NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT
BILTHOVEN, THE NETHERLANDS

Report no. 289202017

Health effects of freshwater bathing among primary school children

Design for a randomised exposure study

I.A. van Asperen, G.J. Medema, A.H. Havelaar,
M.W. Borgdorff

January 1997

This investigation has been performed by order and for the account of the Ministry of Housing, Spatial Planning and the Environment, Directorate-General for Environmental Protection, within the framework of project 289202 "watermicrobiology".

National Institute of Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands, Telephone: +31 - 30 - 274 91 11, telefax: +31 - 30 - 274 29 71

MAILING LIST

1	Directie Drinkwater, Water en Landbouw van DGM
2	plv. Directeur-Generaal Milieubeheer, dr. ir. B.C.J. Zoeteman
3	Hoofdinspecteur Milieuhygiëne
4	ir. W. Cramer DGM/DWL
5	dr. B.J.A.M. Haring, DWL
6	dr. C.J.M. van den Bogaard, HIMH
7	drs. J.K. van Wijngaarden, IGZ
8-29	P. Bokelaar, secretaris Technische Beleidsgroep Zwemwater
30-41	Provincies, Diensten Milieu en Water
42-51	Regionale Inspecties van de Volksgezondheid voor de Hygiëne van het Milieu
52	Landelijke Vereniging voor GGD'en
53-112	Artsen Infectieziektenbestrijding GGD'en
113	Nederlandse Vereniging voor Infectieziekten
114	Nederlandse Vereniging voor Kindergeneeskunde
115	Nederlandse Vereniging voor Medische Microbiologie
116	Gezondheidsraad
117	Voorzitter Gezondheidsraad
118-134	Streeklaboratoria voor de Volksgezondheid
135	dr. F. van Loock, Instituut voor Hygiëne en Epidemiologie, Brussel
136	S. Engelsman, ANWB
137	D. Kay, CREH, UK
138	J. Fleisher, State University New York, USA
139	B. van Maele, Europese Commissie, DG XI
140	D. Eisma, Europees Parlement
141	N. Lightfoot, PHLS Newcastle, UK
142	A. Godfree, North West Water, UK
143	D. Ramaekers, TECHNA Consult, België
144	W. Stoop, GGD West-Veluwe/Vallei
145	G. Natrop, GGD West-Veluwe/Vallei
146	J. Cappon, RIZA
147	H. v.d. Mark, RIZA
148	Depot Nederlandse Publicaties en Nederlandse Bibliografie
149	Directie RIVM
150	Prof. dr. ir. D. Kromhout
151	dr. ir. A.M. Henken
152	ir. A.H.M. Bresser
153-154	Medewerkers MGB
155-185	Medewerkers CIE
186-191	Leden werkgroep gastro-enteritis
192-195	Auteurs
196	SBD/Voorlichting en Public Relations

