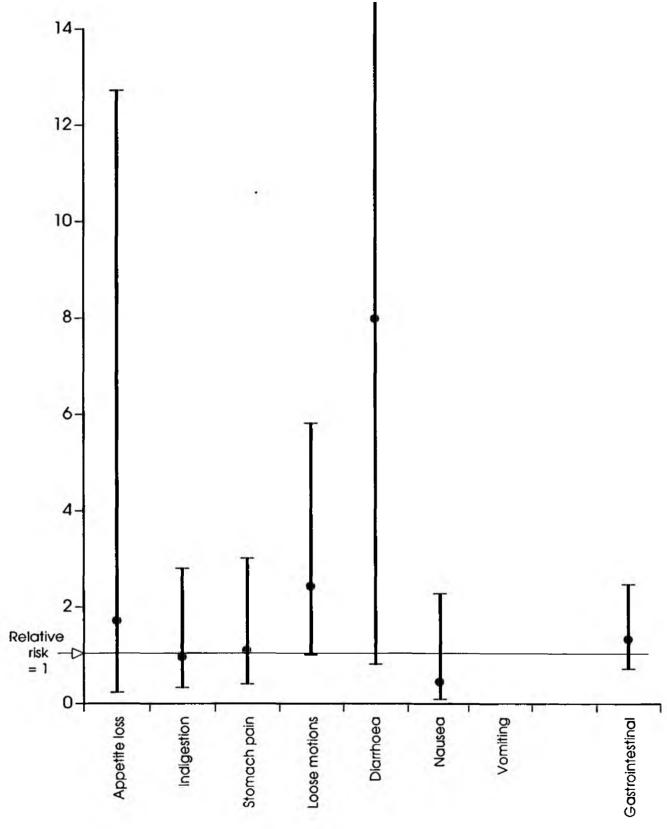


Figure 3.20 Gastrointestinal symptom relative risk controlling for seafood consumption on the exposure day

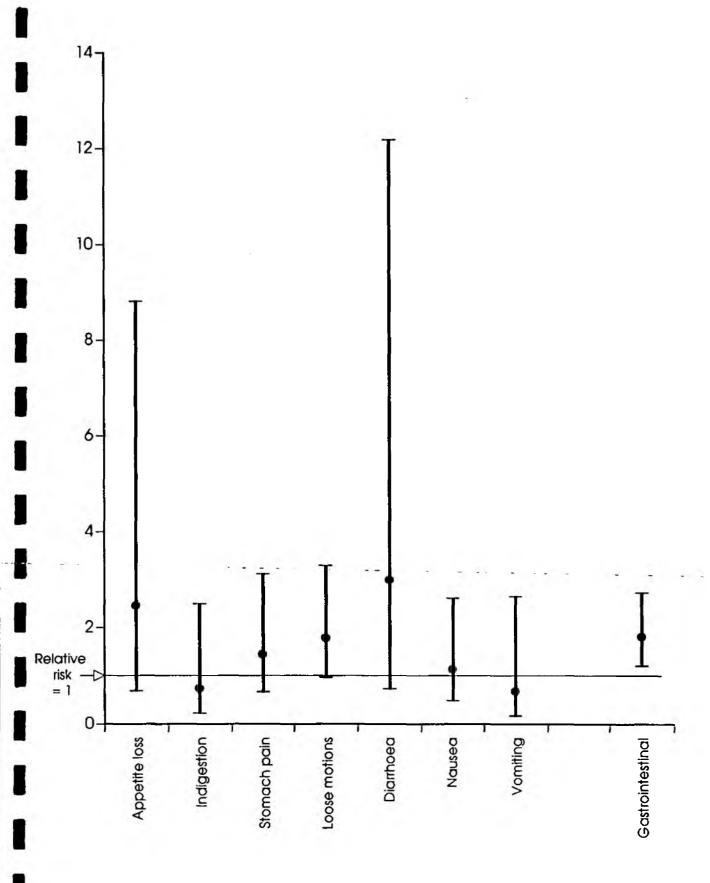


Figure 3.21 Gastrointestinal symptom relative risk controlling for bought sandwich consumption one week post-exposure

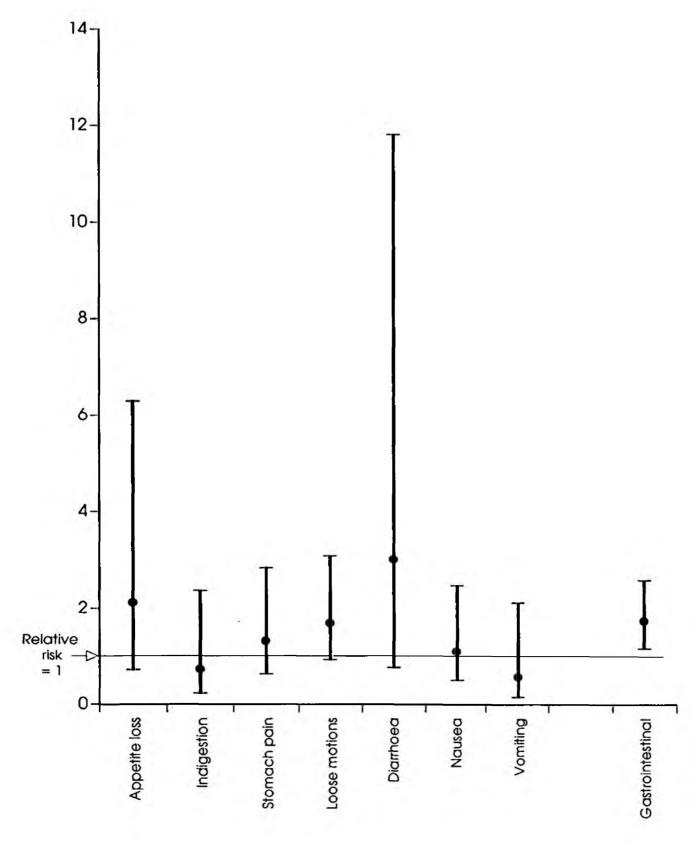


Figure 3.22 Gastrointestinal symptom relative risk controlling for seafood consumption one week post-exposure

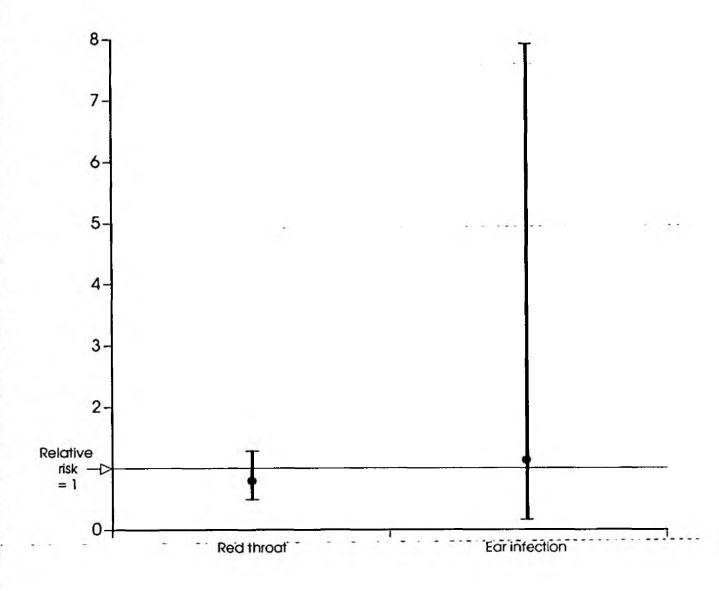


Figure 3.23 Relative risk, medical diagnoses at one week post exposure

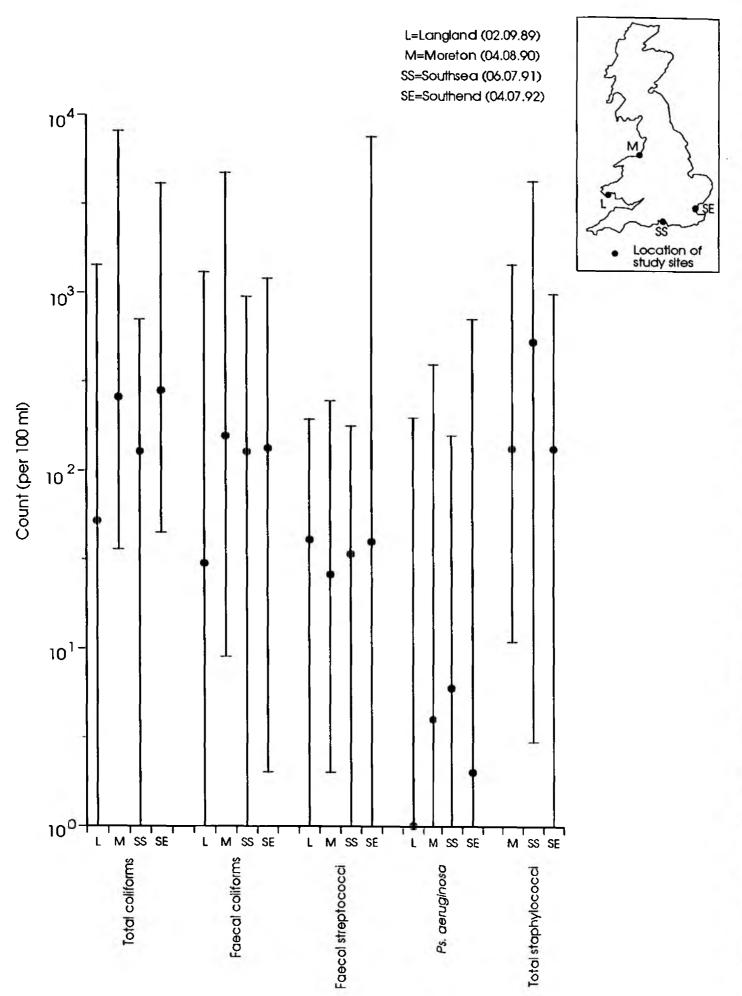


Figure 4.1 Comparison of geometric mean and range of indicator organisms (count per 100 ml) for in-shore samples from the cohort studies

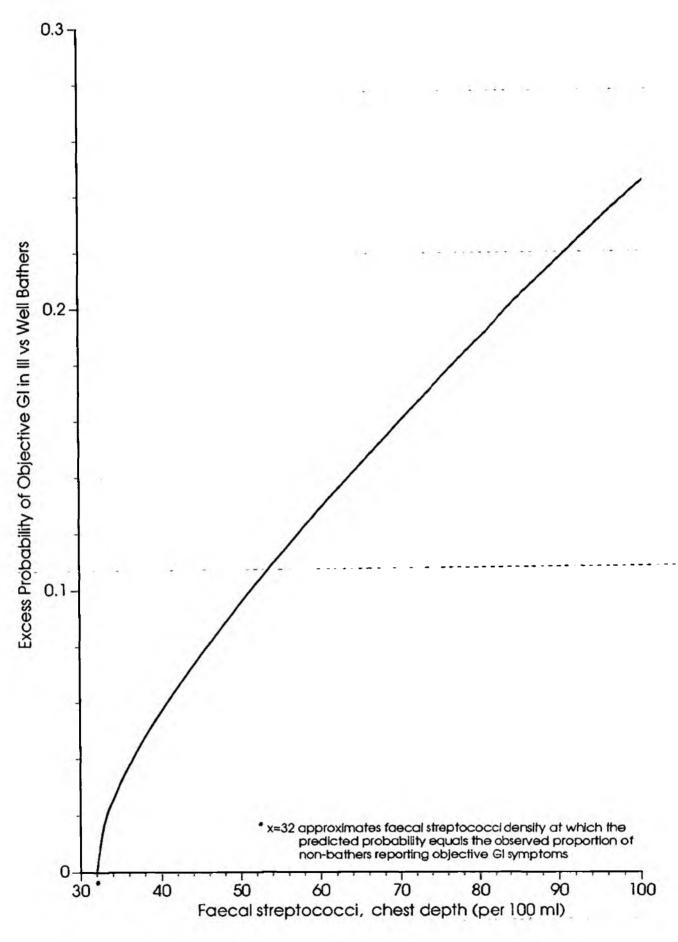


Figure 4.2 Logistic model to predict the excess probability of objective symptoms of gastroenteritis among ill vv well bathers from faecal streptococci density (per 100 ml) at chest depth

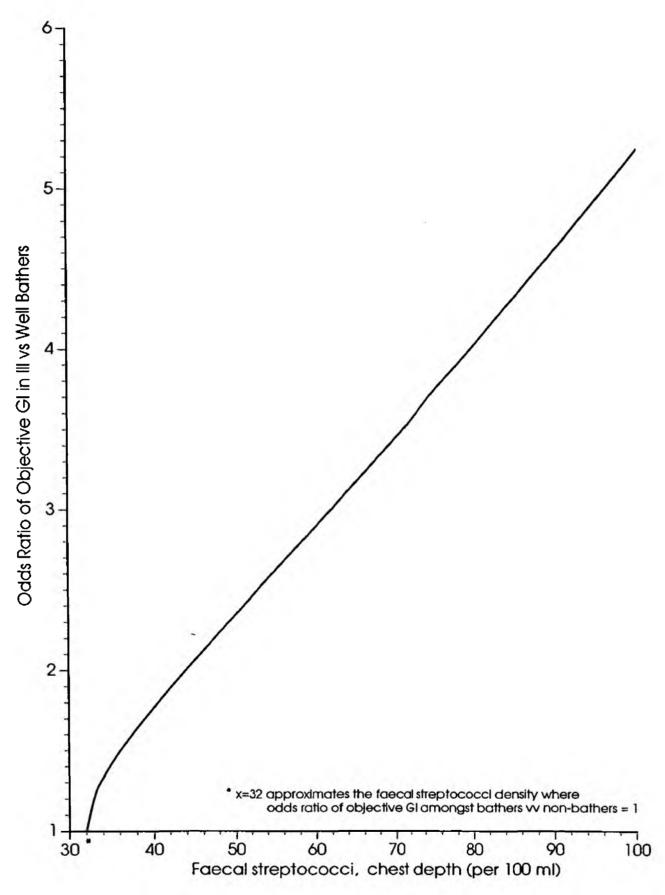


Figure 4.3 Logistic model to predict the odds ratio of acquiring objective symptoms of gastroenteritis among ill vs well bathers from faecal streptococci density (per 100 ml) at chest depth

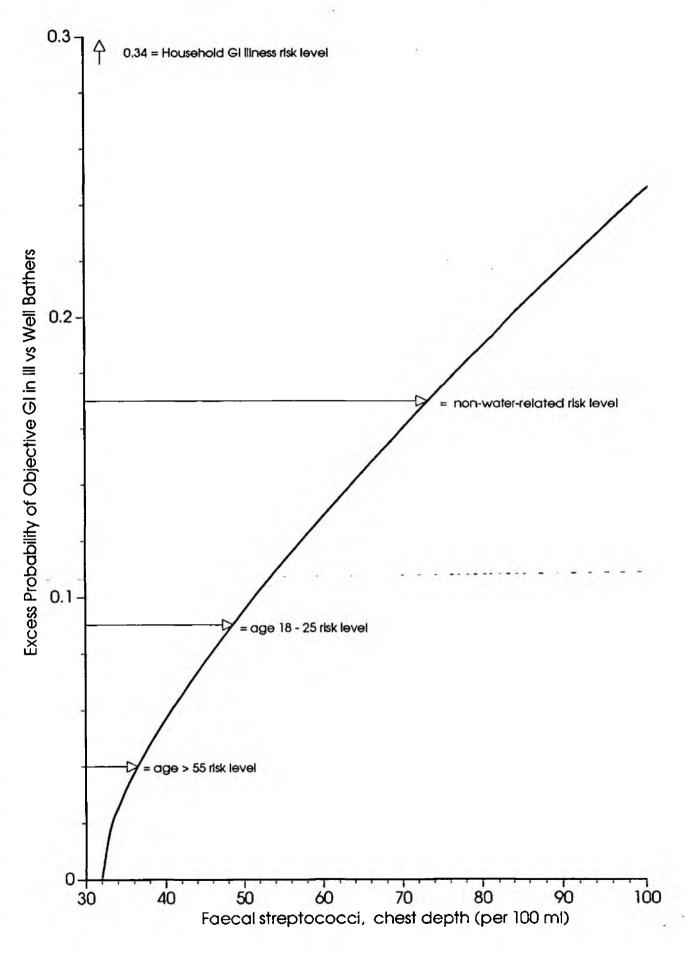


Figure 4.4 Comparison of riskof objective gastroenteritis from different sources with risk associated with exposure to sewage contaminated sea water indexed by faecal streptococci (per 100 ml) at chest depth

Appendix I



SUBJECT INFORMATION SHEET

Study on the Possible Health Effects of Bathing in Waters which Meet EEC Directive Standards

FUNDING AGENCY Department of the Environment

-MANAGEMENT AGENCY Water Research Centre

RESEARCH SUPERVISORS Prof F. Jones, Dr D. Kay (University of Wales)
Dr R. Salmon (CDSC Welsh unit)

1. NATURE OF THE STUDY

1.1 Background

A degree of sewage contamination can be detected at most UK bathing beaches. There is no reliable information, for UK bathing waters, with which to define the minor risks to health caused by bathing in this coastal environment. Britain and our European partners accept the European Bathing Waters Directive standards as one measure of 'acceptable' bathing water quality. However, we do not know if these standards are either too lax or too stringent to ensure that minor diseases will not be contracted by the bathers. It is the objective of this study to answer some of these questions.

1.2 Research Method

This project will involve 400 healthy volunteers. All will be adults over 18 years of age. They will be taken to a beach which has been given a PASS grade on the European bathing water standards. In UK terms this would place the beach in the top 77% of our identified Eurobeaches. The chosen beach will be Southend, Thorpe Bay, and the group of bathers will be taking part in a common leisure time activity practiced by millions of other UK and European citizens (i.e. coastal bathing). The beach has relatively 'good' water quality and has passed the EEC bathing water directive at the Imperative level in recent years. The group of 400 volunteers will be split randomly into two equal groups at the beach. One group will take part in normal beach activities other than water contact pursuits, whilst the other will go into the water. This latter group will each be asked to immerse their heads in the water at least three times during the test, as they might during normal recreational activity.

Every volunteer will have three questionnaire-based assessments to ascertain their state of 'perceived' health, first on the day before exposure, the second about one week later and the third after three weeks. Paralleling this schedule will be the collection of ear and throat swabs by qualified personnel for analysis by the Public Health Laboratory Service. Volunteers will also be required to provide faecal samples for analysis.

2. Health Risks

The Department of Health has indicated that there is only a small risk of illness even if waters are seriously and visibly contaminated. The fact that the study is to be conducted on a beach which meets the standards of the EEC Bathing Waters Directive can give confidence that there is no risk of serious illness. However, previous work in this area, conducted outside the UK, has suggested that there might be a slight risk of contracting minor illnesses such as stomach infections. We cannot guarantee that there is zero risk of volunteers contracting such infections. However, this risk is no greater than that experienced by many millions of coastal bathers each year who use waters which currently meet EEC standards.

3. Insurance Cover

With the exception of volunteers who have been confirmed pregnant by a doctor, all participants in the study will be covered for accidental injury. Exact details of this insurance cover are available for inspection upon request from any of the supervisors listed above. In broad terms, this policy follows the guidelines recommended by the Royal College of Physicians Research on Healthy Volunteers (1986).

4. Expenses

All participants will receive £10 for out of pocket expenses and the inconvenience experienced on the day of exposure and during the associated medical examinations. This token payment is not intended to cover 'risk'.

5. Consent

- (i) I have read and understood sections 1 through to 4 of this subject information sheet.
- (ii) I give my consent for the medical examinations and sample collections outlined and I am willing to be involved in this study.
- (iii) I understand that insurance cover has been arranged by the project supervisors. I understand that I can pull out of this study at any time but I undertake to inform the supervisors immediately if I take such a decision.
- (iv) I am willing to provide information on my medical history to the researchers on the understanding that any such information will be treated in strictest confidence.