197	Bureau Rapportenregistratie
198	Bibliotheek RIVM
199-220	Bureau Rapportenbeheer

TABLE OF CONTENTS

SAMENVATTING

Doel-Het testen van de haalbaarheid en onderzoeksmethoden van een studie bij kinderen die als doel heeft eventuele gezondheidseffecten vast te stellen van recreatie in zoetwaterplassen die voldoen aan de huidige zwemwaternormen.
Onderzoeksopzet-Pilot fase van een gecontroleerde en gerandomiseerde expositiestudie.
Setting-Twee basisscholen in Ede en een zoetwaterplas in Nederland.
Populatie-69 Zwemmers en 74 niet-zwemmers in de leeftijd van 9 tot 14 jaar (groep 6, 7 en 8).
Methoden-In de zomer van 1996, gedurende twee onderzoeksdagen, werden kinderen at random toegewezen aan een groep die deelnam aan een estafette race of trefbal in het water, of aan een groep die deelnam aan sport- en spelactiviteiten op de speelweide. Het zwemwater, dat goedgekeurd was door de provincie, werd op de onderzoeksdag uitvoerig bemonsterd om blootstelling van de zwemmers aan microbiologische waterkwaliteitsparameters vast te stellen. Tevens werd met behulp van vragenlijsten het optreden van gezondheidsklachten na één en drie weken nagegaan en werd informatie verzameld over andere factoren die tot gezondheidsklachten kunnen leiden.
Resultaten-Deel 1 (onderzoeksresultaten): van de elf uitgenodigde scholen namen er twee deel aan het onderzoek (18%). Het percentage kinderen per school dat deelnam aan het onderzoek bedroeg 96% en 66%, respectievelijk voor school A en school B. Ethische bezwaren waren de voornaamste redenen niet mee te doen aan het onderzoek. Na randomisatie werd van 68% van de kinderen bruikbare informatie verzameld over gezondheidsklachten in de week na de onderzoeksdag; onvolledige informatie-verzameling werd voornamelijk veroorzaakt door het uitstellen van de tweede onderzoeksdag in verband met slecht weer en het aanvangen van de zomervakantie. In totaal nam 50% (107/216) van de kinderen in de klas deel aan het onderzoek en vulde in de week na de onderzoeksdag een vragenlijst in. Na drie weken follow-up bedroeg dit ongeveer 30%. Dit percentage kan maximaal verhoogd worden tot 80% als de respons in de verschillende fasen van het onderzoek maximaal is. Blootstelling aan het water was intensiever tijdens de estafette race dan tijdens trefbal, omdat kinderen vaker kopje onder gingen en vaker water binnen kregen. Concentraties van thermotolerante bacteriën van de coligroep en faecale streptokokken voldeden op beide onderzoeksdagen aan de Richtwaarden uit de Europese zwemwaternormstelling en variatie werd voornamelijk waargenomen tussen de onderzoeksdagen en niet gedurende een onderzoeksdag. In de week na de onderzoeksdag had 4,7% van alle kinderen (zwemmers en niet-zwemmers) gastroenteritis, 0,9% luchtwegklachten en 1,4% huidklachten. *Deel II (evaluatie van de onderzoeksopzet):* het onderzoek is tijdrovend en complex en sterk afhankelijk van de weersomstandigheden. Voor toekomstig onderzoek moet daarmee rekening worden gehouden. Ethische bezwaren kunnen substantieel zijn en een meer persoonlijke benadering van scholen en ouders is aangewezen. Overwogen zou moeten worden de onderzoeksgroep te wijzigen door kinderen uit groep 5 in plaats van groep 8 te bestuderen, daar zij waarschijnlijk meer gemotiveerd zijn vragenlijsten in te vullen. Er moet naar worden gestreefd blootstelling van de zwemmers aan het water te lokaliseren en het meten van de blootstelling per kind te intensiveren om zo een betere schatting te krijgen van blootstelling van elk kind aan indicator organismen. *Deel III (steekproef omvang):* op basis van volksgezondheids, statistische en logistieke overwegingen zou toekomstig onderzoek ongeveer 2300 kinderen moeten bestuderen. Daarmee kan een statistisch significant effect van zwemmen worden aangetoond als de basis incidentie ten minste 3,5% en het relatief risico ten minste twee is.

Conclusie-Gezondheidseffecten van recreatie in zoet water kunnen worden bestudeerd bij leerlingen op een basisschool met behulp van een gewijzigde opzet van de gerandomiseerde expositie studie die is uitgevoerd in de zomer van 1996. Het onderzoek is echter tijdrovend en complex en sterk afhankelijk van de bereidheid van scholen en ouders deel te nemen aan het onderzoek, als mede van de weersomstandigheden.

SUMMARY

Objective-To investigate the feasibility and methods of a study among children to determine health effects of bathing in fresh waters that meet current water quality standards.
Design-Pilot phase of a controlled randomised exposure study.
Setting-Two primary schools in Ede and one freshwater lake in the Netherlands.
Subjects-69 bathers and 74 non-bathers aged 9 to 14 years (grade six, seven and eight).
Methods-In the summer of 1996, on two trial days, children were randomly allocated to groups participating in closely supervised water activities (relay race or hit ball) or in non-water related games. Water quality at the beach met European water quality standards. Intensive water quality monitoring was used to estimate exposure to microbiological indicator organisms and pathogens. Detailed questionnaires at one and three weeks post exposure were used to measure health effects and potential confounders.
Results-Part I (research results): of eleven schools invited, two participated (18%). Consent rates at both schools were 96% and 66%, for school A and school B, respectively. Refusal to participate in the study was mainly due to ethical objections. After randomisation, follow-up was only 68% and this was mainly due to delay of the second trial day because of bad weather and the start of the summer holidays. In total, 50% (107/216) of all children in the classrooms participated on the trial day and completed all questionnaires up to one week post exposure. This fell to 30% after three weeks of follow-up. This rate may be increased to 80% if all conditions are favourable. Exposure to the water was more intensive for those who participated in the relay race, as heads were immersed more frequently and more children swallowed water than in the hit ball game. Concentrations of thermotolerant coliforms and faecal streptococci met the European Guide levels on both trial days and they varied between but not within trial days. Of all children (bathers and non-bathers) 4.7% developed gastroenteritis, 0.9% respiratory illness and 1.4% skin complaints within one week of follow-up. *Part II (evaluation of study design)*: the study design is time consuming and complex and strongly dependent on weather conditions. Future studies should be planned with this in mind. Ethical objections may be substantial and more oral communication with schools and parents should be planned. Shifting the study population from grade 6 to 8 to grade 5 to 7 should be considered to enrol children that are more motivated to complete questionnaires in the week after the trial day. Exposure of bathers to the water should be more localised and water sampling should be made more intensive to improve the assessment of exposure to indicator organisms for each individual bather. *Part III (sample size):* based on public health-, statistical- and logistical considerations, a future trial should include about 2300 children (ratio of bathers to non-bathers 2:1). This allows for detecting a significant health effect of bathing if the attack rate in the non-bathing group is at least 3.5% and the relative risk is at least two.
Conclusions-The health effects of freshwater bathing in a recreative population can be studied among primary school children using a modification of the controlled randomised exposure study that was carried out in the summer of 1996. The study, however, will be time consuming and complex and strongly dependent on the ability to find volunteers to participate in the study and on weather conditions.

1. INTRODUCTION

1.1 Background

Both in The Netherlands and in Europe concern for the quality of water used for recreational purposes is growing. Surface waters are used extensively by both resident and tourist populations and good quality bathing waters are important for tourism. With the increased water use, the health effects of bathing are also recognised. In The Netherlands, every summer health complaints occur after bathing in waters that meet current water quality standards. These complaints are reported to Provincial authorities and Regional Health authorities (1, 2, 3). Epidemiological studies in The Netherlands and abroad have shown that bathing in waters that meet current standards is associated with an increased risk of health complaints (4-18)

The Dutch government has set out the policy that, by the year 2010, all national surface waters should be of such a quality that bathing is possible without becoming ill (19). Similar aims have been defined by the European Union in the 5th Environmental Action Programme (20). To evaluate the quality of bathing waters with reference to its impact on public health, information is required about the health risks of bathing in waters that meet current water quality standards. This information can be used to evaluate current standards (both at the Dutch and the European level) and to prepare a cost-benefit analysis of measures to control sewage and non-point source pollutions.

To date, bathing in Dutch fresh waters that meet current water quality standards has been associated with an increased risk of otitis externa among bathers in lakes that contained *Pseudomonas aeruginosa* (17) and with an increased risk of gastrointestinal, respiratory and skin/mucosal complaints among endurance athletes in faecally polluted waters (21) compared to non-bathers. In the latter study it was found that risk of gastroenteritis increased three to five fold after exposure to concentrations of thermotolerant coliforms above the European Guide level (100 cfu per 100 ml) as compared with exposure to lower levels. Comparing the mean thermotolerant coliform concentrations in Dutch fresh waters with the threshold level observed in the athletes study, similar health effects may be expected for about thirty per cent of all Dutch fresh waters (22). This assumes, however, that athletes are comparable with the Dutch recreative population in terms of susceptibility to waterborne health risks. As this may not be true, additional health risk data from a recreative population exposed to faecally polluted fresh waters are needed.

1.2 Objectives

We carried out a pilot study to investigate if a controlled randomised exposure design among primary school children can be used to study, in a recreative population, the health effects of bathing in faecally polluted fresh waters that meet current water quality standards. This report presents the study design and the results of this pilot study. The feasibility of the study was examined by trying out the study design and study methods and investigating which methods need revision. The study was further carried out to obtain information on the proportion of children that develop health complaints within a week after the exposure.

Based on the results of the pilot study it will be decided if future studies will be carried out and how many subjects are needed. The pilot study will not determine significant differences in attack rates between bathers and non-bathers, nor identify indicators of faecal pollution showing a relation with health complaints.