Signed:	Home Address:
Name (Please print):	
Date: 9 2 Number:	Postcodo
Home Phone No:	Postcode:
	Surgery Address:
Work Phone No:	
GP's Name:	
<u></u>	Postcode:



Public Health Laboratory Service

PHLS Communicable Disease Surveillance Centre (Welsh Unit) Abton House Wedal Road

Roath Cardiff CF4 3OX

Fax: 0222 521987 Telephone: 0222 521997

Our Ref

Your ref

IN CONFIDENCE

/ /92

Dear

Re:

This patient has volunteered for a Department of the Environment funded study of the possible minor health risks associated with coastal bathing. A beach currently passing the EC mandatory water quality levels has been selected (Southend, Thorpe Bay). The study should require no additional work on your part. I am simply writing to keep you informed. The chairman of your local medical committee has been consulted and can see no objections to the survey proposal.

During the study day, (Saturday 4th July), your patient will be allocated at random to a swimming or non-swimming group. "Swimming" will consist of entering the water, swimming, splashing etc. for a minimum of ten minutes. The survey will include two medical interviews, ear and throat swabs and faecal samples, as well as questionnaires.

Study participants will be asked about chronic illnesses and recent health history. They have not been told to check with their GP for fitness to take part, as they are only being asked to carry out a normal leisure activity. If you feel there is any reason why this patient should not take part please telephone me on the above number or contact Dr D. Kay at St. Davids University College, Lampeter, by telephoning 0570 424749.

People deemed unfit at the pre-exposure interview will be excluded by one of the doctors on the study team.

A copy of the enclosed information sheet, approved by the Royal College of Physicians ethical committee for Research on Healthy Volunteers, has been signed by your patient.

If I do not hear from you, I shall assume that you are happy for the study team to include your patient in the survey.

Yours sincerely,

Dr. R. L. Salmon, MA, MB, BS, MRCGP, MFPHM Consultant Epidemiologist

Appendix II





STRICTLY CONFIDENTIAL

8. General Practitioner: Name:	Coding only
Address:	
Tel:	
9. Health Authority:	HA.
10. Local Authority:	LA
1. Please list all the members of your household (i.e. all those who live in our home) with their sex and ages: A household means sharing facilities and at least one meal per day emember to include the interviewee in the total household count) Iame (Surname not required) Sex Age Name (Surname not required) Sex	Include the Interviewee in the total count Total in household Total children upto 5 in household Identify the state of
Has anyone in your household been unwell with a possible infection in the past two weeks? Yes No Not sure	Household illness
If <u>yes</u> please give details	Ulness type
Prompt - Diarrhoea, gastric infection (nausea, vomiting etc), sore throat, ear and eye infections	



STRICTLY CONFIDENTIAL	Coding only	
SECTION TWO - GENERAL HEALTH		
12. Do you have any longstanding illness, disability or infirmity? (Anything that has troubled you over a period of time or is likely to affect you over a period in the future).		
Yes No o Not sure,	Long, ill	17
if no, turn to question 14, page 5		
If yes, please indicate the nature of the problem by ticking all the boxes that apply from this list on this and the following page. Use the space at the bottom of page 4 to describe any circumstances not covered by the available boxes. Prompt for each - have you ever had		
1. ARTHRITIS: specify	Arthritia	18
2. BACK PAIN (include: aches (lumbago) disc problems)	Back Pain	19
3. raised BLOOD PRESSURE	Blood Pressure	20
4. CHEST PROBLEMS (include asthma/bronchitis)	Chest	21
5. DIABETES *	Diabetea	
6. DIGESTION PROBLEMS (e.g. dyspepsia (ulcar)): specify	Digestion Problem type	
7. BOWEL PROBLEMS (e.g. constipation, calitis,	Bowel Problem	
irritable bowel syndrome): specify	problems type 25	26
8. HEARING LOSS / EAR PROBLEMS : specify	Hearing/ear Problem Type	1 28
9. HEART DISEASE (include angina) *	Hoart	29
10. HEPATITIS / LIVER DISEASE	Hopmicis Type	
If Yes which type of hepatitis? Infectious Type A / B	A = 1 B = 2 other = 3 not known =	9
Infective jaundice (type A) or Serum Hepatitis (type B) Other type (Non infectious etc.)		

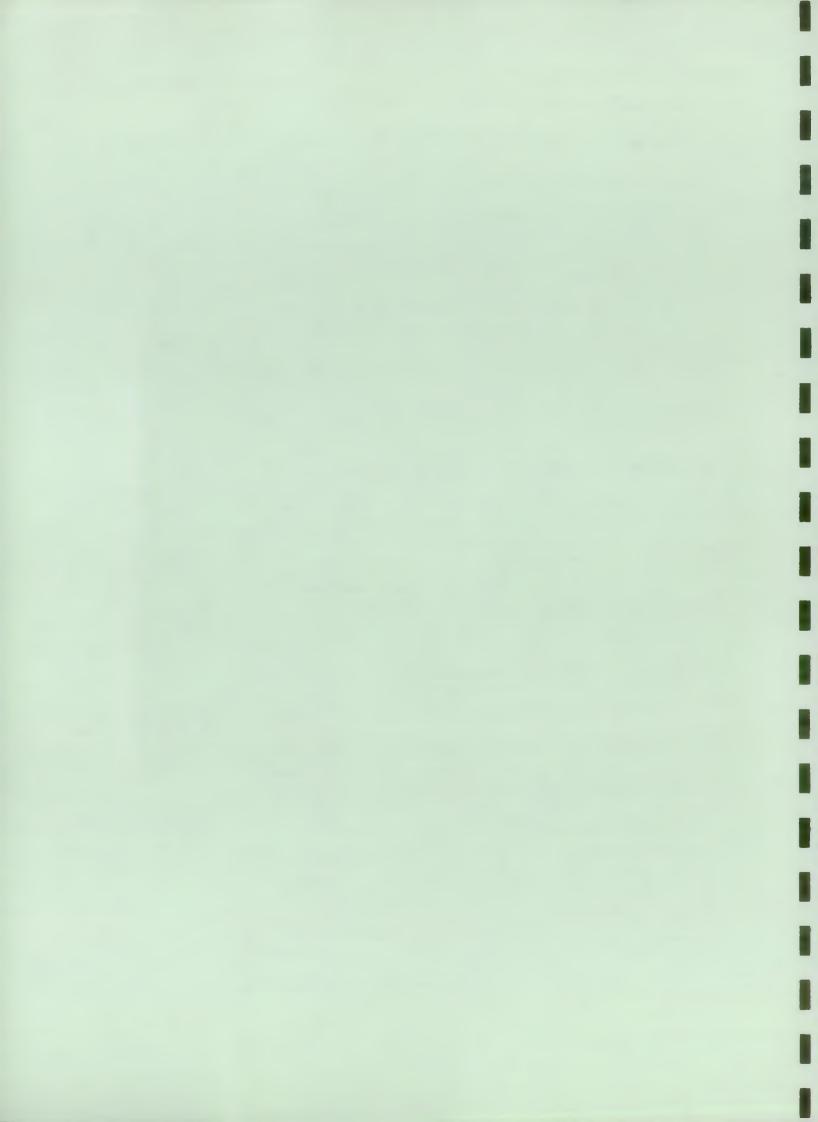


Coding only

STRICTLY CONFIDENTIAL

GENERAL HEALTH - CONTINUED

11. Problems due to INFECTION specify infection and problem:		Infection Infection Type 32
12. Problems resulting from INJURY OR ACCIDENT: specify: the problem		Injury/Accident
13. KIDNEY or BLADDER problem: specify:		Kidney/ Problem type 35
14. NEUROLOGICAL Condition: specify (e.g. strokes / epilepsy / paralysis / neuralgia / migraine)		Neurological Problem type 37
15. HAYFEVER		Hay fever 39
16. SKIN Problems: specify		Skin Problem type 41
(e.g. eczema psoriasis)		
17. STRESS / ANXIETY (For which you require medical treatment)		Streas / Anxiety 42
18. POOR VISION/ EYES: specify:		Eyes Problem type 44
Short sight = 1 Long sight = 2 Glaucann = 3 Detatched retine Include frequent eye irritation - will eyes = 5	2 = 4	
19. OTHER PROBLEMS: Please give a brief description		Other Problem type



STRICTLY CONFIDENTIAL	Coding only
GENERAL HEALTH - CONTINUED	
13. Do you see a doctor regularly for any of these problems?	
Yes No Not sure	Doctor seen
If yes, is this your GP, a hospital specialist, or both	
GP Hosp Both Other *	Doctor Details
* Give details	
14. How many times a year do you have diarrhoea?	
(An increase over your normal bowel habits equal to runny stools lasting at least 24 hours)	
Often Sometimes Rarely Hardly ever Never Not 1-2 a 3-11 <2 <1 Sure	
month a year a year	Diambora
15. Have you in the past 6 months had an illness which caused you	*
to stay home from work, miss normal activities or go to hospital?	_
Yes No Not Sure	Ill in last 6 months
If no go to Question 16, next page	
If yes please complete the following section.	lliness lliness
Diagnosis of illness	5t type 5
Were you admitted to Hospital? Yes No 0	Diners Hosp adm.
How long were you sick / off Weeks Days work?	Illne as (MWD)
If more than 1 illness record details of the most serious / recent illness	
Month illness started Jan Feb Mar Apr May Jun Jul	Illness start (M)
Are any of these illnesses / Is this illness still giving you symptoms? Yes No Not Sure	Illness still giving symptoms ?



Coding only

STRICTLY CONFIDENTIAL GENERAL HEALTH - CONTINUED

16. In the last 3 weeks, please answer whether you have had any of the following symptoms, persisting for more than 24 hours.

(Answer Yes, No or Not sure for every symptom) EVERYONE TO ANSWER THIS SECTION

FRIGHER THIS GEOTION		
'Flu / cold symptoms Lasting 24 hours on		
	Not date - Jun Duration Yes No Sure / Jul in days symptom Onset	Duration
1. Fever (Hot and cold, shivers)	Fever Pever	57
2. Severe / unusual headache	Headache Headache	58
3. Aching arms, legs, joints	Aching Immbs	59
4. Sore throat	Size throat	60
Chest symptoms Lasting 24 hours or more		
	To bail fill days	Duration
5. Chest pains / aches	Chest pains	61
6. Dry cough	Dry cough	62
7. Productive cough (phlegm / sputum)	Prod. cough	63
8. Wheezing / Shortness of breath	Breathing diff.	64
9. Runny nose	Runny nose	65
Ear / eve symptoms Lasting 24 hours or	sore Onset	
	Not date - Jun Duration Yes No Sure / Jul in days Symptom Onset	Duration
10. Ear infection (sore, discharge)	Yes NO Sure / Jul In days Symptom Onset Ear infection	
11. Eye infection (sore red eyes, discharge)	Bye infection	67
12. Blurred vision (difficulty with eye sight)	Vision Vision	68
	Onset	
Gut symptoms Lasting 24 hours or mo	Ves No Sure / Jul in days	Duration
13. Loss of appetite	Appetite Onset	Durandi
14. Indigestion	Indigestion I	75
15. Stomach cramps (colic / lower abdominal pain / griping)	Stomach pain	
16. Loose bowel motions (looser than normal)	Loose bowels	72



STRICTLY CONFIDENTIAL GENERAL HEALTH - CONTINUED

16. Continued	Onset	
Gut symptoms continued	Not date - Jun Duration	
17. Diarrhoea (3 or more runny stools in 24 hours)	Yes No Sure / Jul in days	Symptom Onset Dention Diarrhoea
18. Nausea (feeling sick)		Names 74
19. Vomiting (being sick)		Vomiting 75
Skin symptoms Lasting 24 hours or more	Onset	
	Yes No Sure / Jul in days	
20. Skin rash on body		Skim rash
21. Skin ulcer / sore		Skm ulcer/
22. Itching (irritation)		Itching 78
Other symptoms Lasting 24 hours or m	are a	
Other Symptoms Labourg 24 House Of the	Onset Not date - Jun Duration	
	Yes No Sure / Jul in days	
23. Excessive tiredness (unusual		Lassinde
fatigue, lassitude)		Dizziness 79
24. Dizzy or giddy		80
25 Ding and needles / tingling		Pins and
25. Pins and needles / tingling		nocdes 81
26. Muscle cramps (e.g. cramp in arm or leg)		Muscle cramps 82
27. If you have had any symptoms for list, please describe them, listing the		
	onset date and duration.	Other 1
1.		
2.		Other 2
		Other 3
3.		85
17. Do you smoke cigarettes at all? Pro	mpt for pipe smoking	
Yes No O	Pipe or any other kind of Smoker Not Sure	Sesoker?
If no go to question 19, next page		
If yes how many cigarettes do you smok	e per day?	No. cigarettee
(Include cigars and 'roll your own' as cigone counts as 1 cigarette.)	arettes. Each	



STRICTLY CONFIDENTIAL GENERAL HEALTH - CONTINUED

18. If you are an ex smoker how long is it since you gave up? Non smokers tick appropriate box Years Months Days	Gave up
19. How often, if ever, do you drink alcohol? At least once Less than Never drink Not a week once a week alcohol sure If the answer to 19 is 'never' skip to question 22 page 9	Alcohol
20. Approximately how many units of alcohol have you consumed in the past seven days? One unit = Half a pint of beer, lager, citier, stout, etc. a single measure of spirits; whisky, vodka, gin, rum, etc. a small glass of martini, port, sherry, wine, etc. a glass of wine	units of alcohol
Ask the volunteer to try and remember where they were and who they were with each day - it may help them to recall what they drank. 21. Would you say that last week was fairly typical of what you usually drink in a week?	
No - volunteer volunteer usually drinks less more	Normal dranking 90



GENERAL HEALTH - CONTINUED

22. Have you taken any tablets or medicines	in the last four weeks?
(Include regulari chronic prescriptions as v	
from the chemists! supplied by the clinic)	
	Not

	Yes	No Sure
Tick which:		Name of Tablet/Medicine
1. Antibiotics *		
2. Steroids *		
3. Laxatives		
4. Stomach remedies		
(e.g. Milk of magn	esia, antae	eids etc.)
5. Other		

SECTION THREE - VISITS AT HOME AND ABROAD

23. In the past 4 weeks have you spent any nights away from home, e.g. for a holiday or to visit relatives?

Yes

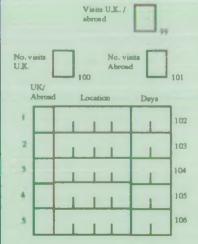
If yes was this in the	U.K. or abi	road?		
	U.K.	Abroad	Both	
		L 2	Ш,	
Please give the date(s) and place	e(s) visited be	elow:	
In the past 4 weeks or	nly			
Place(s)	Da	ate(s)	Duration of	sta

No

Not Sure

Place(s)	Date(s)	Duration of stay

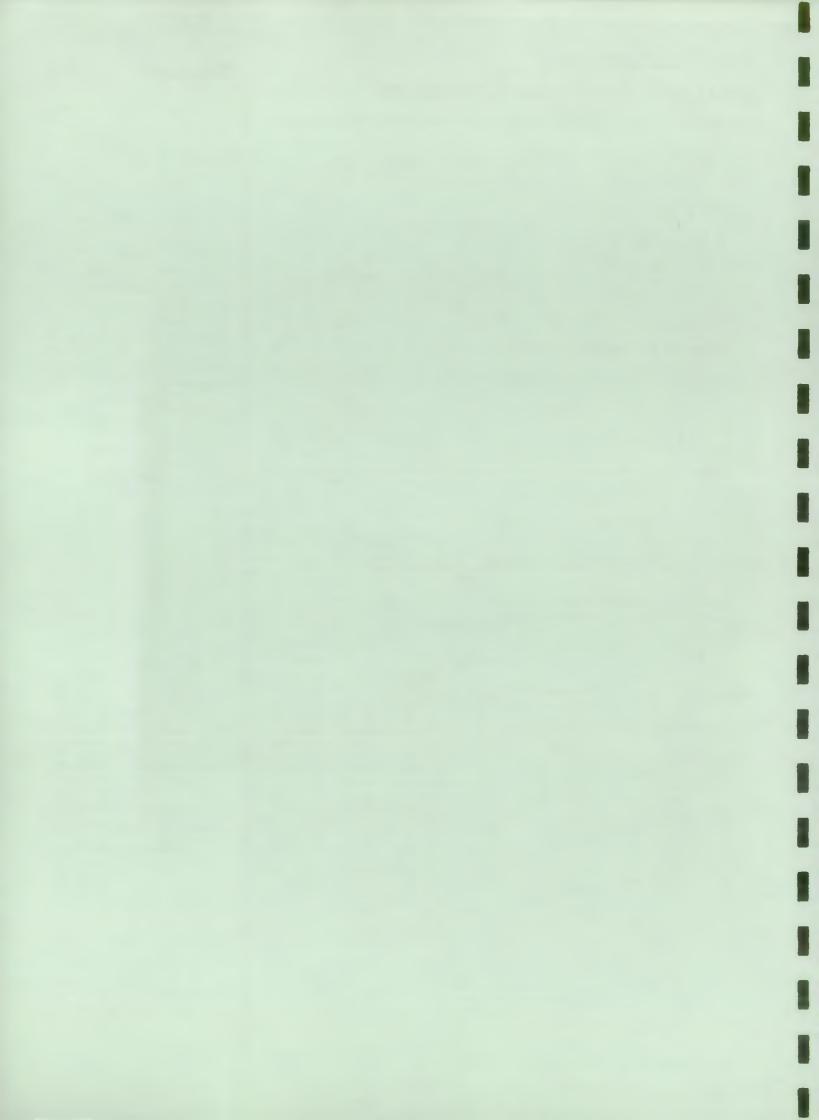
	Drags	ot .
	Antibiotics	92
	Latatives	
		93
	Laxatives	
		L 94
	Stomach remedies	95
Other 96	Other type	97



Visits away from home



STRICTLY CONFID	DENTIAL					Coding only	10
VISITS AT HOME	AND ABR	OAD - CON	TICLUJOIT	2		I	
24. Apart from short h time in your life? (Excluding 2 week	olidays, ha	No [any time		as at any	Tripa overseas	
holidays)	l l	<u></u> , L	_ 0	L],	107	
If no go to section 4, ti	his page.						
If yes how long? Up to 1 month	1 mo to 1 yr	1-3 yr	>3 yr	Born abroad	Not Sure	Time overment 108	
Please list the coun	try / countr	ies:					
						Country visited I Country visited 2 Country visited 3 Country visited 4	109 110 111 112
ECTION FOUR - GE 25. In an average month following activities at the	n, how ofte	en do you tak					
1. Pub/ Drinking club 2. Party 3. Leisure centre 4. Church / religious meeting		Occasional 1-3 Times	Not at all	Not Sure	No. of times in last month	Pub Prequency 113 Party 115 Leisure Prequency Centre 117 Church Prequency	116
9		1	-0			119	120



GENERAL LEISURE ACTIVITIES - CONTINUED

26. In the summer months, how often do you take part in the following water related sports / activities?

(Please give average/typical exposure in times per month during the summer period with reasonable weather, and in fresh/sea water)..

	Frequent O >3 Times	ccasional 1-3 Times	Not at Not all Sure		No. of times in last month	
1. Dinghy sailin Canoeing				, [], [],		Ca
2. Speed / motor boating / rowing	or	□,		. 🗆, 🗆,		5
3. Subaqua / diving / snorkeling				, 0,0,		Si
4. Surfing / water skis / jet skis				, 🗆, 🗖,		Su
* Include : wind su	rfing, sailboar	ding etc.				
5. Fishing				9 0 0 1		F
6. Paddling / wading				, , , ,		P= /\
7. Other				9 1 1		
*Specify:						
27. How often of (Please give ave summer period	erage/typica	l exposure	e in times per	month during		
Frequent		nal			No. of imes	
>3 Times	1-3 Times	Not a	Not Sure		n last month	
				\Box , \Box ,		Bat
If yes how far	do you usua	lly swim?				