2. METHODS

2.1 Study design

The study is designed as a randomised controlled exposure survey. Volunteers are randomised to a bathers and a non-bathers group. Bathers are exposed on a trial day to fresh water at a beach that meets current Dutch and European water quality standards. Non-bathers participate in games at the beach on the same trial day. Intensive water quality monitoring is carried out at the moment of bathing to estimate exposure to faecal indicator organisms and faecal pathogens. Detailed questionnaires are used to collect data on the occurrence of health complaints and potential confounding factors before and after the trial day.

The study design is based on recommendations by the World Health Organisation in 1972 (23) and was adopted during a four-year study (1989-1992) in sea water in the UK (16). Although a randomised design is more complex than its counterpart, the so-called beach survey in which bathers and non-bathers are approached at the beach of their choice, it gives more valid results. Beach surveys have been carried out in several countries in the 70's and 80's (5, 6, 8, 9, 10, 11, 14, 15) but they have some major drawbacks. Self selection of bathers and non-bathers, misclassification of exposure data due to crude assessment of concentrations of indicator organisms, biased risk estimates due to inadequate control for other potential risk factors and proxy interviews may have influenced the study outcomes.

In a randomised controlled design these drawbacks can be overcome. Randomisation, or random assignment of subjects to exposure categories, prevents self-selection bias. In a controlled design, bathers can be asked to bathe in a pre-defined area. Concomitant sampling of the bathing area controls the effects of temporal and spatial variation of concentrations of indicator organisms and prevents misclassification of exposure assessment. Information on potential confounding risk factors can be obtained in a separate interview before randomisation (and is therefore not influenced by exposure status) and can be subsequently controlled for in the analysis.

Although volunteers are asked to participate in an activity that has potential adverse health effects (i.e. occurrence of minor health complaints), the study exposure is equivalent to recreational bathing as performed by millions of people every summer. Only in the framework of an experiment, in which this activity is closely supervised, water quality is extensively monitored and health complaints, if any, are systematically registered, it is possible to measure its potential health effects and the relation with water quality. Ethical clearance for the study was granted by the external Ethics Committee of the RIVM.

2.2 Study population

Primary school children in grade 6, 7 and 8, aged 9 to 14 years (mean 11 years), were studied. We chose to study children in stead of adults as children are more frequent swimmers, they are more intensively exposed to water than adults and they may be more susceptible to infections. Young age (up to 20 years) has been associated with an increased risk of waterborne infections (10, 11, 13, 24) and if children are the group at highest risk, health based standards derived from studies among children provide a safe margin if applied to adults.

The study population was further restricted to children that are supposed to be able to participate in trial day activities and to give valid answers in the self-administered questionnaires. We therefore chose children in grade 6, 7, and 8. After grade 8 children leave primary school and go to secondary school.

All children went to primary school in Ede, a town in the middle eastern part of the country. Ede was chosen because it is located in an area with few fresh and sea waters, thus minimising opportunities for exposure to surface waters in the days before and after the study exposure. We have considered to include children in a city in the neighbourhood of freshwater lakes and to request them not to bathe during the study period. This option was rejected as it may result in bathing episodes that are not mentioned to the investigators. Ede was also chosen because it was located within one hour driving from the freshwater study site.

2.3 Study size of the pilot investigation

As the objectives of the pilot investigation were to test the study design and study methods, and not to determine whether bathers developed significantly more or less health complaints than non-bathers, the study size should be adequate to test logistics; we therefore aimed to study 100 bathers and 100 non-bathers on one trial day. Dependent on the number of children in each school this implied that two or three primary schools should participate in the pilot study.

2.4 Fresh water study site

The fresh water study site was recreation area 'de Byland' in Herwen, near Lobith (Figure 1). The site was located in the eastern part of the country, about forty kilometres to the south east of Ede. The fresh water was a 202 m^3 lake connected to the river Rhine. The river Rhine enters The Netherlands in Lobith. The major source of pollution in the river Rhine are effluents of treated sewage. The study site was selected according to the following criteria:

1. Location in fresh water that had met the Dutch and EU Imperative level for thermotolerant coliforms (≤ 2000/100 ml) in 1993, 1994 and 1995. Data were provided by the Province of Gelderland (C. Collé).
2. Sewage effluents (not disinfected) as major source of contamination. Minor influence of other sources (birds or ducks, other bathers, run-off of agricultural waste).
3. Safe bathing area with minimal size of 100 metres and maximum depth of 1m20 at 50 metres off shore. Safe non-bathing area, sewered toilets, no signs of the presence of rats.