I I metres

Prompt for an answer in metres

I length of a pool = 25 m

Coding only

Code positive response for sea / fresh water as 1 and negative response as 0.
Sea Fresh Frequ 121 122 123
Speed boat 124 Pregu 125 126
Sub Sca Presh Presh 127
Surfing Sca Fresh Frequence.
Sea Fresh Fishing 133 Sea Fresh Padding Padding Frequ Frequ Frequ
136 137 138 138 139 140 141



GENERAL LEISURE ACTIVITIES - CONTINUED

27		. 0	1
27.	COL	TILL	ned
41.	COL		uvu

Please specify where you have bathed in the past three weeks:

Place(s)	UK	Abroad	No. of visits
1			
2			
3			
4			
5			

28.	How	often	do	you	use	a	swimming	pool?
-----	-----	-------	----	-----	-----	---	----------	-------

1. PUBLIC swimming pool

Frequent	Occasional					No. of times
>3	1-3	Not at	Not	Sea	Fresh	in last
Times	Times	all	Sure	water	water	month

2. OTHER swimming pool (e. g. a private pool or lide)

Frequent	Occasional					No. of times
>3	1-3	Not at	Not	Sea	Fresh	in last
Times	Times	all	Sure	water	water	month

29. How often do you visit a beach without going into the water?

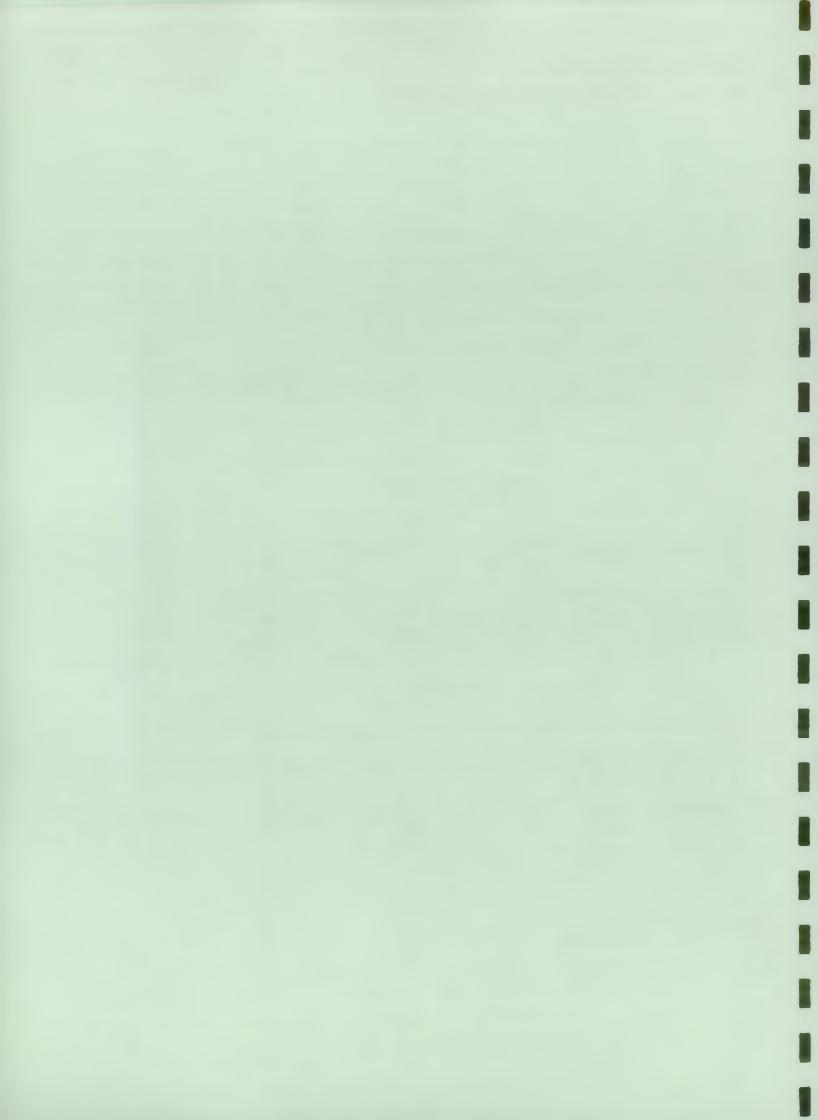
Frequent >3 Times	Occasional 1-3 Times	Not at all	Not Sure	Sea Fresh water water	times in last month
	□,			\square , \square ,	

	UK?	Location	Days	
1				146
2				147
3				148
4				149.
5_				150

		Sea	Presh		
Public pool				Prequ	
	151			152	153

		Sea	Presh		
Other pool				Prequ	
	154	_	_	155	 156

	Sea	Fresh		
Beach without			Prequ	
bathing1	57	_	158	1.50



160

161

162

STRICTLY CONFIDENTIAL

GENERAL LEISURE ACTIVITIES - CONTINUED

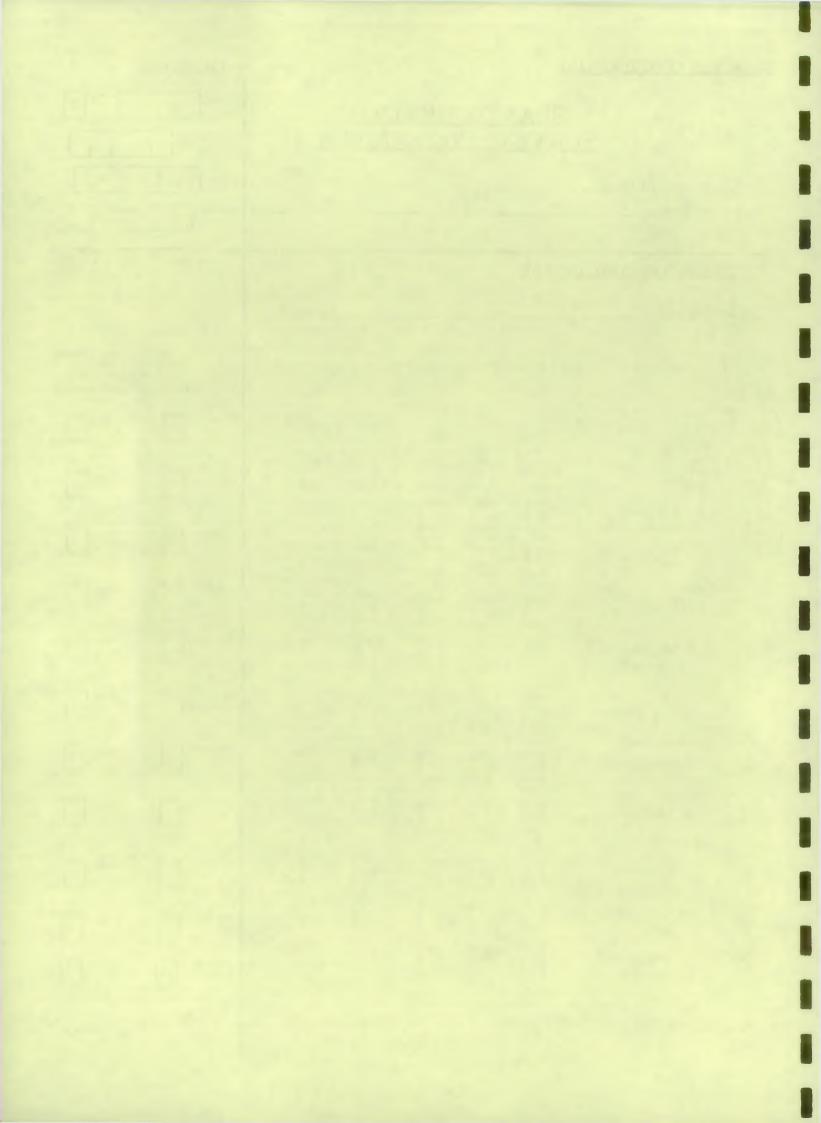
30. In the last month have you been to a theme / leisure park and used any water rides? (e.g. log rides, water shutes).	
Yes No Not Sure	Water rides
If yes was the site at home or abroad? U.K. Abroad	UK?
Please give the name of the site:	Park Location
31. Are you able to swim competently? (Can you swim approximately 2 lengths in a swimming pool?) 32. Additional comments. Use the space below to add any other information that you feel will help this study (Information about general health, travel work abroad, and leisure activities relating to recreational use of water)	Swimmer?
resistant de l'article a l'article per l'article de l'article per l'arti	Other comments 1
	Other comments 2
	Other comments 3



Coding only

DICAL SHEET - to be com	pleted by th	e examini	ng doctor		
Doctor's initials:				Doctor No.	167
If the volunteer is female is	she pregnant	t?			
	Yes	No O		Voluniteer pregnant	168
Appearance of Throat:	Normal	Red	Infected	Appearance of throat	169
Evidence of any middle ear infection:	Yes	No O	Not Sure	Evidence of ear infection	170
Have the medical sections of See Page 1 for details	this question Yes	nnaire beer	n checked?	Micdscal sections checked ?	
Do you recommend exclusion	Yes	unteer from	the study?	Exclusion	171
If <u>yes</u> please state the reason a	and any med	ical finding	s briefly below:	recommended 7	172





STRICTLY CONFIDENTIAL Coding only FOOD INTAKE - CONTINUED Proposed Purchased or brought Not Yes Sure from home resort 12. Any Take-away food 13. Sea food * (e.g. shellfish, cockles etc.) Sea food Specify: SECTION TWO-HEALTH 3. In the last 3 days, including today, please tick whether you have had any of the following symptoms. (Answer Yes, No or Not sure for all, or None on next page) Read out the section headings and ask if they have had any symptoms of that type Code response to no symptoms (see page 3) as 0 m all boxes Not 'Flu / cold symptoms Sure Yes No 1. Fever (Hot and cold, shivers) Headache Headache 235 3. Aching arms, legs, joints 4. Sore throat Any chest problems? Chest symptoms 5. Chest pains / aches Dry cough cough 7. Productive cough (phlegm / cough sputum) Breathing

Chest symptoms

Any chest problems?

5. Chest pains / aches

6. Dry cough

7. Productive cough (phlegm / sputum)

8. Wheezing / shortness of breath

9. Runny nose

Ear / eve symptoms

Any ear or eye problems?

10. Ear infection (sore, discharge)

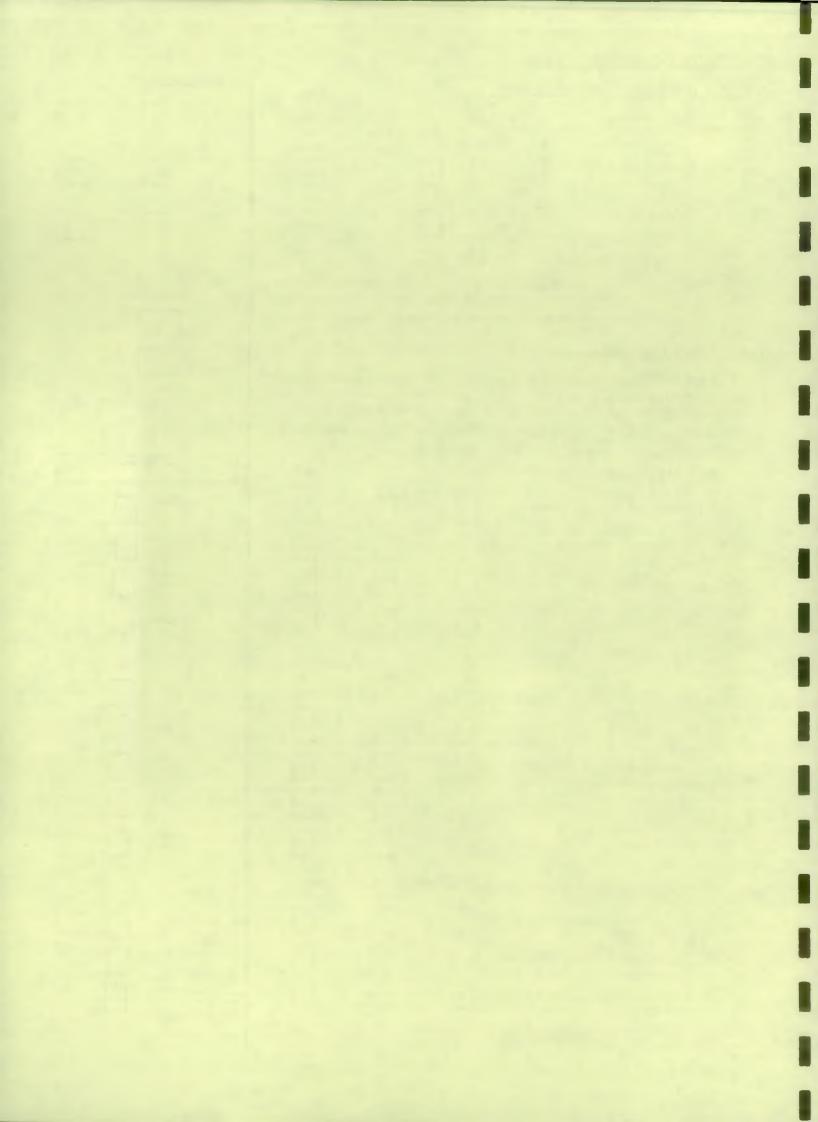
11. Eye infection (sore red eyes, discharge)

12. Blurred vision (difficulty with

Ear infection 243
Eye infection 244
Vision 245

List continued on following page

eye sight)

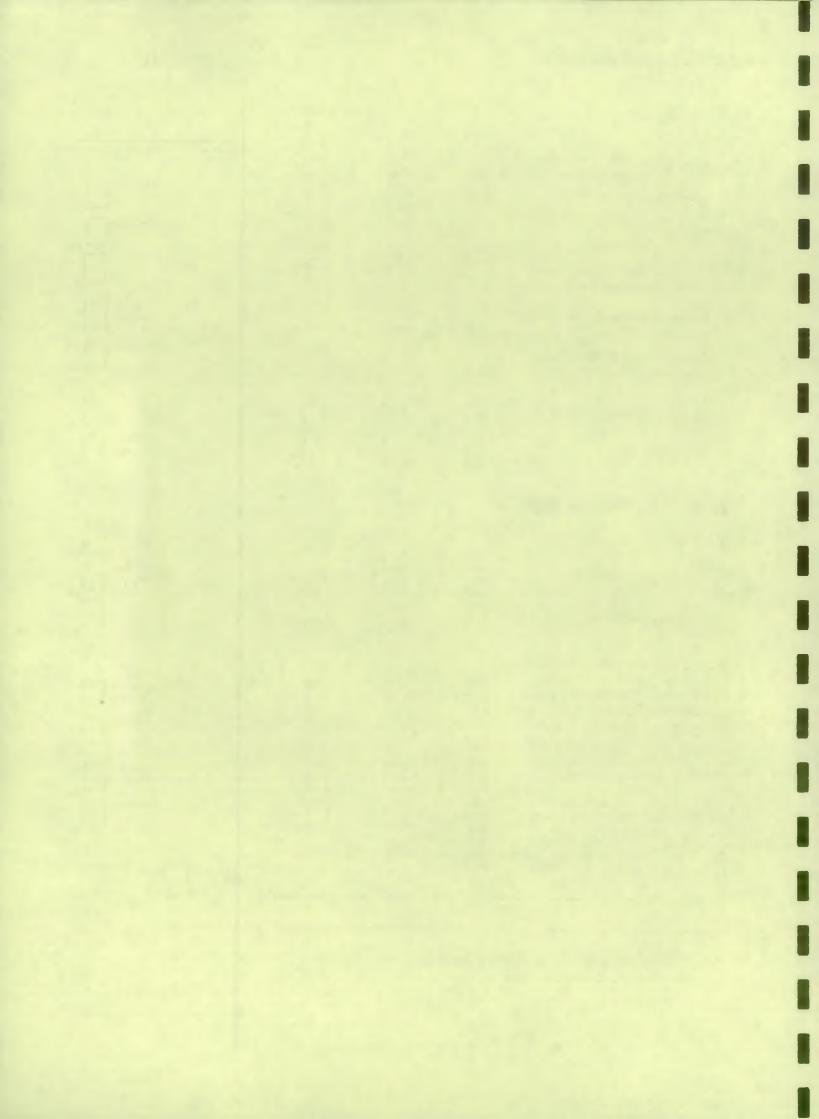


3. 3	Sym	ptoms	continu	ied
------	-----	-------	---------	-----

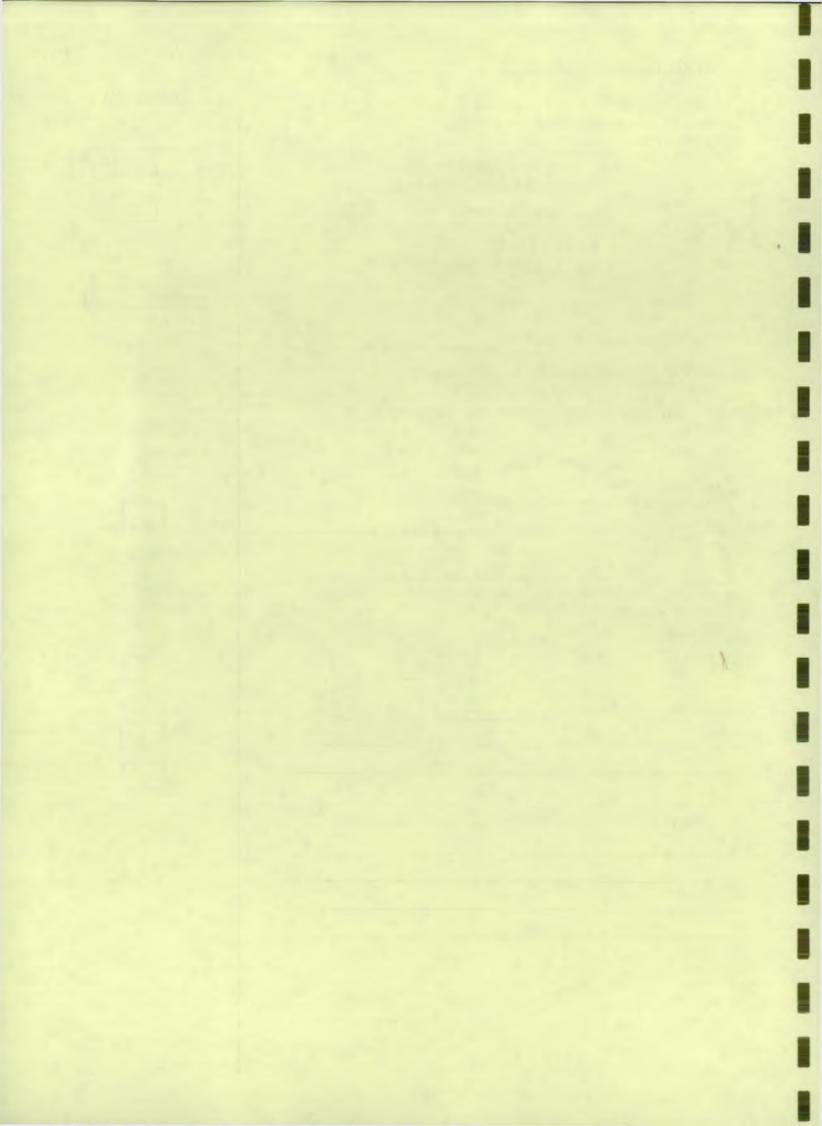
Gut	symptoms Any stomach i bow			Not
	POSTARS S	Yes	No	Sure
13.	Loss of appetite	Ш,		□,
14.	Indigestion			
15.	Stomach pain (colic / lower abdominal pain / griping)			Ο,
16.	Loose bowel motions (loose than normal)	er 🔲		□,
17.	Diarrhoea (3 or more runny stools in 24 hours)			Π,
18.	Nausea (feeling sick)			□,
19.	Vomiting (being sick)			Π,
Skir	n symptoms Any skin problems	7		
20.	Skin rash on body			
21.	Skin ulcer / sore			
22.	. Itching (irritation)			
Oth	er symptoms Any other proble	ms ?		
23.	Excessive tiredness (unusu fatigue, lassitude)	lal		
24.	Dizzy or giddy			
25.	Pins and needles / tingling			
26.	Muscle cramps (e.g cramp in arm or leg)			□,
	. If you had any symptoms n the space below:	ot on this list,	please write	them
	No symptoms recorded	I in the last 3 d	ays 🗍	

If NO illness go to Question 7, next page

Code response to no symptoms as 0 in all bones Appetite 247 Stomach pain 250 251 252 Skin sleer/ 254 liching. 255 Dizziness



	Coding only
4. Ring all days on the calendar on which the symptoms occured:	
(When did the lliness start, when did it finish and how long did it last?)	Date of 0 7 9 2 262
June / July 1992 TWTFSS	Duration - days 263
30 1 2 3 4 5	Code as per symptom numbers in
5. What was the first symptom?	the list on pages 2 and 3 First symptom 264
Number as per the symptom list on pages 2 and 3	
6. Have you seen your doctor about these symptoms? Yes No	
	Doctor seen 265
If yes has an illness been diagnosed? Diagnosis	Diagnosis 266
7. Apart from this study, have you been swimming, taken part in any water sports / water leisure activities, or visited a beach since the interview with the GREEN FORM	
Yes No Not sure	
	Water activities 267
If yes please give details:	Activity
	type
8. Do you have any other information you would like to add?	
	Other information



	Supervisor Name Supervisor No.		···						atior iviti											mei	rsion	ŀ									
wallowe	d		* Location	-	1	2	3	1	2	3	1	2	3	1	2	3	1	· .	3	1	2	3	1	2	3	1	2	3	1	2	3
water?			** Activity	•	1	2	3	1		3		2		1	2]3	1		3			3	1	2_	3	1	2	3	1		3
Y/N	Name / No.	No.	time in	Time		0-10 min			10-2 min			20-3 min			30-4 min			10-50 min:			50-6 mir		L	60-7 min			0-80 mins			80-90 min	
		1		Loc.						-	_	_				_				L	ļ	<u> </u>	_	<u> </u>		_		4	\dashv		
		_		Loc.				_		0	-					 				一		_			_			ㅓ	-		
		2		Act.						E .																					
		3	!	Loc.			_				-	<u> </u>				_	_			-								4			
		4		Loc.						i	┢		-			-	┢			┝		<u> </u>	┢					ᅱ	+		
		4		Act.						2																					
		5		Loc.				_		3		 				 	<u>L</u> .			L	_	 	<u> </u>					4	_		_
				Loc.						:	 						\vdash			_	_		-					\dashv	\dashv	\dashv	_
		6		Act.					Ė	i																					
		7		Loc.							_					_					<u> </u>						_	\dashv	_	_	_
-+				Act.							_			\vdash			_			_		Н				_	_	{	4	\dashv	_
		8		Loc.						-										_	-						\dashv	\dashv	\dashv	\dashv	_
		_		Loc.								×																コ			
		9		Act.																							_	_	_	_	
		10		Loc.	_									_												_		4	_	_	_

3

Coding only

Volunteer

SEASID	E HEALTH
SURVEY:	1992 SAMPLE

Study no Post exposure interview (1 week after bathing) date 0. Interviewer Interviewer name: _ non both SECTION ONE-FOOD INTAKE 1. Subject name: Code No as 0 in all boxes, code not sure as 9 in all boxes 2. Have you eaten any of the following foods during the past week? Not Yes No Sure 1. Ice cream 2. Bought sandwiches 3. Chicken Eggs 4. Eggs Mayonnaue 5. Mayonnaise (fresh) Hot dogs 6. Hot dogs Hamburgers 7. Hamburgers Salad 8. Salad Raw milk 9. Raw milk (i.e. green top) 315 10. Cold meat / Cold meat / pâté pâte 316 11. Meat pies / Meat pies / pasties pasties

List continued on following page



STRICTLY CONFIDENTIAL Coding only FOOD INTAKE - CONTINUED Not Yes No Sure Take away 12. Any Take-away food 13. Sea food * Sea food (e.g. shellfish, cockles etc.) Specify: SECTION TOWO-HEALTH 3. Since the bathing day, please tick whether you have had any of the following symptoms. (Answer Yes, No or Not sure for all, or None on next page) Read out the section headings and ask if they have had any symptoms of that type. Show calendar provided to help ascertain the onset date and duration of each symptom. Code response to no symptoms (see page 3) as 0 in all boxes Onset 'Flu / cold symptoms Not **Duration** date -Yes No in days Sure Jul Fever 1. Fever (Hot and cold, shivers) 2. Headache Aching limbs 3. Aching arms, legs, joints 4. Sore throat Chest symptoms Any chest problems? Chest 5. Chest pains / aches 6. Dry cough cough Prod. 7. Productive cough (phlegm / sputum) 8. Shortness of breath 9. Runny nose

Ear / eve symptoms Any ear or eye problems?

10. Ear infection (sore, discharge)

11. Eye infection (sore red eyes)

12. Blurred vision (difficulty with eye sight)

Vie

Eye infection

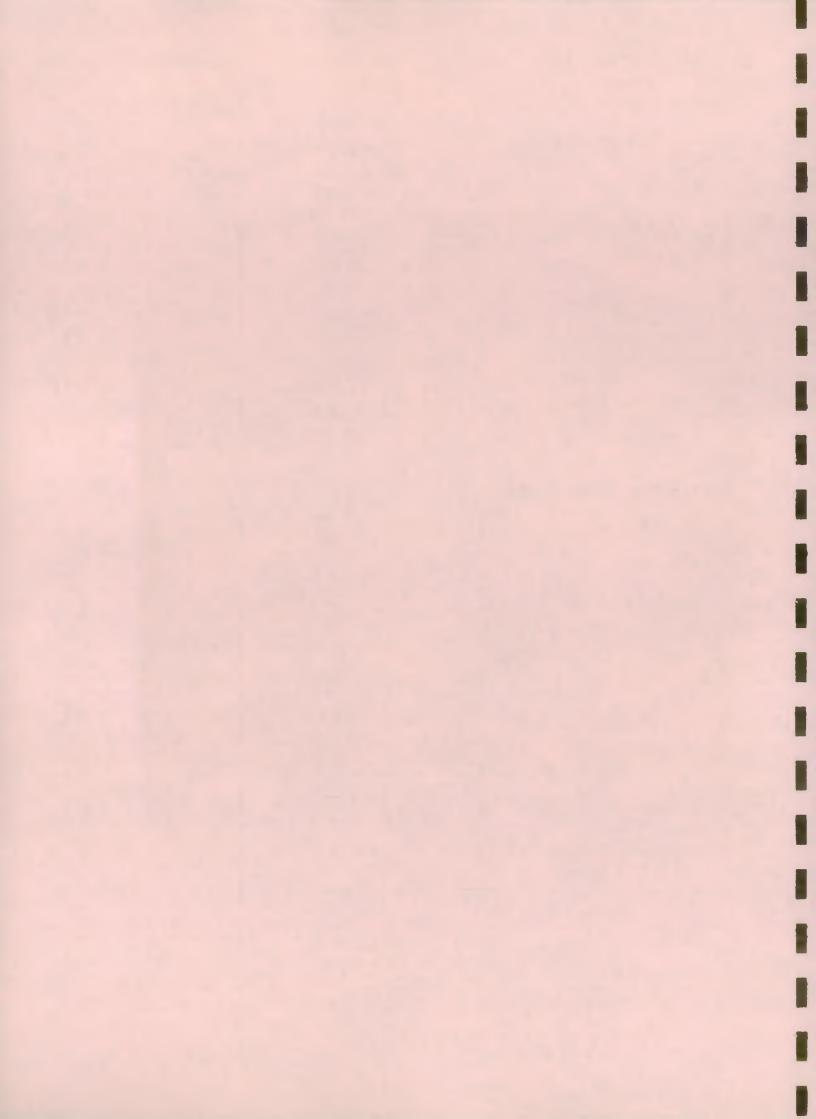
Vicen 33

List continued on following page

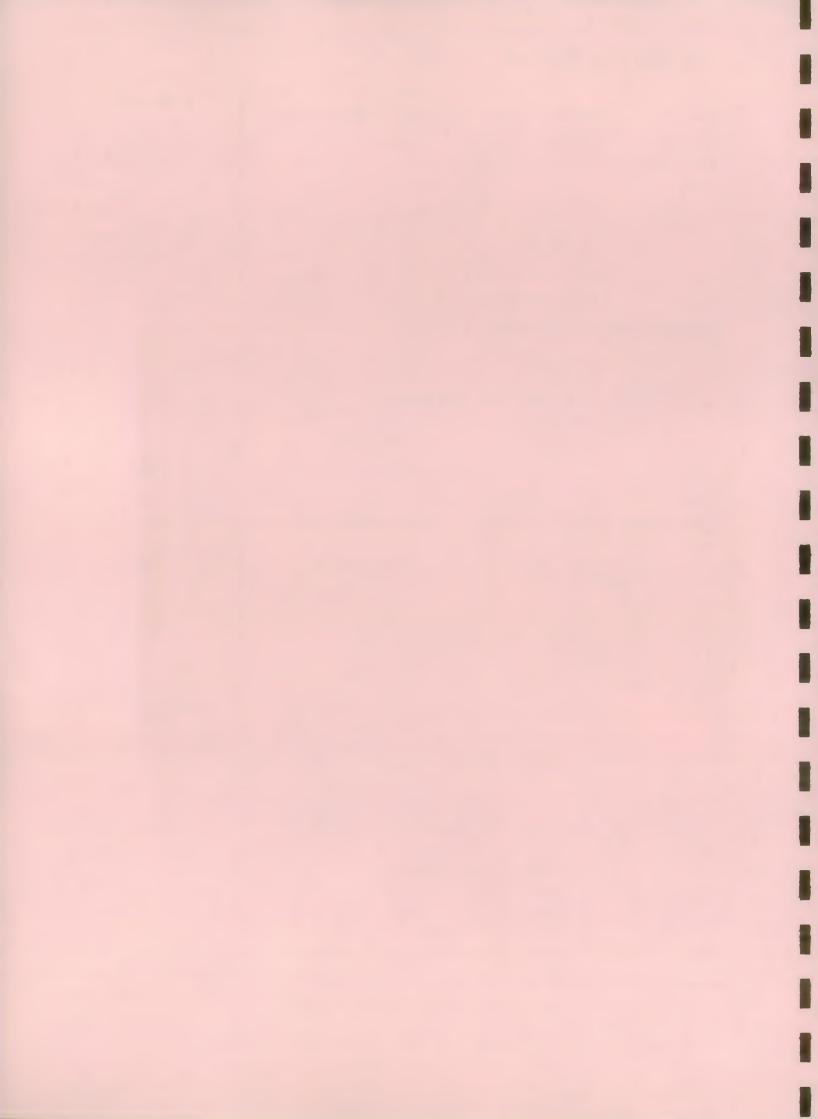


3. Sym	ptoms cont	inued								
Guts	symptoms	Any stomach / bowel		Not	Onset date -	Duration	Code respon	nee to no sy	mploms as 0	
		problems?	Yes No	Sure	Jul	in days	Appetite	mptom	Onset	Duration
15.	Loss of ap	petite		, \square ,			Indigestion			333
14.	Indigestion	1		, \square ,						334
15.	Stomach p abdominal			, ,			Stomach pam			335
16.	Loose bow	rel motions (looser					Loose bowels			336
17.	Diarrhoea stools in 24	(3 or more runny hours)					Diarrhoea			337
18.	Nausea (fe	eling sick)					Nausca			338
19.	Vomiting ((being sick)					Vomiting			339
Skin	symptoms	Any skin problems?								
20.	Skin rash						Skin rash			340
21.	Skin ulcer	/ sore		, [],			Skin ulcer /			341
22.	Itching (ir	ritation)		, 🛛 ,			Itching			342
Oth	er sympton	ns Any other problems	?							
23.	Excessive fatigue, l	tiredness (unusual assitude)		. [].			Lassitude			543
24.	Dizzy or g	iddy		0 .			Dizzinesa			344
25.	Pins and n	eedles / tingling					Pins and needles			345
26.	Muscle cra in arm or	amps (e.g. cramp leg)					Muscle crainpa			346
	If you had he space be	any symptoms not on low:	this list, p	lease wr	ite them	_	Other	347	Туре	348
W	nen did thes	se symptoms start?			many day	ys 🔲	Other- onnet		Other- duration	350
	No s	symptoms recorded in	the last 3 c	lays [

If NO illness go to Question 7, next page



4. Ring all days on the calendar on which any of the symptoms occured:	Coding only
(When did the illness start, when did it finish and how long did it last?)	Dute of 0 17 9 2 351
June / July 1992 M T W T F S S 29 30 1 2 3 4 5	Duration - days
6 7 8 9 10 11 12	
(Code first day of onset and total duration)	
5. Have you seen your doctor about these symptoms? Yes No	
	Doctor acen
If yes has an illness been diagnosed?	Diagnosis 1
Diagnosis	354
6. How many days work / normal activities did you miss because of this illness / symptom?	Diagonsus 2
days work / activities	Days lost 355
Were you admitted to hospital? Yes No	Hospital admission
If yes which hospital:	
7. Apart from this study, have you been swimming, taken part in any water sports / water leisure activities, or visited a beach since the bathing day	
Yes No Not sure	
	Water activities 357
If yes please give details:	
	Activity type 356
	130

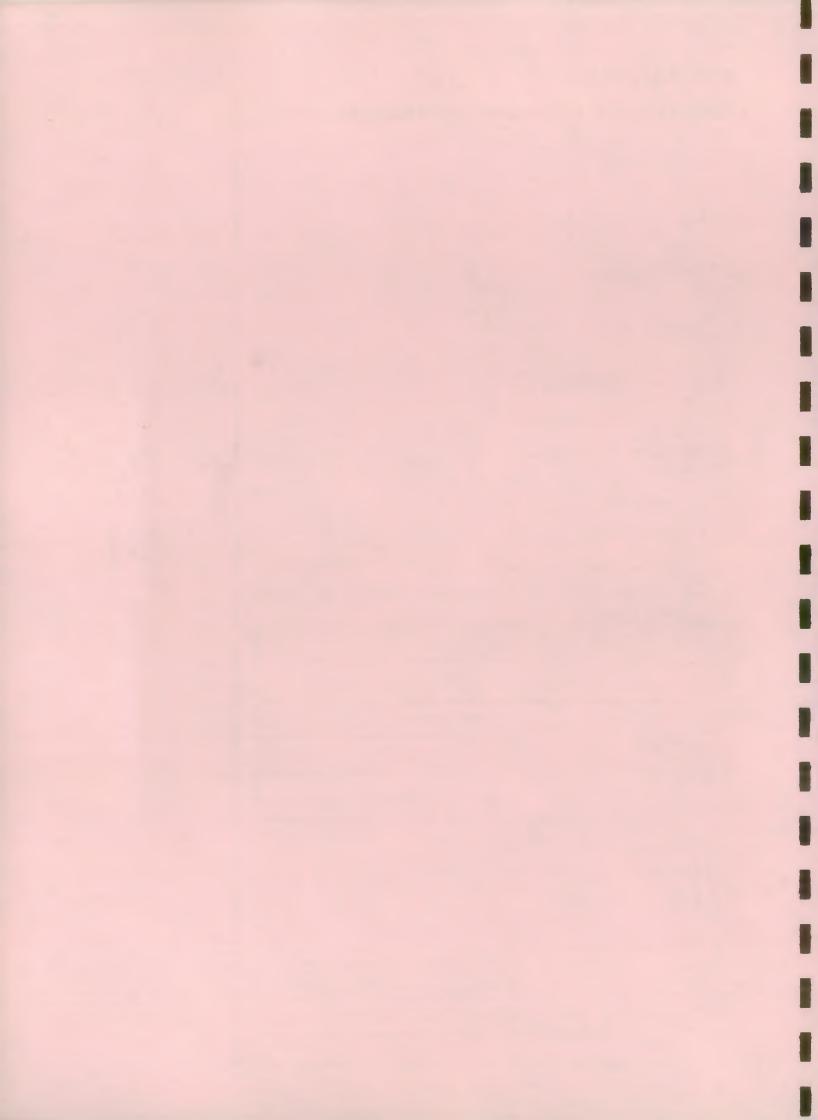


Coding only

STRICTLY CONFIDENTIAL

MEDICAL SHEET - to be completed by the examinining doctor

Doctor's initials:				Doctor No.	359
Faecal sample presented:	Yes	No Do		Faccal sample 1 presented ?	100
Throat swab taken:				Throat swab taken ?	361
Ear swab taken:				Ear swab Taken ?	362
Appearance of Throat:	Normal	Red	Infected	Appearance of throat	363
Evidence of any middle ear infection:	Yes	No D	Not Sure	Evidence of ear infection	164
If <u>yes</u> please give brief details	5:				
If the volunteer shows eviden suspected diagnosis:					
Те					
Action taken . Advised to see G. P. if symp	Action takes				
Letter sent to G. P.		365			
Telephone call to G. P.					
Other*					
* Specify:					



dob

SEASIDE HEALTH SURVEY: 1992 SAMPLE

Post exposure postal questionnaire

Please read through this form and then answer the questions carefully. Please answer every question, circling items, or ticking boxes e.g.:

June / July 1992 M T W T F S S 29 30 1 2 3 4 5

29 30 1 2 3 4 5 6 (7 8 9 10 11) 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 YES

NO

or providing written answers, as necessary.

Please do not write in the section for coding at the right hand side of the form.

The completed form should be returned in the envelope provided as soon as possible.

Study no 403

date 9,2

interviewer 4,1

Bather / non bather 402

1. Name:

2. Date of Birth

Day Month Year

3. Sex : MALE

FEMALE ____

4. Home address:

Postcode _

5. Work/study address

Telephone no. (home):

6. Contact details for follow-up (address etc. over next three months).



7. In the last three weeks (since 4th July symptoms? Please answer YES, NO or YES to any symptom please give the day each symptom started and how many day 'Flu / cold symptoms	NOT ate, as	SUR far a	E for eass you can	ch. If y	ou answer	
Fig / cold symptoms			NOT	Date	Uour long it	
(1.) Fever (Hot and cold, shivers, raised temperature)	YES	NO	SURE	Started	How long it lasted (days)	Pever Durstion Headache
(2.) Severe Headache						
(3.) Aching arms, legs, joints						Aching limbs 411
(4.) Sore throat						Sore throat
Chest symptoms			NOT	Date	How long it	
(5.) Chest pains / aches	YES	NO	SURE ,	Started	How long it lasted (days)	Chest pains Onset Duration 413
(6.) Dry cough						Dry
(7.) Cough with phlegm / mucus						Prod. cough
(8.) Wheezing / Shortness of breath						Breathing diff.
(9.) Runny nose						Runny nose 417
Ear / eve symptoms			NOT	Date	How long it	
(10.) Ear infection (sore and / or	YES	NO	SURE		lasted (days)	infection
discharge) (11.) Eye infection (sore red eyes and / or discharge)						Eye
(12.) Blurred vision (difficulty with eye sight)						Visino 420
Stomach / bowel symptoms			NOT	Date	How long it	
(13.) Loss of appetite	YES	NO	SURE	Started	lasted (days)	Appetite Obsert Duration
(14.) Indigestion						dipertion 421
(15.) Stomach pain (colic / lower abdominal pain / griping)						Stomach paint 422
Symptom list continued on the fo	llowin	g pa	ge			



	ns continued ach / bowel symptoms continued	d					
Stollia				NOT	Date	How long it	
(16.)	Loose bowel motions	YES	NO O	SURE	Started	lasted (days)	Loose bowels Duration
(17.)	Diarrhoea (3 or more runny stools in 24 hours)			Ο,			Diarrhoes 425
(18.)	Nausea (feeling sick)						Names .
(19.)	Vomiting (being sick)						Vomting 426
Skin	symptoms			NOT	Date	How long it	
(20.)	Skin rash on body	YES	NO	SURE .	Started	lasted (days)	Skim rash
(21.)	Skin ulcer / sore						Skin ulcei / sore
(22.)	Itching (irritation)						biching 43
<u>Oth</u>	er symptoms			NOT	Date	How long it	
(23.)	Excessive tiredness (unusual fatigue, lassitude)	YES	NO		Started	lasted (days)	Lassitude
(24.)	Dizzy or giddy						Dizzinem 432
(25.)	Pins and needles / tingling			. 🗆 ,			Pins and needles
(26.)	Muscle cramps (e.g. cramp in arm or leg						Muscle cramps
	If you had any symptoms not on space below:	on this					
III tiic	s space below.			Date Started	How long lasted (da		Other:
1.						_	435
2.			-				2 436
3.						_	3
	ou had <u>NO SYMPTOMS AT</u> page 6	ALL	pleas	se skip (to Questi	on 16	