Selection criteria were discussed with staff members of the Provinces of Gelderland, Flevoland and Utrecht. These provinces were selected because of their geographical situation (near Bilthoven). They were asked to report all eligible lakes to the RIVM. This resulted in three potential study sites. From these the Byland was chosen because it met all criteria and there were no dead corners or small beaches, in contrast to the other two sites. Additional information provided by the Province showed that no particular problems concerning the faecal pollution of the water had been reported in the past years.

2.5 Approaching the schools

Schools were primarily selected according to number of pupils in grade 6, 7, and 8 (high numbers were preferred). Schools were selected and approached in collaboration with the Local Health Authority in Ede.

One long letter, one short letter and a leaflet about the study were developed to approach schools. The long letter included information about the study, the time schedule and the procedure of getting informed consent. Informed consent should be given by the school director, teachers of grade 6, 7 and 8, a representative advisory body or parents' committee and the parents of all individual children. The short letter included only information about the time schedule of the study. The leaflet was designed especially for the parents and contained all relevant information about the study, such as

aims of the study, potential health risks of participation, practical information about the trial day and questionnaires, maintaining confidentiality, reporting of the study and a telephone number where further information could be requested (at the RIVM and the Local Health Authority).

Twelve schools were contacted to participate in the study. Three schools were contacted using the long letter and the leaflet. One of them participated (further referred to as school A). Three schools were contacted by telephone. One of them requested further information (long letter and leaflet), but none of the schools participated. The short letter and the leaflet were sent to six schools of which one participated (further referred to as school B). Potential participation of one school was cancelled by the research team as the required number of children would probably be reached in the two participating schools. This meant that the overall participation rate was 18% (2/11). At both participating schools informed consent was given by the school director, teachers of grades 6 , 7 and 8, and a representative advisory body (including parents and teachers) during one or two meetings at school. At both schools approval of the representative advisory body was coupled with some ethical objections, but these were not a reason for refusal. It took two months to enrol schools. Both schools informed the Inspectorate of Education about participation in the study.

2.6 Approaching the parents

Information about the study was given to the parents of children in grades 6, 7 and 8. This included a study leaflet, a baseline questionnaire, an informed consent form and two letters. One letter was signed by the school director or a teacher and the other by a medical doctor of the RIVM research team and a staff member of the Local Health Authority. The latter turned out to be the father of a child in school B, but this was not known by the RIVM team in advance. Information was handed out in classrooms four weeks before the trial day. At school B this happened to occur just before a school break of one week.

Written informed consent (and a completed baseline questionnaire) or refusal was obtained three weeks before the trial day. Twelve parents requested further information. They were invited by letter to attend a specially arranged meeting at school two weeks before the trial day; only two of them came eventually.

2.7 Questionnaires

Five different questionnaires were developed for the study: a baseline questionnaire, daily health form, disability questionnaire, follow-up questionnaire and non-response questionnaire[1]. Figure 4 presents the time schedule of the questionnaires.

The baseline questionnaire had to be completed by the parents of participating children three weeks before the trial day. It obtained information on age, sex, general health, swimming certificates and country of birth of participating children as well as on the country of birth and the educational level of the parents. The latter questions were included as they are indicators of social status and are known to be related to general health.

The daily health forms had to be completed by the participating children in the classroom in the morning at seven occasions: on the day before the trial day, on the trial day itself, and during the next five days including the Saturday in the week after the trial day. They provided information on the presence of health complaints, exposure to swimming pools, sea- and freshwaters and consumption of foods in the two days before the trial day and on the six subsequent days. Each questionnaires consisted of a double sided form with questions about health complaints on one side and questions about

[1] All questionnaires are available from the first author

swimming behaviour and food consumption on the other side. All questions referred to the day before, so the children had to remember only complaints, swimming and foods of the day before. The following types of complaints were registered: gastrointestinal complaints (nausea, vomiting, stomach ache, diarrhoea (and number of loose stools), and fever), respiratory complaints (coughing, sneezing, runny nose, sore throat), skin complaints (itching, rash), ear complaints (ear ache, itching, discharge) and eye complaints (ache, itching, inflammation). All children were asked whether complaints were present during morning, noon, evening and/or night. On the day before the trial day children were taught intensively how to complete the questionnaire. This was done by a person with pedagogical and research expertise. All forms were completed in the presence of this person; this took about ten to fifteen minutes every day. Children who were absent from school were interviewed at home. As the summer holiday started one day after the trial day at school A, health forms at this school were given to the pupils to complete at home.