If you answered <u>YES</u> to <u>ANY SYMPTOMS</u> please answer the Questions on the next page



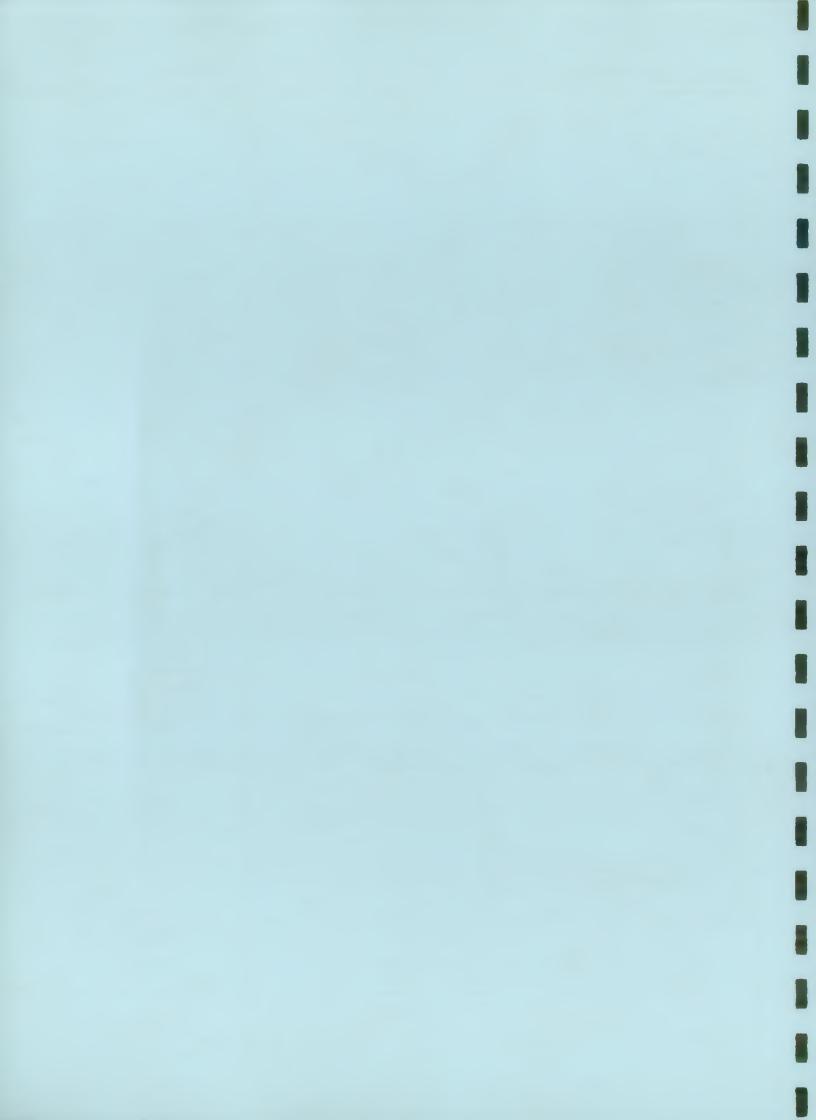
8. Were the symptoms you ticked part of one illness?	
YES - One illness	Single illness?
NO - I had more than one illness	438
NOT REALLY AN ILLNESS - 1 was not unwell	
2	
UNSURE	
9. On the calendar below, please circle all the days on which you were	Date of O 9,2
unwell or had these symptoms	Duration 439
June / July / August 1992 M T W T F S S	of illne as (days)
29 30 1 2 3 4 5	
6 7 8 9 10 11 12	
13 14 15 16 17 18 19	
20 21 22 23 24 25 26	
27 28 29 30 31 1 2	
3 4 5 6 7 8 9	
To help you remember, the bathing day is shaded	
10. What was the first symptom of your illness?	Code 01 - 26 as per symptom list
	First Symptom
	441
11. If you had more than one illness please give details below,	
especially the date each illness started	



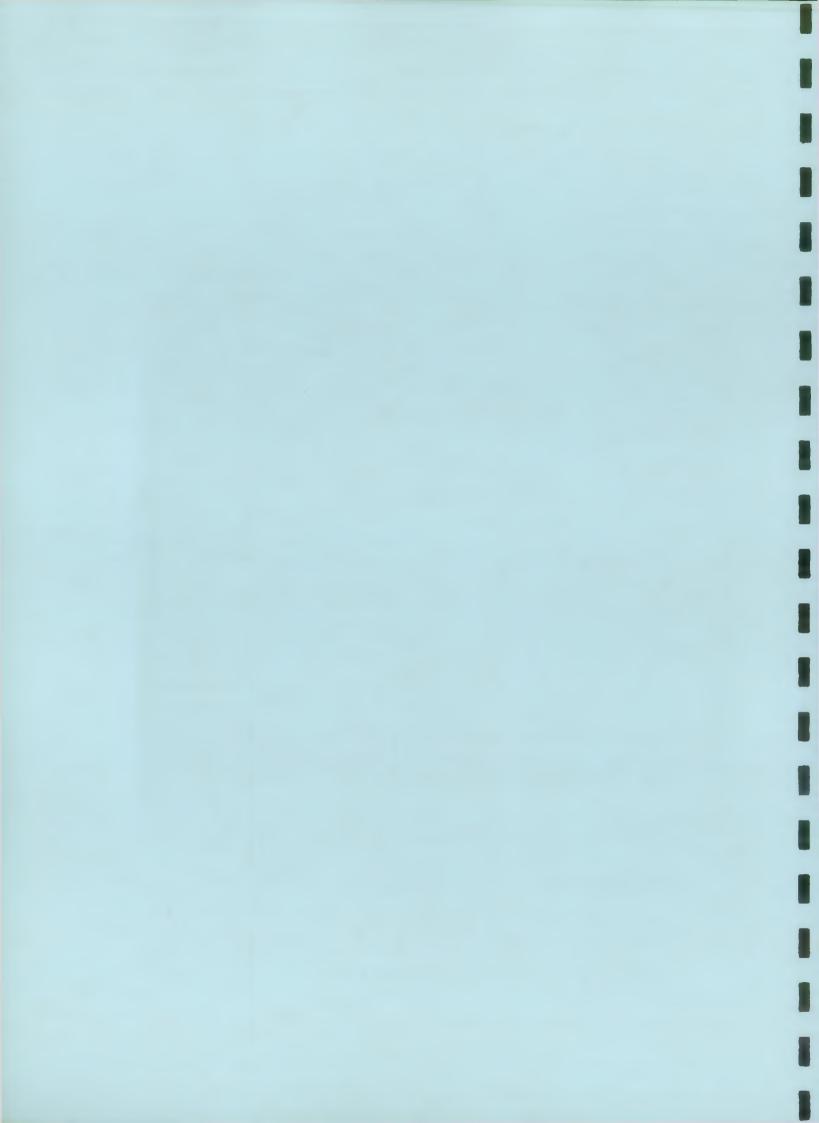
12. Was this illness diagnosed by your G.P.? YES NO If YES, what was the diagnosis?	Diagnosis illness 1
Please tick if we may approach your doctor for more information, if necessary: YES NO O O O O O O O O O O O O	Doctor may be consulted ?
13. Did you take any drugs or medicines for your illness, PRESCRIBED BY YOUR DOCTOR? YES NO If YES please list:	Prescr. Drug type 445
14. Have you received hospital treatment for any illness since the bathing day? YES NO If YES which hospital did you attend?	Hospital beatment 46
15. How many days did you have away from work or normal activities because of this illness? ONE 2-7 7-14 MORE NONE DAY DAYS DAYS THAN 14 NOT ONLY DAYS SURE	Working days lost



16. Have you	ever become ill s	oon after bath	ing in waters i	n the U. K. ?	
		YES	NO D		Any alkness from UK bathing
If YES, what than one):	was it any of the	following illi	neses (You can	tick more	
Headache	Toothache	Earache	Diarrhoea	Vomiting	Type of silness
Fever	A common cold	Sore throat	Eye irritation	Other*	
* Please s	pecify:				
17. Have you	ever gone to the	beach feeling	ill?		
		YES	NO D		Visits to beach feeling all
If YES, what than one):	t was it any of the	e following illi		n tick more	450
Headache	Toothache	Earache 3	Diarrhoea	Vomiting	Type of illness 451
Fever	A common cold	Sore throat	Eye irritation	Other*	
* Please s	specify:				
18. Did feeling	g ill on these occa	asions prevent	you from ente	ring the water?	
	YES	NO Do	NOT SURE		Ilinesa prevent bathing! 452



19. How often do you get sunburned while at the beach?	
Always Frequently Rarely Never	Sauhum?453
20. How often do you apply some sort of medication or home remedy to a sun burn aquired at the beach?	
Always Frequently Rarely Never Sure	Sunburn medication?
21. Are you prone to motion sickness while travelling in automobiles, buses or trains?	
Always Frequently Rarely Never	Motion sickness?
22. Has anyone else in who lives in your household been unwell with a possible infection in the last 3 weeks? (The household includes only the people you live with or with whom you share facilities, such as a kitchen or toilet).	
YES NO SURE	Household Disease 456
If NO - no new illnesses in last 4 weeks, go to question 25 on page 8	
23. If <u>YES</u> - did any illness in your household / family start before yours? NOT	
YES NO SURE	III beliere 457



24. Pl	ease give details bel	ow:			
Fo	or type of illness wri	te:			
	'D' 'S' 'EAR' 'EYE' 'F' 'T' 'O'	for Stoffor an for Fe for a s	carrhoea comach upsets (e.g. feltomach upsets) EAR infection (e.g. exertion (e.g. some series) EYE infection (e.g. some series) EYE	ar ache) re red eyes)	
	g. if a child had diagon would write: 'D,		upset stomach and an for type of illness	ear infection	
	Name or initials	Age	Type of illness	Date illness started	Illness Onset Age Type Date
					1 458
					2
					3 1 1
					4
					461
					5 462
			which could help e.g. ut the illness and symp		
					Other details 1
					Other
					detaile 2
25. H	Have you taken part on 5th July?	in any wa	ter sports since the visi	t to the beach	
		YES	NO SUR		Water activities
				19	
	If NO go	to QUEST	FION 26, on PAGE 9		

If **YES** please continue on the next page

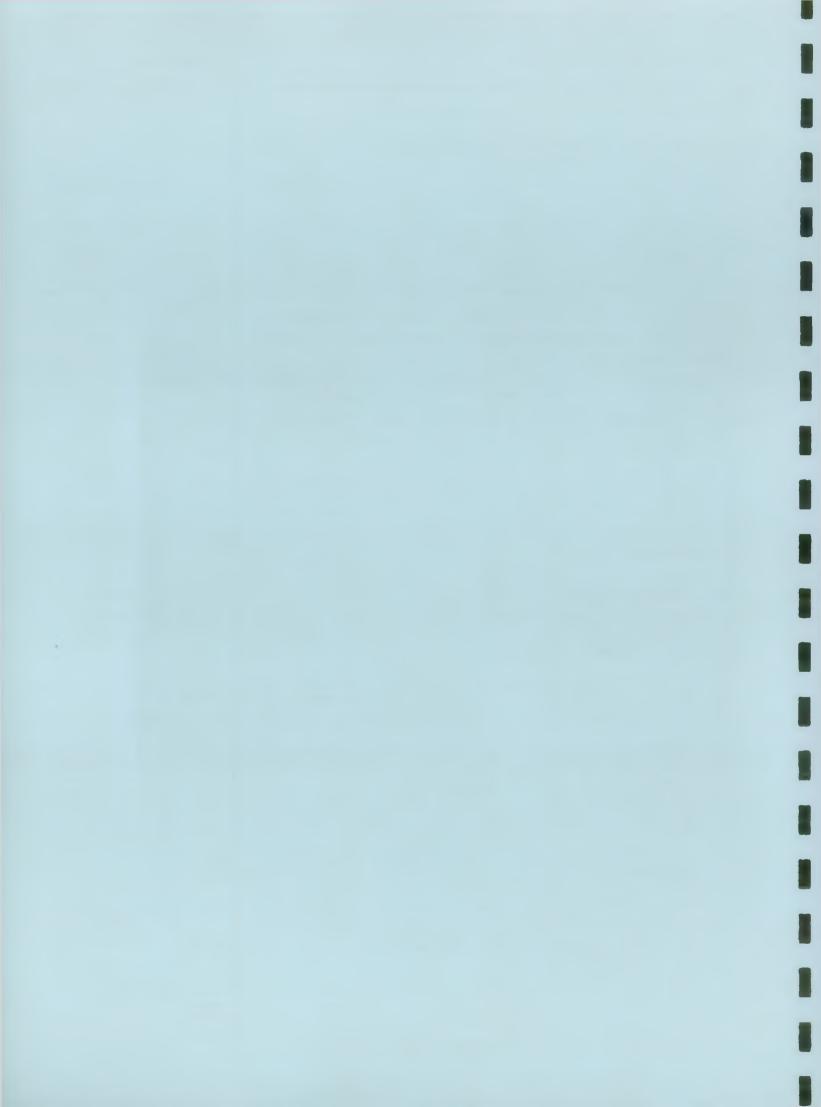


QUESTION 25 CONT	INUE	D					
If YES please answer the for each of the activities the activities listed:			ist, i.e. pl	lease an		of	
		YES		NOT SURE	TIME SINCE 4		
Public swimming	pool				SINCE 4.		Public Prequency pool
Other swimming	pool						Other pool Frequency 468
For each of the water sp sea or fresh water (tick) Lakes and reservoirs etc	both if						Code positive response for sea / fresh water as 1 and negative response as 0.
	YES	NO	NOT SURE		If YES, tic type of wat		Sea Freah
(1.) Dinghy sailing/ Canoeing			9	SEA	FRESH		Dinghy / Canoe 470
(2.) Speed / motor boating / rowing	□,			SEA	FRESH		speed boat Sea Fresh
(3.) Subaqua / diving / snorkeling				SEA	FRESH		sub aqua 474 475
(4.) Surfing / water ¶ skis / jet skis				SEA	FRESH		Surfing etc. Sea Fresh
¶ Include: wind surfi	ng, sail	boarding 6	etc.				
(5.) Fishing				SEA	FRESH		Finking Sea Presh
(6.) Paddling / wading				SEA	FRESH		Pishing Sea Fresh 481
(7.) Other				SEA	FRESH		Sea Fresh
Details of other was	ter spor	t:					
26. Do you consider water	er relate	ed activitie	s danger	ous?			

QUESTION 26 IS CONTINUED ON THE NEXT PAGE

YES

NO



QUESTION 26 CONTINUED

If <u>YES</u> which of the following water-related activities do you consider dangerous? (You can tick more than one):

Dinghy sailing	Canoeing	Wind sufing / sailboating	Scuba / snorkeling
Water skiing	Surfing	Swimming / bathing	Other*

* Please specify:

27. Since the day at the beach have you spent any nights away from home, e.g. for a holiday or to visit relatives?

YES	NO	SUR
Ш,		

If YES was this in the U.K. or abroad?

U.K.	ABROAD	вотн

Please give the date(s) and place(s) visited below:

Place(s)	Date(s)	Duration of stay
1.		
2.		
3.		
4.		

28. Have you been swimming in the sea, or in a lake or river since the day at the beach?

YES	NO	NOT SURE	SEA WATER	LAKE / RIVER	NUMBER OF TIMES SINCE 4 JULY
		Π,			

QUESTION 28 IS CONTINUED ON THE NEXT PAGE

Dangerous activity 485

Visits away from home

Visits U.K./

		487		
No. U.K	visits	No. vii Abroad		489
	JK/ Abroac	Location	Date of return	
1				490
2				491
3				492
4				

Code positive response for sea / fresh water as and negative response as 0.

Sea Presh

	Sea	Presh	
Bathing 1		Prequ	
	0.1		
	1910	495	496

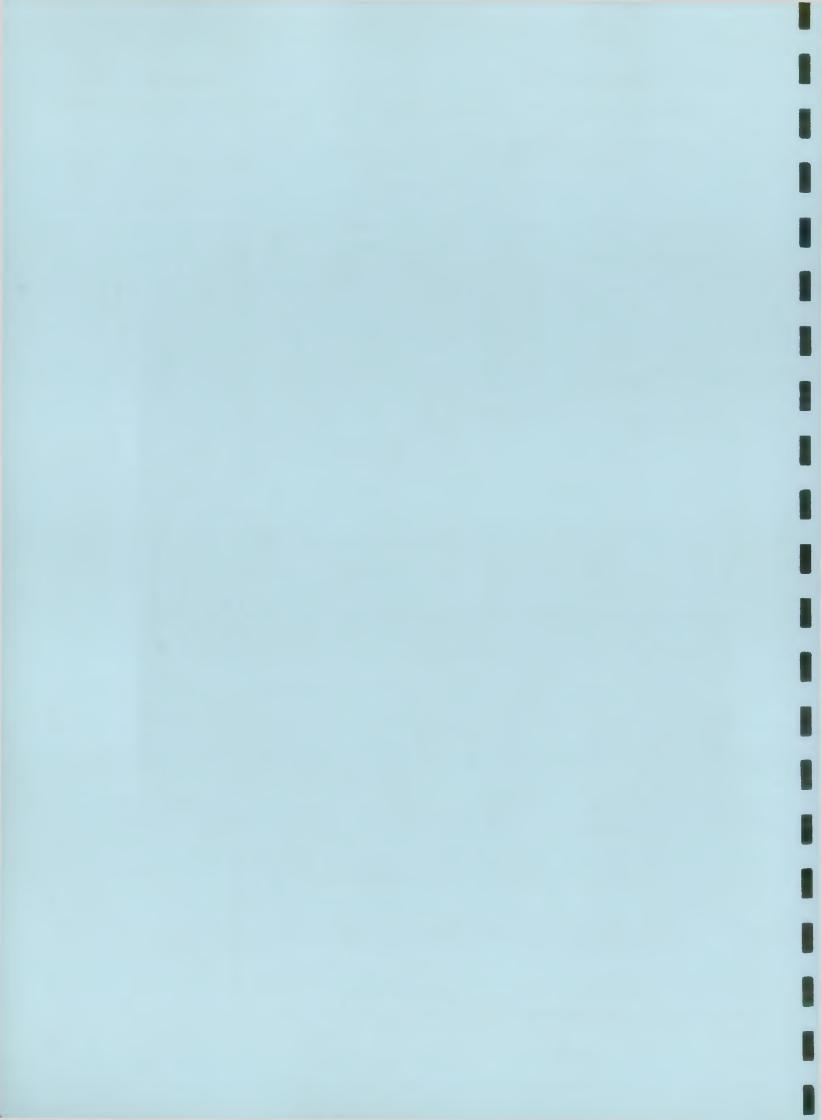


UESTION 28 CONT				Addres .	- The same	47
If YES please list w in the United Kingdo	here you have be om or abroad and	en swimmin specifying	g, ticking whether dates if possible:			
Place(s)	UK A	broad	Date(s)	ហ	K? Location	No. of visits
1.				1	1 1	488
2.				2	111	1
3.				3		489
4.				4		490
29. Have you visited the day at the best YES If YES please list ar United Kingdom or Place(s) 1	NOT SURE o o o o	SEA WATER d, ticking what the state of the	NUMBER LAKE / TIMES RIVER SINCE 4 JU nether in the		Sea Fresh Pre- 492 493	No. of vasts
3.				- 3		496
4.				- 4		497
30. When you visit		athe or enter	the water:			498
Every visit	Most visits	Rarely	Never		Water 499	
31. How frequently						
Always	Frequently	Rarely	Never		end mmers join 500	

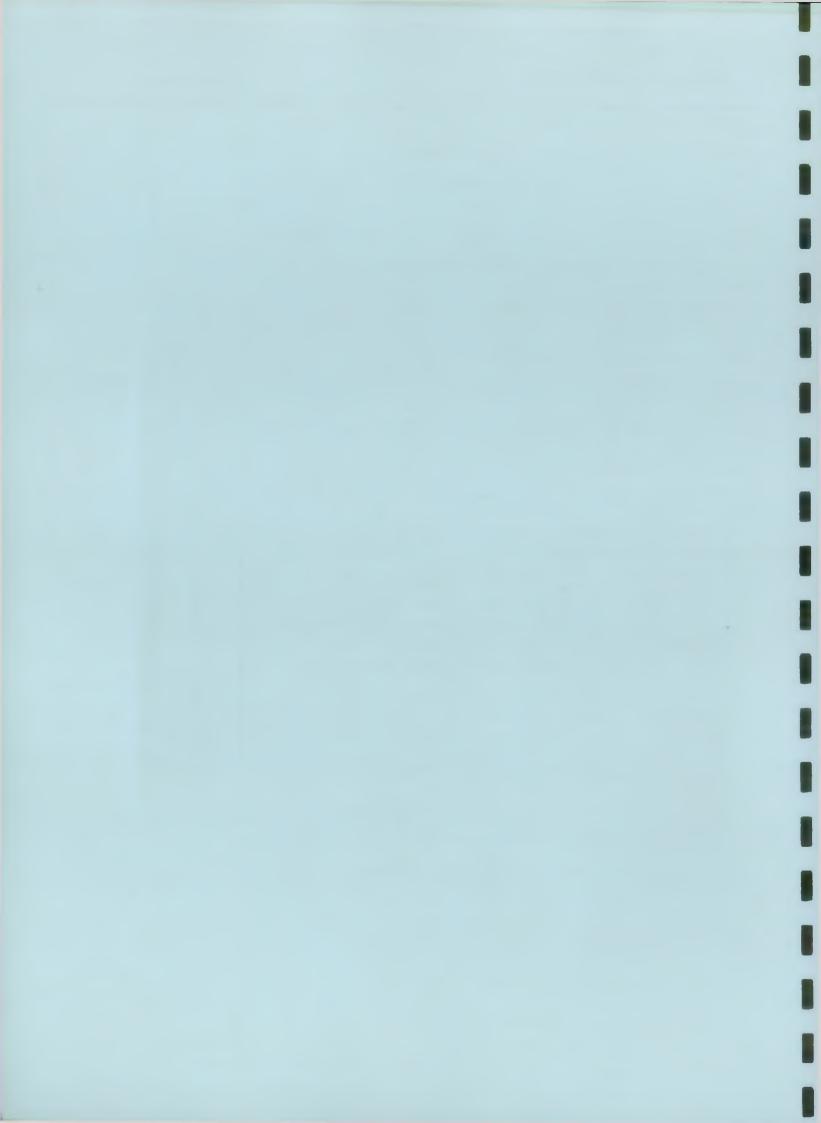


32. Since the day at the beach, have you been to a theme / leisure park and used any water rides? (e.g. log rides, water shutes).	
YES NO NOT SURE	
	Water rades 501
If YES was the site at home or abroad?	
U.K. ABROAD	UKT
	Park Location 502
Please give the name of the site:	503
33. Have you heard anything regarding the way beaches are maintained in th U. K.?	
YES NO	State of UK beaches
	504
If NO go to QUESTION 34, below	
II NO go to QUESTION 34, below	
If <u>YES</u> has this information been <u>positive</u> or <u>negative</u> :	
Positive Negative	
	+/-UK beach mainstenance 505
If NEGATIVE, how often do you worry about this issue?	
Not at all Somewhat much Sure	
	Head mimeration 506
34. Have you heard anything regarding the cleanliness of bathing waters in the U. K.?	
YES NO	State of UK beaches
	507
If NO go to QUESTION 35, next page	

If YES continue on next page



QUESTION 34 CONTINUED:	
If <u>YES</u> has this information been <u>positive</u> or <u>negative</u> :	
Positive Negative	+/-UK beach cleandmeas 508
If <u>NEGATIVE</u> , what specific problems have you heard about? (You can tick more than one box):	
Floating Health Oil spills objects risk	beach problems 509
Chemical Sewage pollution pollution Other *	
* Please specify:	
35. Have you ever refused to go bathing for any of the following reasons:	
Beach too Water too Surf / waves Fear of dirty dirty too rough illness	refusal to bathe 510
36. How did you first hear about this study? (Please tick one box)	
From a From a On In a friend / recruiter television newspaper Other * relation	Source of
	infoS11
* Please specify:	
37. Have you seen any news / media coverage of the study?	
YES NO	Media
	512
38. Are you a member of an environmental organisation?	
YES NO	
	Environmental organisation

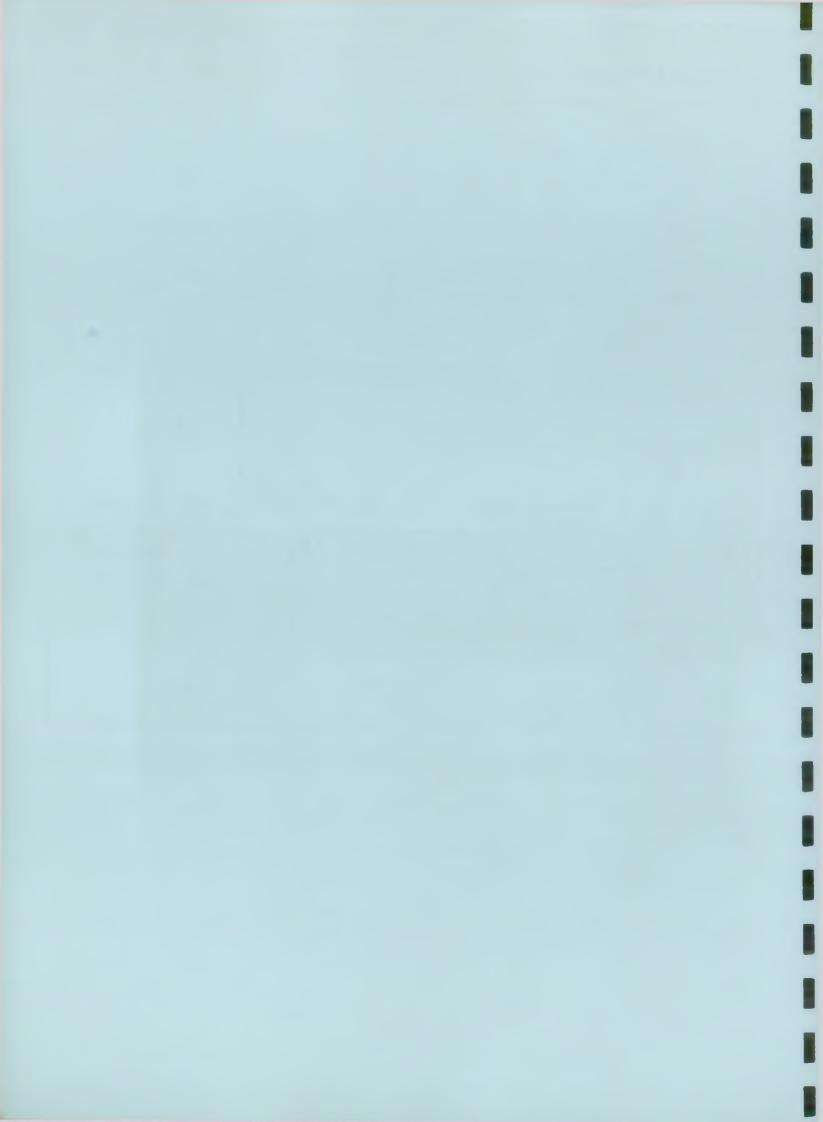


Thankyou for taking the time and trouble to fill in this form. Please return the completed form as soon as possible in the envelope provided.

Also, please remember to send away your final specimen in the container and stamped addressed box supplied. Address for correspondence:

Dr. D. Kay,
Department of Geography,
St. David's University college,
Lampeter, Dyfed
SA48 7ED,
Wales.

Tel. 0570 424749



Appendix III



APPENDIX III Environmental microbiology

A. Environmental Bacteriology

Introduction

Water quality was assessed during the period of the study by examining samples taken at three depths and four sampling stations, at 20m intervals, along the beach. Immediately after collection, samples were delivered to a mobile laboratory situated immediately adjacent to the control centre. Samples were examined for total coliforms, faecal (thermotolerant) coliforms, faecal streptococci, *Pseudomonas aeruginosa* and total staphylococci. Composite samples were analysed for *Salmonellae* and *Cryptosporidium*.

Materials and Methods

Sampling

Water samples were collected into each of two sterile polystyrene containers (Northern Media Ltd) to provide a combined volume of 300 ml. All sample containers were pre-labelled with a unique reference denoting the sample station, depth and run number. Samples were transported immediately after collection as Indicated above. On receipt samples were checked for completeness and placed in the laboratory to await analysis. Laboratory analysis was carried out as soon as possible and within six hours of collection in all cases. Meteorological and environmental conditions at the time of sampling were recorded on a *pro forma*.

Membrane filtration techniques (MF) were used for microbial enumeration. Volumes of sample analysed were determined from data produced from routine monitoring undertaken by NRA (Southern Region) for the immediately preceding weeks. Small volumes of sample (<10 ml) were added to approximately 50 ml of sterile distilled water prior to filtration. The MF apparatus consisted of polycarbonate filter funnels, the bases of which were held in a three place manifold (both Gelman-Sciences Ltd). Filter funnels were sterilised by autoclaving for 15 minutes at 121°C before use and by immersion in a boiling water bath between samples. Membrane filters of 47 mm diameter having a pore size of 0.45 µm were used throughout (GN6 Grade, Gelman Sciences Ltd). Depending upon the organisms sought, absorbent pads (Gelman Sciences Ltd) soaked in an excess of liquid broth or the appropriate agar medium was used in Petri dishes of 55 mm diameter.

Analytical techniques

Total and faecal coliforms were enumerated using membrane lauryl sulphate broth (Oxoid MM615), incubating for 14 hours at 37°C and 44°C respectively following an initial incubation period of four hours at 30°C for both (Anon., 1983). Faecal streptococci were enumerated using Slanetz & Bartley agar after incubation for 44 hours at 44°C, following an initial incubation period of four hours at 37°C(Anon., 1983).

Ps. aeruginosa were enumerated on a modification of King's A broth (Anon., 1983), solidified by the addition of agar (1.5% w/v) which was sterilised by autoclaving at 121°C for 15 minutes, allowed to cool to 50°C before the addition of filter sterilised ethanol. The complete medium was poured into 55 mm Petri dishes and allowed to solidify. Membranes were incubated at 37°C for 48 hours and colonies producing a diffusible green pigment counted as Ps. aeruginosa, identification being assisted by viewing under long wave UV illumination.

Total staphylococci were determined using a membrane filtration procedure as described by Stengren and Starzyk (1984). Membranes were placed grid uppermost on plates of M-5LSMA Plates were incubated at 37°C for 48 hours, following which all typical colonies were enumerated. Confirmation of staphylococci was made on the basis of cell morphology and Gram staining.

Volumes of 0.1, 1.0 and 10 ml were examined for total coliforms with 1.0 and 10 ml aliquots being used for faecal coliforms. All other assays took place using 10 ml and/or 50 ml volumes of sea-water. In the case of coliform counts all dilution's were counted and the final result expressed as the weighted average of all plates producing a value. Assays for faecal coliforms and faecal streptococci were performed in triplicate.

The residual sample remaining after completion of all membrane procedures was retained (approximately 100 ml). These retained samples were pooled and examined for *Salmonellae* and *Cryptosporidium*. A single volume of 1.5 litres was examined for the presence of *Salmonellae* using standard procedures (Anon, 1982). Analysis for *Cryptosporidium* was performed on a composite of 15 litres. The sample was concentrated by centrifugation. Procedures for the isolation and identification of *Cryptosporidium* oocysts followed the standard method (Anon, 1989).

Quality control

On each run, quality control samples consisting of duplicated samples were collected and examined along with that batch. During the exposure period 7 such samples were taken for comparison with actual samples for the analyses of total coliform, faecal coliform, faecal streptococci, *Pseudomonas aeruginosa* and total staphylococci. Geometric mean values for the samples and their matching replicates were tested for significant differences using a two tailed paired sample t-test (Table 1). The only significant difference was found for analyses of streptococci duplicates. Quality control was also undertaken by analysis of prepared samples from PHLS containing total coliforms and *E. coli.* Results from the contractors laboratory were within ranges specified by PHLS.

Table 1 Paired t-test results for quality control samples taken at Southend-on-Sea, 04.07.92

Variable	Geo. Mean S	log ₁₀ itd. dev	Std. error	N	t	2-tail p
Total coliform ¹	301.761	0.356	0.135	7	-0.22	0.832
Total coliform ²	320,000	0.261	0.099	7		
Faecal coliform ¹	112.058	0.320	0.121	7	-1.80	0.122
Faecal collform ²	137.900	0.343	0.129	7		
Faecal streptococci 1	49.957	0.469	0.177	7	5.50	0.002†
Faecal streptococcl ²	13.408	0.312	0.118			
Large Faecal strep. ¹	31.779	0.082	0.031	7	4.41	0.005†
Large Faecal strep. ²	12.487	0.254	0.096			
Pseudomonas aeruginos	a1 2.483	0.637	0.241	7	1.00	0.356
Pseudomonas aeruginos		0.370	0.140	7		
Total staphylococci ¹	99.254	0.436	0.218	4	3.19	0.05
Total staphylococci ²	20.404	0.476	0.238	4		

¹ Samples, 2 Duplicates, † Significant difference at α < 0.05, DF = N-1

B. Environmental virology

Isolation of Enteric Viruses from large volumes of water

Although enteric viruses are present initially in very high concentrations in sewage contaminated with stools from infected individuals, the subsequent dilution of sewage/sewage effluent in waters into which it is discharged, ensure that the final concentration of viruses in the aquatic environment is considerably less than the initial concentration in faeces. Thus, the isolation of enteric viruses from the aquatic environment involves the concentration of large volumes (10-20 litres) of water into small workable volumes (10 ml) which can then be assayed for the presence of viruses using tissue culture or an appropriate assay for viral particles or antigens.

A variety of methods for the concentration of low numbers of viruses from large volumes of water have been described (Gerba et al., 1978; Ramia and Sattar, 1980). The method chosen for this study is the one used routinely by Acer Environmental and is suitable for the isolation of both enteroviruses and rotavirus. It involves a two-stage concentration procedure, adsorption and elution of viruses on microporous filters, followed by organic flocculation.

In aqueous solution, viruses behave as amphoteric, hydrophilic colloids and the net charge is a function of pH, ionic composition and ionic strength of the solution (Morris and Waite, 1981). These properties are exploited in the concentration of viruses from large volumes of water. At low pH in the presence of cations, viruses adsorb by virtue of their surface charge to a variety of media, including cellulose nitrate and glass fibre. Elution from this initial adsorptive phase is achieved using an organic material at high pH, resulting in a primary eluate of more manageable volume. Further concentration of viruses is achieved by a secondary concentration step. This procedure, known as organic flocculation (Katzenelson et al., 1976), utilises the property of organic materials to precipitate or flocculate when the pH of the solution is lowered near the isoelectric point of the material. Viruses are effectively adsorbed to this denovo precipitate, which forms spontaneously upon lowering the pH of the solution. The precipitate and associated viruses are subsequently collected by low speed centrifugation. Viruses are then recovered for assay by dissolving the precipitate in a suitably small volume of moderately alkaline buffer.

Materials and Methods - Concentration of sample

Adsorption

10 litre samples of water were collected in sterile pots from fixed stations along the designated beach and transported to the Virology laboratory for processing within 24 hours of sampling.

The sample was acidified to pH 3.5 with concentrated HCI. Then aluminium chloride, to a final concentration of 0.0005M, was added to enhance virus adsorption (Goyal and Gerba, 1982). The sample was then filtered through a polypropylene cartridge pre-filter (pore size 75 μ m) and then through a glass fibre cartridge filter (pore size 8 μ m) using a peristaltic pump. The pre-filter prevented the pores of the membrane from becoming clogged with sand and fine silt commonly found in marine water samples. After all the water had passed through the filtration apparatus adsorbed virus was eluted from the filters by passing 500 ml of 1% beef extract in 0.05M glycine (adjusted to pH 9.5 by addition of 1M NaOH) using a peristaltic pump.

Flocculation

IM glycine (pH 2.0) was added dropwise to the filter eluate until a fine brown precipitate began to form at around pH 4.0, the isoelectric point of beef extract, which generally coincided with the formation of a dense brown precipitate. The eluate was transferred to a refrigerator at 4°C. After 1 hour, the precipitate took on a flaky appearance forming a "floc". This floc was centrifuged at 2800g for 20 minutes and the resultant pellet was resuspended in 10 ml 0.15M Na₂HPO₄ buffer. The pH of the concentrate was adjusted to 7.5 before dividing it into two equal aliquots and storage at

-70°C until the samples were assayed for enteroviruses (aliquot 1) and rotavirus (aliquot 2).

Assay for enteroviruses

Buffalo Green Monkey kidney (BGM) cells (passage numbers 101-103) were used in the assay for enteroviruses. These cells are fibroblastic in morphology and have reported viral sensitivity to poliovirus types 1, 2 and 3, echovirus types 3, 6, 7, 9, 11, 12 and 27, coxsackie virus types A9 and B1, B2 and B3 and reovirus type 1. The BGM cell cultures were propagated serially in growth medium (HMEM) supplemented with 50% Leibovitz L15 medium and 10% Foetal Calf Serum (Gibco Laboratories Ltd).

The samples were assayed for enteroviruses using the suspended cell method in vented petri dishes. The 5 ml concentrate derived after concentration of water samples was divided into 1 ml aliquots and each aliquot was added to one petri dish. 1 ml of BGM cell suspension containing approximately $3x10^7$ cells and 10 ml agar overlay medium were also added to each petri dish and the three constituents were When the agar was set, the petri dishes were inverted and mixed thoroughly. incubated in a CO2 incubator in the dark for up to 5 days. The agar overlay medium contains the vital dye, neutral red, which specifically stains live cells. Virus-infected cells are apparent macroscopically as areas in the monolayer where the vital dye has not been taken up by the cells. These areas of dead cells (plaques), which usually correspond to the number of infectious units of virus in the sample, were noted, and after their confirmation as plaques (and not artefacts) using the inverted light microscope to detect cytopathic effect (CPE), were counted and for each sample expressed as plaque-forming units (pfu) per 5 litres. This figure was then multiplied by 2 to obtain the estimated level in the original 10 litre sample. Results were then expressed as plaque-forming units (pfu) per 10 litre sample. Poliovirus 2 was included as a control each time and batch of sample concentrates were assayed for the presence of enterovirus by the plaque assay.

Assay for rotavirus

Unlike the enteroviruses described above, human rotavirus cannot be cultivated directly in vitro by current organ or tissue culture techniques. However, if the virus is centrifuged at low speed on to a preformed monolayer of cells, the cells become more susceptible to infection and in the presence of trypsin and absence of serum, the virus undergoes an incomplete replicative cycle, producing viral antigens in the cell. Although the infection is abortive and yields little or no infectious virus (Thouless *et al*, 1977), the viral antigens that are produced can be detected using immunofluorescent antibodies.

The immunofluorescence technique is based on the antibody-antigen reaction in which the antibody-antigen complex is made visible by incorporating a fluorochrome in the antibody molecule. Fluorescence is then detected by darkground illumination using uttra-violet light or visible blue light. In this way, individual fluorescent foci (cells) are recorded and are quantified as infectious units.

Rhesus Monkey kidney (LLC-MK2) cells (passage number 240-245) were used for assay for rotavirus. These cells are susceptible to infection by both human and a variety of animal rotaviruses (McNulty *et al.*, 1977; Thouless *et al.*, 1977) and are used widely for immunofluorescence assays. The LLC-MK2 cultures are propagated serially in growth medium (HMEM) supplemented with 50% Leibovitz L15 medium and 10% foetal calf serum.

The sample concentrates were assayed for rotavirus as follows: LLC-MK2 cells were removed from maintenance culture flasks by trypslnisation with 0.005% trypsln-EDTA solution. After addition of growth medium, the resultant cell suspension was centrifuged at 800 g for 5 minutes. The supernatant was discarded and the cell pellet was resuspended in serum free medium (SFM) containing 0.5 mg ml $^{-1}$ trypsin (without EDTA). Cells were seeded in 96-well microtitration plates at a rate of 5 x 10 4 cells/100 μ l/ well (Figure 2). The plates were incubated for 1 hour at 37°C with high C02 concentration and then for a further 1.5 hours with low C02 concentration. 100 ml of the sample concentrate was then added to each well and the plates were centrifuged at 1400 g for 60 minutes. The plates were then incubated at 37°C for 1 hour, when the sample was removed and replaced with 150 ml SFM (without trypsin). The plates were then incubated overnight at 37°C in 5% C02/air atmosphere.

After overnight Incubation, the medium was removed and each well was washed once with phosphate buffered soline (PBS). The cells were then fixed in ice cold methanol at 4°C for 10 minutes, rehydrated with PBS and then incubated at room temperature for 10 minutes. The plates were then air-dried and 100 ml rabbit-antirotavirus antiserum (1:40 dilution in PBS) was added to each well and, after shaking for 5 minutes, the plates were incubated for 1 hour at 37°C. Each well was washed 3 times with PBS (with shaking) and 100 ml FITC conjugated goat-anti-rabbit antiserum (1:40 dilution in PBS) was added to each well. After shaking for 5 minutes, the plates were incubated for 1.5 hours at 37°C.

Each well was washed 3 times with PBS and 50 ml of 1% solution amido black was added to each well. After shaking for 10 minutes at room temperature each well was washed three times with PBS, and then the plates were air-dried. The number of fluorescing cells (fluorescing foci (ff)), which usually corresponds to the number of infectious rotavirus particles in the sample, were then counted using a Nikon "Diaphot" inverted microscope at an excitation wavelength of 495 mm. The results were then expressed as fluorescing foci per 10 litre sample. Human rotavirus extracted from stools from infected individuals, was used as a control and was included each time a batch of sample concentrates were assayed for rotavirus by the immunofluorescence test.

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C. Clinical sample analysis - swabs and faeces

Escherichia coli / Coliform

Single colonies were picked from MacConkey agar to purity plates and tested for production of glucoronidase enzyme,

giucoronidase producer

E.coli

glucoronidase negative

- Coliform

Pseudomonas aeruginosa

Single colonies were picked from MacConkey agar and tested to determine whether they were oxidase positive or negative. Oxidase positive colonies were then tested for resistance to cetrimide and production of pyocyanin and pyoverdin.

Staphylococcus aureus

Single colonies were emulsified in Wellcome Staphaurex latex suspension. Colonies causing latex agglutination were identified as Staphylococcus aureus.

Streptococcus faecalis

Single colonies were picked from blood agar and MacConkey agar onto a pyruvate containing medium and incubated anaerobically for 24 hours. Streptococci which fermented pyruvate were confirmed by Group O antigen detection - (Wellcome - Streptex). Fifteen isolates selected at random were confirmed by using the API 20 strep typing system.

Haemolytic streptococci

Single colonies were picked from anaerobic blood agar to blood agar purity plates. The streptococcal group was then determined using the Wellcome streptex grouping system.

Salmonella

Colonies were picked from MLCB and/or XLD agars (Oxold) to MacConkey purity plates. Cultures were then identified or excluded serologically.

Shigella

Colonies were picked from XLD agar (Oxoid) onto MacConkey purity plates. Cultures were then identified or excluded serologically.

Campylobacter

Single colonies were picked from Charcoal selective Campylobacter medium to microaerophilic blood agar purity plates. Oxldase positive organisms were tested for aerobic growth, biotyped and phage typed.

E.coli 0157

Up to five non-sorbitol fermenting colonies were picked and tested with *E.coli* 0157 antisera (PHL Colindale).

Appendix IV



Appendix IV Details of statistical analyses and attack rates

A. Clinical results

Ear swabs

	Bath	ers	Non-bo	thers				
Determinand	pos	neg	pos	neg	RR	95% (CI	P
Haem. strep.	0	150	0	172		-,-		-,-
S. faecalls	1	149	0	172	-,-			0.932*
Collform	16	134	14	158	1.31	0.66	2.59	0.558
E. coli	1	149	4	168	0.29	0.03	2.54	0.462*
Ps. aer	3	147	5	167	0.69	0.17	2.83	0.878*
S. aureus	3	147	6	166	0.57	0.15	2.25	0.646*
Any determinand	23	127	26	146	1.01	0.61	1.70	0.919

^{*} Fisher's exact test, 2x1 talled p

Throat swab s

	Batt	ers	Non-bo	thers				
Determinand	pos	neg	pos	neg	RR	95% (CI	P
Haem, strep	3	148	7	165	0.49	0.13	1.85	0.453*
S. faecalis	43	108	60	112	0.82	0.59	1.13	0.266
Coliform	24	127	28	144	0.98	0.59	1.61	0.954
E. coli	4	147	5	167	0.91	0.25	3.33	1.000*
S. aureus	2	149	6	166	0.38	0.08	1.85	0.377*
Any determinand	66	85	83	89	0.91	0.71	1.15	0.480

^{*} Fisher's exact test, 2x1 tailed p

Either swab

	Batt	ners	Non-bo	thers :				
Determinand	pos	neg	pos	neg	RR	95% (CI	P
Any determinand	75	75	95	77	0.91	0.73	1.12	0.409

Ear swab result attack rates (‰)

Determinand	Bathers	Non-bathers
Haemolytic streptococci	0.000	0.000
Streptococcus faecalis	6.667	0.000
Collform	106.667	81.395
Escherichia coll	6.667	23.256
Pseudomonas aeruginosa	20.000	29.070
Staphylococcus aureus	20.000	34.884
Any determinand	153.333	151.163

Throat swab result attack rates (‰)

Determinand	Bathers	Non-bathers		
Haemolytic streptococci	19.868	40.698		
Streptococcus faecalis	284.768	348.837		
Coliform	158.940	162,791		
Escherichia coli	26.490	29.070		
Staphylococcus aureus	13.245	34.884		
Any determinand	437.086	482.558		

Either swab attack rates (‰)

Determinand	Bathers	Non-bathers
Any both swabs	500.000	552.326

B. Questionnaire results

Pre-exposure

	Bat	hers	Non-ba	thers				
Symptom	(C)	well	#11	Well	RR	95%	CI	P
Fever	2	162	9	183	0.26	0.06	1.19	0.115
Headache	10	154	6	186	1.95	0.72	5.25	0.275
Aching Ilmbs	19	145	12	180	1.85	0.93	3.70	0.112
Sore throat	17	147	27	165	0.74	0.42	1.30	0.371
Chest pains	3	161	3	189	1.17	0.24	5.72	1.000*
Dry cough	12	151	6	186	2.36	0.90	6.14	0.116
Productive cough	7	157	15	177	0.55	0.23	1.31	0.245
Breathing diff.	7	157	8	184	1.02	0.38	2.76	0.828
Runny nose	25	138	25	167	1.18	0.70	1.97	0.637
Ear infection	6	158	2	189	3.49	0.71	17.08	0.195*
Eye infection	3	161	5	187	0.70	0.17	2.89	0.903*
Blurred vision	4	160	1	191	4.68	0.53	41.49	0.281*
Appetite loss	5	159	9	183	0.65	0.22	1.90	0.603
Indigestion	5	159	3	187	1.93	0.47	7.96	0.568*
Stomach paln	9	154	5	187	2.12	0.72	6.20	0.257
Loose motions	18	146	12	180	1.76	0.87	3.54	0.159
Diarrhoea	12	151	7	185	2.02	0.81	5.01	0.189
Nausea	7	157	8	184	1.02	0.38	2.76	0.828
Vomiting	6	158	5	187	1.40	0.44	4.52	0.790
Skin rash	8	156	11	181	0.85	0.35	2.07	0.905
Skin sore	6	158	0	192	••		+	0.018*†
Itching	13	151	12	180	1.27	0.60	2.70	0.682
Lassitude	8	156	13	178	0.72	0.30	1.69	0.588
Dizziness	1	163	8	183	0.15	0.02	1.15	0.063*
Pins `n needles	3	160	0	192	• • •	-,-	-,-	0.192*
Muscle cramps	3	161	0	192	•••	7.7	-,-	0.194*
Upper respiratory	39	125	42	150	1.09	0.74	1.59	0.764
Lower respiratory	44	120	39	-153	1.32	0.91	1.93	0.186
Ear/eye Infection	12	152	7	184	2.00	0.80	4.95	0.198
GastroIntestinal	32	131	27	165	1.40	0.87	2.23	0.207
Skin	19	145	16	176	1.39	0.74	2.61	0.396
Other	9	149	19	172	0.57	0.27	1.23	0.209
Any symptom	86	76	83	109	1.23	0.99	1.53	0.081
Any bathing symp	85	78	77	115	1.30	1.04	1.63	0.031†

^{*} Fisher's exact test, 2x1 tailed p t significant at α =0.05

On exposure day

	Bath	ers	Non-ba	ithers				
Symptom	H	well	110	well	RR	95%	CI	p
Fever	2	162	2	188	1.16	0.17	8.13	1.000*
Headache	21	142	32	158	0.76	0.46	1.27	0.374
Aching limbs	15	149	- 8	182	2.17	0.95	4.99	0.096
Sore throat	11	153	18	172	0.71	0.34	1.46	0.452
Chest pains	2	162	2	188	1.16	0.17	8.13	1.000*
Dry cough	9	155	8	181	1.30	0.51	3.28	0.764
Productive cough	5	159	14	175	0.41	0.15	1.12	0.116
Breathing diff.	3	160	7	182	0.50	0.13	1.89	0.472*
Runny nose	17	146	23	167	0.86	0.48	1.56	0.744
Ear Infection	7	157	2	188	4.05	0.85	19.25	0.113*
Eye Infection	3	161	5	185	0.70	0.17	2.86	0.891*
Blurred vision	2	162	1	189	2.32	0.21	25.32	0.890*
Appetite loss	4	160	2	188	2.32	0.43	12.49	0.552*
Indigestion	7	157	7	182	1.15	0.41	3.22	0.998
Stomach pain	7	157	7	182	1.15	0.41	3.22	0.998
Loose motions	17	147	7	183	2.81	1.20	6.62	0.023†
Diarrhoea	8	156	1	189	9.27	1.17	73.33	0.021*†
Nausea	3	161	6	184	0.58	0.15	2.28	0.658*
Vomiting	2	162	0	190	-,-		-,-	0.428*
Skin rash	5	159	5	185	1.16	0.34	3.93	1.000*
Skin sore	4	160	0	190	**			0.090*
Itching	9	155	5	185	2.09	0.71	6.10	0.271
Lassitude	7	157	5	185	1.62	0.52	5.01	0.580
Dizziness	3	161	2	188	1.74	0.29	10.27	0.862*
Pins 'n needles	4	160	2	188	2.32	0.43	12.49	0.552*
Muscle cramps	6	157	2	188	3.50	0.72	17.09	0.195*
Upper respiratory	37	126	43	147	1.00	86.0	1.48	0.911
Lower respiratory	26	136	37	152	0.82	0.52	1.29	0.472
Ear/eye infection	11	153	7	183	1.82	0.72	4.59	0.294
GastroIntestInal	24	140	17	173	1.64	0.91	2.94	0.133
Skin	14	150	8	182	2.03	0.87	4.71	0.144
Other	9	148	7	180	1.53	0.58	4.02	0.538
Any symptom	72	89	78	110	1.08	0.85	1.37	0.618
Any bathing symp	71	91	76	114	1.10	0.86	1.40	0.537

 $^{^{\}circ}$ Fisher's exact test, 2x1 talled p † significant at α =0.05

One week post -exposure

	Batt	ners	Non-bo	ithers				
Symptom	III	well	ŧII	well	RR	95%	CI	p
Fever	6	157	2	190	3.53	0.72	17.27	0.190*
Headache	25	138	34	158	0.87	0.54	1.39	0.649
Aching Ilmbs	8	155	7	185	1.35	0.50	3.63	0.746
Sore throat	32	131	26	166	1.45	0.90	2.33	0.161
Chest pains	5	158	3	189	1.96	0.48	8.09	0.552*
Dry cough	10	152	7	184	1.68	0.66		0.397 -
Productive cough	14	149	8	184	2.06	0.89	4.79	0.133
Breathing diff.	2	161	3	188	0.78	0.13	4.62	1.000*
Runny nose	22	141	21	171	1.23	0.70	2.16	0.566
Ear Infection	13	150	3	189	5.10	1.48	17.60	0.008†
Eye Infection	4	159	4	188	1.18	0.30	4.64	1.000*
Blurred vision	1	162	0	192			-,-	0.918*
Appetite loss	8	155	4	188	2.36	0.72	7.68	0.241
Indigestion	4	158	7	185	0.68	0.20	2.27	0.743
Stomach pain	14	149	11	179	1.48	0.69	3.18	0.416
Loose motions	24	139	16	176	1.77	0.97	3.21	0.084
Dlarrhoea	7	156	3	189	2.75	0.72	10.46	0.219*
Nausea	10	153	10	182	1.18	0.50	2.76	0.884
Vomiting	3	160	5	187	0.71	0.17	2.91	0.910*
Skin rash	8	155	4	188	2.36	0.72	7.68	0.241
Skin sore	4	158	2	190	2.37	0.44	12.78	0.533*
Itching	9	154	5	187	2.12	0.72	6.20	0.257
Lassitude	14	149	7	185	2.36	0.97	5.70	0.082
- Dizziness	7 -		- 6 -	100	1:37	0:47	4.01	0.763
Pins `n needles	4	159	4	188	1.18	0.30	4.64	1.000*
Muscle cramps	1	162	2	190	0.59	0.05	6.44	1.000*
Upper respiratory	53	110	54	138	1.16	0.84	1.59	0.434
Lower respiratory	40	122	32	160	1,48	0.98	2.24	0.083
Ear/eye Infection	17	146	7	185	2.86	1.22	6.73	0.020†
GastroIntestInal	48	115	31	160	1.81	1.22	2.71	0.004†
Skin	19	143	9	183	2.50	1.16	5.38	0.025†
Other	17	141	12	175	1.68	0.83	3.40	0.210
Any symptom	100	63	83	109	1.42	1.16	1.74	0.001†
Any bathing symp	97	66	83	109	1.38	1.12	1.69	0.003†

 $^{^{\}circ}$ Fisher's exact test. 2x1 tailed p † significant at α =0.05

Three weeks post-exposure

	Bath	ers	Non-bo	ithers				
Symptom	ill	well	III	well	RR	95%	CI	P
Fever	13	144	6	175	2.50	0.97	6.42	0.082
Headache	17	139	22	160	0.90	0.50	1.64	0.864
Aching limbs	14	140	9	172	1.83	0.81	4.11	0.204
Sore throat	44	111	32	150	1.61	1.08	2.41	0.025†
Chest pains	4	153	3	179	1.55	0.35	6.80	0.838*
Dry cough	11	145	10	172	1.28	0.56	2.94	0.715
Productive cough	13	144	12	170	1.26	0.59	2.67	0.701
Breathing diff.	8	149	5	175	1.83	0.61	5.49	0.413
Runny nose	26	131	24	159	1.26	0.76	2.11	0.459
Ear Infection	17	140	5	177	3.94	1.49	10.44	0.005†
Eye Infection	7	150	2	181	4.08	0.86	19.36	0.110*
Blurred vision	2	155	4	178	0.58	0.11	3.12	0.828*
Appetite loss	9	147	3	178	3.48	0.96	12.63	0.083
Indigestion	5	152	7	176	0.83	0.27	2.57	0.981
Stomach pain	11	146	10	172	1.28	0.56	2.92	0.726
Loose motions	29	127	22	162	1.55	0.93	2.59	0.120
Dlamhoea	12	143	6	179	2.39	0.92	6.21	0.109
Nausea	14	142	11	173	1.50	0.70	3.21	0.397
Vomiting	7	148	5	180	1.67	0.54	5.16	0.544
Skin rash	6	149	7	178	1.02	0.35	2.98	0.809
Skin sore	1	155	2	182	0.59	0.05	V	1.000*
Itching	12	143	7	178	2.05	0.83	5.07	0.178
Lassitude	17	139	11	173	1.82	0.88	3.77	0.148
Dizziness	11	145	3	181	4.32	1.23	15.23	0.026†
Pins 'n needles	4	152	3	181	1.57	0.36	6.92	0.819
Muscle cramps	7	149	5	179	1.65	0.53	5.10	0.558
Upper respiratory	56	98	50	133	1.33	0.97	1.82	0.096
Lower respiratory	43	114	36	146	1.38	0.94	2.04	0.128
Ear/eye infection	24	133	10	172	2.78	1.37	5.64	0.005†
Gastrointestinal	47	109	38	143	1.44	0.99	2.08	0.072
Skin	16	139	10	175	1.91	0.89	4.09	0.135
Other	23	129	12	168	2.27	1.17	4.41	0.020†
Any symptom	98	57	85	98	1.36	1.12	1.66	0.003†
Any bathing symp	98	58	84	100	1.38	1.13	1.68	0.002†

^{*} Fisher's exact test, 2x1 tailed p t significant at α =0.05

Pre-exposure symptom attack rates (%)

Symptom	Bathers	Non-bathers
Fever	12.195	46.875
Headache	60.976	31.250
Aching limbs	115.854	62.500
Sore throat	103.659	140.625
Chest pains	18.293	15.625
Dry cough	73.620	31.250
Productive cough	42.683	78.125
Breathing diff.	42.683	41.667
Runny nose	158.374	130.208
Ear Infection	36.585	10.471
Eve Infection	18,293	26.042
Blurred vision	24.390	5.208
Appetite loss	30,488	46.875
Indigestion	30.488	15.789
Stomach paln	55.215	26.042
Loose motions	109.756	62.500
Dlamboea	73.620	36.458
Nausea	42.683	41.667
Vomlting	36.585	26.042
SkIn rash	48.780	57. 292
Skin sore	36.585	0.000
Itching	79.268	62.500
Lassitude	48.780	68.063
Dizziness	6.098	41.885
Pins in needles	18.405	
Muscle cramps	18.293	0.000
Upper respiratory	237.805	218.750
Lower respiratory	268.293	203.125
Ear/eye Infection	73.171	36.649
Gastrointestinal	196.319	140.625
Skin	115,854	83.333
Other	56.9 62	99.476
Any symptom	530.864	432.292
Any bathing symp	521.472	401.042

Symptom attack rates on the exposure day (%)

Symptom	Bathers	Non-bathers
Fever	12.195	10.526
Headache	128.834	168.421
Aching limbs	91.463	42.105
Sore throat	67.073	94.737
Chest pains	12.195	10.526
Dry cough	54.878	42.328
Productive cough	30.488	74.074
Breathing diff.	18.405	37.037
Runny nose	104.294	121.053
Ear Infection	42.683	10.526
Eye Infection	18.293	26.316
Blurred vision	12.195	5.263
Appetite loss	24.390	10.526
Indigestion	42.683	37 .037
Stomach pain	42.683	37.037
Loose motions	103.659	36.842
Diamhoea	48.780	5.2 63
Nausea	18.293	31.579
Vomiting	12.195	0.000
Skin rash	30.488	26.316
Skin sore	24.390	0.000
Itching	54.878	26.316
Lassitude	42.683	26.316
Dizziness	18.293	10.526
Pins 'n needles	24.390	10.526
Muscle cramps	36.810	10.526
Upper respiratory	226.994	226.316
Lower respiratory	160.494	195.767
Ear/eye Infection	67.073	36.842
GastroIntestinal	146.341	89.474
Skin	85.366	42.105
Other	57.325	37.433
Any symptom	447.205	414.894
Any bathing symp	438.272	400.000

One week post-exposure symptom attack rates (%)

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Symptom	Bathers	Non-bathers
Fever	36.810	10.417
Headache	153.374	177.083
Aching limbs	49.080	36.458
Sore throat	196.319	135.417
Chest pains	30.675	15.625
Dry cough	61.728	36.649
Productive cough	85.890	41.667
Breathing diff.	12.270	15.707
Runny nose	134.969	109.375
Ear infection	79.755	15.625
Eye Infection	24.540	20.833
Blurred vision	6.135	0.000
Appetite loss	49.080	20.833
Indigestion	24.691	36.458
Stomach paln	85.890	57.895
Loose motions	147.239	83.333
Dlarrhoea	42.945	15.625
Nausea	61.350	52.083
Vomiting	18.405	26.042
Skin rash	49.080	20.833
Skin sore	24.691	10.417
Itching	55.215	26.042
Lassitude	85.890	36.458
Dizziness	42.945	31.250
Pins 'n needles	24.540	20.833
Muscle_cramps	6.135	
Upper respiratory	325.153	281.250
Lower respiratory	246.914	166.667
Ear/eye infection	104.294	36.458
GastroIntestinal	294.479	162.304
Skin	117.284	46.875
Other	107.595	64.171
Any symptom	613.497	432.292
Any bathing symp	595.092	432.292

Three week post-exposure symptom attack rates (‰)

Symptom	Bathers	Non-bathers
Fever	82.803	33.149
Headache	108.974	120.879
Aching limbs	90.909	49.724
Sore throat	283.871	175.824
	20007	., 5,52 ,
Chest pains	25.478	16.484
Dry cough	70.513	54.945
Productive cough	82.803	65.934
Breathing diff.	50.955	27.778
Runny nose	165.605	131.148
Ear Infection	108.280	27.473
Eye Infection	44.586	10.929
Blurred vision	12.739	21.978
Appetite loss	57.692	16.575
Indigestion	31.847	38.251
Stomach pain	70.064	54.945
Loose motions	185.897	119.565
Diarrhoea	77.419	32.432
Nausea	89.744	59.783
Vomiting	45.161	27.027
Skin rash	38.710	37.838
Skin sore	6.410	10.870
ltchin g	77.419	37.838
Lassitude	108.974	59.783
Dizziness	70.513	16.304
Pins 'n n oo dles	25.641	16.304
Muscle cramps	44.872	27.174
Upper respiratory	363.636	273.224
Lower respiratory	273.885	197.802
Ear/eye Infection	152.866	54.945
Gastrointestinal	301.282	209.945
Skin	103.226	54.054
Other	151.316	66.667
Any symptom	632.258	464.481
Any bathing symp	628.205	456.522
-		

C. Water ingestion and GI illness

One week post-exposure - Ingestion vv control

	Bathe wi swallo seaw	ho wed	Ne batt	on ners				
Symptom	m	Well	ŧII	well	RR	95%	CI	P
Appetite loss	7	74	4	188	4.15	1.25	13.78	0.036*†
Indigestion	3	77	7	185	1.03	0.27	3.88	1.000*
Stomach pain	9	72	11	179	1.92	0.83	4.45	0.201
Loose motions	13	68	16	176	1:93	0.97	3.82	0.094
Dlarrhoea	6	75	3	189	4.74	1.22	18.50	0.044*†
Nausea	6	75	10	182	1.42	0.53	3.78	0.652*
Vomiting	2	79	5	187	0.95	0.19	4.79	1.000*
GastroIntestinal	29	52	31	160	2.21	1.43	3.41	0.001†

^{*} Fisher's exact test, 2x1 talled p t significant at α =0.05

Three weeks post-exposure - Ingestion vv control

	Bathe W swalio seaw	no wed	No batt					
\$ymptom	ill	well	111	well	RR	95% (CI	P
Appetite loss	6	71	3	178	4.70	1.21	18.32	0.045*†
Indigestion	_ 2	76	7-	⁻ 176 ⁻	0.67	0.14 -	3.16 -	-0.928°
Stomach paln	7	71	10	172	1.63	0.65	4.13	0.443
Loose motions	15	62	22	162	1.63	0.89	2.97	0.163
Diarrhoea	9	68	6	179	3.60	1.33	9.78	0.022*†
Nausea	8	69	11	173	1.74	0.73	4.15	0.322
Vomiting	6	71	5	180	2.88	0.91	9.17	0.135*
GastroIntestinal	27	50	38	143	1.67	1.10	2.53	0.026†

^{*} Fisher's exact test, 2x1 tailed p

[†] significant at α =0.05

One week post-exposure - no ingestion vv control

Bathers who did not swallow Non bathers seawater **Symptom** well Ш well RR 95% CI p Appetite loss 1 80 4 188 0.59 0.07 5.22 1.000* 80 185 Indigestion 1 7 0.34 0.04 2.71 0.516* 0.38 Stomach paln 5 76 11 179 1.07 2.97 1.000* Loose motions 11 70 16 176 1.63 0.79 3.36 0.269 Diarrhoea 80 189 0.79 0.08 1 3 7.48 1.000" Nausea 4 77 10 182 0.95 0.31 2.94 1.000* Vomiting 80 187 0.06 3.99 1 5 0.47 0.851* Gastrointestinal 19 62 31 160 1.45 0.87 2.40 0.217

Three weeks post-exposure - no ingestion vv control

Bathers who did not swallow Non **bathers** seawater Ħ well 111 well RR 95% CI **Symptom** p 76 178 Appettte loss 3 3 2.29 0.47 11.11 0.522" Indigestion 76 7 176 0.99 0.26 3.74 3 1.000* Stomach pain 75 10 172 0.92 0.30 2.85 1.000* 4 Loose motions 14 65 22 162 1.48 0.80 2.74 0.293 75 179 4.62 1.000* Dlarrhoea 3 6 1.19 0.30 0.49 Nausea 73 11 173 1.27 3.32 0.830 6 77 180 0.06 0.851* Vomiting 5 0.47 3.99 1 Gastrointestinal 20 59 38 143 1.21 0.75 1.93 0.543

^{*} Fisher's exact test, 2x1 tailed p

^{*} Fisher's exact test, 2x1 tailed p

D. Diet and G1 illness

Dietary habits on the exposure day

	Bati	hers	Non B	athers	
Food type	yes	no	yes	no	P
Ice cream	66	98	62	126	0.193
Sandwiches	40	123	39	151	0.439
Chicken	65	98	71	119	0.709
Eggs	92	71	104	86	0.831
Mayonnaise	30	134	14	175	0.003†
Hot dogs	9	154	8	182	0.746
Hamburgers	24	140	28	162	0.902
Salad	120	44	130	60	0.389
Raw milk	4	160	3	185	0.850*
Cold meat / pâté	78	86	78	112	0.313
Meat pies	35	129	37	152	0.781
Take away food	46	118	52	137	0.994
Seafood	24	139	11	178	0.009†

 $^{^{\}circ}$ Fisher's exact test, 2x1tailed p t significant at $\alpha{=}0.05$

Dietary habits 1 week post exposure

	Ва	thers	Non I	Bathers	
Food type	yes	no	yes	no	p
Ice cream	98	65	101	90	0.207
Sandwiches	71	92	63	129	0.049†
Chicken	90	73	111	80	0.659
Eggs	108	53	131	59	0.796
Mayonnaise	17	146	12	180	0.216
Hot dogs	10	152	11	181	0.960
Hamburgers	31	131	38	154	0.984
Salad	130	33	151	41	0.900
Raw milk	6	157	4	188	0.557*
Cold meat / pâté	101	62	99	93	0.063
Meat pies	55	107	47	145	0.065
Take away food	60	103	65	127	0.639
Seafood	40	123	2 5	167	0.008†

^{*} Fisher's exact test, 2x1tailed p t significant at α =0.05

Stratified analyses - food consumption and gastrointestinal symptoms on the exposure day

		Ate n	navonnal	S O	Dic	L not ec	t mayoni	naise				
	Bat	hers	Non B	athers	Bati	hers	Non Bo	athers				
Symptom	131	well	FII	Well		well	M	well	RR*	95%	CI	p
Appetite loss	3	27	0	14	1	133	2	173	1.75	0.24	13.03	0.895
indigestion	3	27	1	13	4	140	6	168	0.92	0.32	2.70	0.892
Stomach pain	1	29	1	13	6	128	7	168	1.00	0.37	2.67	0.790
Loose motions	4	26	2	12	13	121	5	170	2.44	1.08	5.55	0.046†
Diarrhoea	2	28	0	14	6	128	1	174	9.30	1.07	81.03	0.033†
Nausea	1	29	0	14	2	132	6	169	0.56	0.13	2.44	0.656
Vomiting	1	29	0	14	0	134	0	175	•••	-,-	-,-	0.6 9 6
Gastrointestinal	5	25	2	. 12	18	116	15	160	1.50	0.83	2.71	0.243

^{*} Mantel-Haenszet welghted RR of disease given exposure to sea bathing

		Ate se	a food		Dic	not ec	t sea foc	<u>id</u>				
	Bat	hers		athers	Bat	hers	Non B	athers				
Symptom .	III	well	in .	Well	11)	well	ill	well	RR*	95%	CI	p
Appetite loss	3	21	0	11	1	138	2	176	1.72	0.23	12.72	0.912
Indigestion	3	21	1	10	4	135	6	171	0.96	0.33	2.80	0.841
Stomach pain	1	23	1	9	6	133	6	172	1.10	0.40	3.01	0.923
Loose motions	6	18	1	10	11	128	6	172	2.43	1.01	5.82	0.063
Dlarrhoea	4	20	0	11	4	135	1	177	7.99	0.82	78.19	0.070
Nausea	2	22	0	11	1	138	6	172	0.45	0.09	2.28	0.517
Vomiting	ī	23	Ō	11	0	139	0	178			•,•	0.689
Gastrointestinal	9	15	2	9	14	125	15	163	1.34	0.73	2.48	0.433

^{*} Mantel-Haenszel weighted RR of disease given exposure to sea bathing

Stratified analyses - food consumption and gastrointestinal symptoms one week post-exposure

		Ate_s	andwiche	<u>s</u>	Did	not ea	t sandwi	ches				
	Bat	hers	Non B		Bath	ners	Non B	athers				
Symptom	101	well	[[]	well	III e	llew	ln	well	RR*	95%	CI	P
Appetite loss	5	66	0	63	3	89	4	125	2.46	0.69	8.82	0.252
Indigestion	1	70	1	62	3	88	6	123	0.74	0.22	2.50	0.861
Stomach pain	7	64	4	59	7	85	7	120	1.45	0.67	3.13	0.455
Loose motions	11	60	4	59	13	79	12	117	1.79	0.98	3.30	0.082
Diarrhoea	3	68	0	63	4	88	3	126	3.00	0.74	12.19	0.197
Nausea	4	67	5	58	6	86	5	124	1.14	0.49	2.62	0.944
Vomiting	1	70	3	60	2	90	2	127	0.68	0.17	2.65	0.838
Gastrointestinai	23	48	9	54	25	67	22	106	1.82	1.21	2.73	0.005†

^{*} Mantel-Haenszel weighted RR of disease given exposure to sea bathing

		Ate_seatood			Did	l not ec	nt seatoo	d				
	Bati	hers		athers	Batt	hers	Non B	athers				
Symptom	m	well	Ħ	Well	iil	well	ÐĮ	well	RR*	95%	CI	P
Appetite loss	1	39	2	23	7:	116	2	165	2.12	0.72	6.30	0.275
Indigestion	0	40	1	24	4	118	6	161	0.73	0.23	2.37	0.835
Stomach pain	5	35	3	21	91	114	8	158	1.33	0.63	2.84	0.593
Loose motions	7	33	3	22	17	106	13	154	1.70	0.93	3.09	0.116
Diarrhoea	1	39	0	25	6	117	3	164	3.02	0.77	11.83	0.180
Nausea	1	39	4	21	9	114	6	161	1.11	0.50	2.47	0.977
Vomiting	1	39	3	22	2'	121	2	165	0.57	0.15	2.12	0.630
Gastrointestinal	11	29	7	17	37	86	24	143	1.75	1.18	2.59	0.007†

^{*} Mantel-Haenszel weighted RR of disease given exposure to sea bathing

E. Serious iliness

GP visits, one week post-exposure

Status	Bathers	Non Bathers	
No	154	184	•
Yes	8	7	
			p=0.744

Days of normal activity lost, one week post-exposure

Status	Bathers	Non Bathers	
None	158	188	
1-4	3	4	
5+	1	0	
			p=0.546

Hospital admission, one week post-exposure

Status	Bathers	Non Bathers
No	161	192
Yes	1	0
		p=0.915*

^{*} Fisher's exact test, 2x1 tailed p

GP visits, three weeks post-exposure

Status	Bathers	Non Bathers	
No	142	172	
Yes	12	10	
			p=0

0.510

Days of normal activity lost, three weeks post-exposure

Status	Bathers	Non Bathers	
None	145	173	
1-4	12	9	
			p=0.423

Hospital admission, three weeks post-exposure

Status	Bathers	Non Bathers	
No	155	182	
Yes	1	1	ž.
	ant test. Oul tailer		p=1.000*

^{*} Fisher's exact test, 2x1 tailed p

F. Medical diagnoses

Appearance of throat

Category	Bathers	Non-bathers
Normal	121	145
Red	30	27

RR=0.79 (0.49-1.28) p=0.414

Appearance of ear

Category	Bathers	Non-bathers	
Normal	145	164	
Infected	2	2	

RR=1.13 (0.16-7.92) p=1.000*
* Fisher's exact test, 2x1 talled p

Appendix V



Appendix V Social, demographic, health, chronic illness, alcohol, smoking, and recreational water use

A. Social and Demographic

Gender

Group	Male	Female
Bathers	87	77
Non Bathers	92	100

p=0.390

Age distributions

Group	18-24	25-34	35-44	45-54	55-64	65+	Unknown
Bathers	36	47	38	19	11	13	0
Non Bathers	30	60	50	20	20	11	

p=0.475

Employment

Status	Bathers	Non Bathers
Student	16	10
Housewife	24	33
Employed	61	69
Part Time	15	26
Self Employed	15	17
Unemployed	19	19
Retired -	13	15
Other	1	3

p=0.618

Total Number in Household

Status	Bathers	Non Bathers
<2	59	63
3-4	81	100
5-8	24	29

p = 0.819

Children under 5 years

Status	Bathers	Non Bathers
None	118	140
1	25	31
2	16	17
≥3	5	4

p=0.927

Household Illness

Status	Bathers	Non Bathers
No	132	159
Yes	30	32
Unknown	2	1

p=0.769

Type of Houshold Illness

Status	Bathers	Non Bathers
Gastrointestinal Upper Respirator Non-specific Vira		5 21 1
Other	7	5

B. Health

Chronic Illness.

	Bathe	rs	Non B	athers	
Illness	yes	no	yes	no	p
Chronic Illness	121	43	131	59	0.377
Arthritis	11	153	14	178	0.994
Back Pain	24	140	29	163	0.980
Blood Pressure	8	156	9	183	0.869
Chest	9	155	12	180	0.937
Diabetes	1	163	1	191	1.000"
Digestive	4	160	6	186	0.925*
Bowel	5	159	9	183	0.603
Ear	10	154	12	180	0.872
Hepatitis	1	163	3	189	0.746"
Infection	7	157	8	184	0.828
Accident	14	150	10	182	0.300
Kidney	5	159	3	189	0.558*
Neurological	8	156	9	183	0.869
Hayfever	34	129	42	150	0.918
Skin	26	138	24	168	0.450
Stress	10	154	10	182	0.895
Eye	65	99	64	128	0.262
Other	11	153	15	177	0.845

^{*} Fisher's exact test, 2x1 talled p

Chronic medical treatment

Status	Bathers	Non Bathers	
No	137	161	_
Doctor Seen	25	31	
Unknown	2	0	p=0.970
G.P.	14	18	
Hosp. Spclst	3	8	
Both	4	2	
Other	3	1	
Unknown	3	2	
Admitted to Hos	pital 6	7	

Illness in Last 6 Months

Status	Bathers	Non Bathers	
No	129	154	_
Yes	35	38	p=0.819

Time Off in Last 6 Months

Status	Bathers	Non Bathers		
None	131	157		
1-9 days	22	28		
≥ 10 days	11	7	p=0.414	

Frequency of Diarrhoea

Status	Bathers	Non Bathers	
Never	25	35	_
<1 yr	63	63	
<2 yr	44	64	
3 -11 yr	24	25	
1-2 mnth	7	4	
Unknown	1	1	p=0.403

Prescription drug use

	Bathers		Non Bathers		
Туре	yes	no	yes	no	p
Prescribed Drugs	84	80	101	90	0.837
Antiblotics	9	155	5	187	0.261
Steroids	7	157	8	184	0.828
Laxatives	1	163	4	188	0.478*
Stomach Remedies	4	160	5	187	1.000*

^{*} Fisher's exact test, 2x1 talled p

C. Alcohol and Smoking

Alcohol Consumption (number of times per week).

Status	Bathers	Non Bathers	
Never	14	13	
<1	59	76	
>1	91	103	

p=0.206

Alcohol consumption distributions

Group	0-2	2-4	4-8	8+
Bathers	130	21	12	$\overline{}$
Non Bathers	162*	23	6	0

p=0.617

Smoking

Group	\$moker	Non Smoker
Bathers	46	118
Non Bathers	48	143

Amount of cigarettes smoked per day

Status	Bathers	Non Bathers
None	118	146
<20	40	37
<20 20-40	4	8
40-60	1	0
40-60 >60	1	1
Other	0	1
Unknown	0	1

p=0.551

^{• 1} Unknown

D. Leisure Activities

General Leisure

Iliness	Bather	Non Bather	
Pub			
Never	47	59	
Occasional	50	68	
Frequent	66	65	
Unknown	1	0	p=0.416
Party			
Never	79	99	
Occasional	77	81	
Frequent	8	11	
Unknown	0	1	<i>p</i> =0.679
Leisure Centre			
Never	89	103	
Occasional	31	27	
Frequent	44	62	
Unknown	0	0	p=0.339
Church			
Never	116	152	
Occasional	25	16	
Frequent	23	23	
Unknown	0	1	p=0.091

Water Sports

\$tatus	Bathers	Non Bathers	
Dinghy			
Never	137	177	
Occasional	16	4	
Frequent	10	11	
Unknown	1	0	<i>p</i> =0.007†
Boating			
Never	145	17 1	
Occasional	13	7	
Frequent	6	14	
Unknown	0	0	p=0.083
Sub-Aqua			
Never	152	189	
Occasional	10	3	
Frequent	2	0	
Unknown	0	0	p=0.022†
Surfing			
Never	141	161	
Occasional	11	11	
Frequent	11	20	
Unknown	1	0	p=0.454
Fishing			
Never	153	178	
Occasional	8	10	
Frequent	3	3	
Unknown	0	1	p=0.972
Paddling			
Never	87	93	
Occasional	44	60	
Frequent	33	38	
Unknown	0	1	p=0.617
Other			
Never	153	174	
Occasional	2	5	
Frequent	3	2	
Unknown	6	11	p=0.527

Frequency of Sea or Freshwater Bathing

Status	Bathers	Non Bathers	
Never	67	81	_
Occasional	51	52	
Frequent	46	58	
Unknown	0	1	p=0.716

Use of Swimming Pools

Status	Bathers	Non Bathers	
Public Pool			
Never	59	69	
Occasional	61	77	
Frequent	41	45	
Unknown	3	1	p=0.653
Other Pool			
Never	139	163	
Occasional	16	19	
Frequent	7	6	
Unknown	2	4	p=0.871

Visits to Beach without Water Contact

Status	Bathers	Non Bathers	
Never	43	35	_
Occasional	56	66	
Frequent	64	91	
Unknown	1	0	p=0.135

Water Rides

Status	Bathers	Non Bathers	
No	151	179	
Water Rides	13	13	p=0.831

Swimming ability (2 lengths of a swimming pool)

Status	Bathers	Non Bathers	
No Swim Competer	14 offy 147	21 168	_
Unknown	3	3	p=0.567

E. Miscellaneaous

Time Overseas.

Bathers	Non Bathers	
92	118	-
72	74	
1	0	p=0.359
21	22	
24	18	
11	12	
9	12	
6	10	
	92 72 1 21 24 11	92 118 72 74 1 0 21 22 24 18 11 12 9 12

Visits Away from Home

Status	Bathers	Non Bathers	
None	91	117	
Away	73	75	p=0.551
U.K.	61	58	
Abroad	8	13	
Both	4	4	