Parents of children who reported incident health complaints in the week after the trial day were sent a disability questionnaire after the first week. Copies of the daily health forms completed by their children were included to validate reported health complaints. Disability was estimated by asking whether the subjects remained in bed, stayed away from school, sought medical advise or used any drug.

A follow-up questionnaire was designed to collect information on the occurrence and severity of health complaints between one and three weeks after the trial day, visits abroad, swimming habits and illness in the household or among friends. It had to be completed by the parents of the children. At school B the questionnaire was handed out in the classroom 2½ week after the trial day; this was one day before the start of the summer holiday. At school A it was sent to the parents 2½ week after the trial day; this was in the summer holiday.

A non-response questionnaire was taken by telephone three weeks after the trial day from parents who had not given consent for the study. This questionnaire was taken mainly to provide information on reasons for refusal that can be reconsidered in future studies. Besides, information was obtained on age, sex, general health and swimming habits of the child.

All questionnaires were developed using experience from earlier national and international bathing water surveys. The daily health forms, however, were designed to be understood by children (i.e. questions were sometimes accompanied by written explanations). They were pre-tested among 55 primary school children and needed minor revision. All other questionnaires were seen by colleagues (epidemiologists) and have been pre-tested among ten parents.

2.8 Randomisation procedures

To be eligible for the study children had to meet the following criteria: at least one swimming certificate and absence of chronic ear complaints or sinusitis. Children who did not meet these criteria and those who were not allowed by their parents to bathe were assigned to the non-bathers group and were excluded from further analysis. All others were equally divided into a bathers or a non-bathers group using an alphabetically class name list and systematic random sampling. The first child on the list was at random assigned to the bather or non-bather group, the second child was then assigned to the other group etc. Randomisation was carried out one week before the trial day and parents were informed about the randomisation status in order to arrange proper clothing for their children.

2.9 Trial days

Two trial days were organised, on 25 June 1996 for school B and on 16 July 1996 for school A. They were organised on a Tuesday to have the possibility to collect pre-and post-exposure information at school and to measure absenteeism. Absenteeism can not be measured on the third and fourth day after the trial if trial days are organised on Thursday or Friday. If trial days are organised on Monday it is less easy to inform parents and children in time if a trial day has to be postponed at the last moment. Trial days could not be organised on a Wednesday as primary school children do not go to school on Wednesday afternoons. Trial days had to be organised in June or the beginning of July because of the summer holiday (usually in July and August). September would have been another possibility although weather conditions may be less favourable and growth of *Pseudomonas aeruginosa* or cyanobacteria in the lakes may be more widespread giving rise to additional health risks.

On the first trial day weather conditions were bad: air temperature 16 °C, overcast, no rain, water temperature 16.5 °C. Therefore the following criteria were defined for the second trial day: expected mean air temperature ≥ 19 °C, probability of sunshine ≥ 40%, probability of rain ≤ 20% and expected wind force ≤ 6 Beaufort. The second trial day had to be postponed twice before these criteria were met on 16 July 1996. On this date the air temperature was 19 °C, sunny, no rain and water temperature was 19 °C.

On the trial days children were taken to the freshwater site by bus during which trip they received a refrigerated lunch. Lunches were prepared by the catering of the National Institute and eaten by all children. They consisted of low-risk foods, e.i. rolls with jam or cheese, currant loaf, piece of fruit and orange juice or coke. The children arrived at the site at 1.00 p.m. and were assigned to a supervisor immediately. Each supervisor was responsible for six bathers or six non-bathers either from the moment the children arrived at the beach until they left. Supervisors were enrolled from a sports institute and had been trained for the trial days. Both supervisors and children had been informed about their groups in the morning of the trial day. Children wore T-shirts with their name on it; supervisors wore name plates.

Non-bathers participated in games at a restricted area of the beach. Games were organised by the supervisors. Supervisors had to make sure that non-bathers did not enter the water.

Bathers participated in relay races or hit ball in the water. Exposure took place in a pre-defined area. Three exposure settings have been tested in order to investigate which setting produced the most intensive exposure: four short relay races on 25 June, two long relay races on 16 July and one hit ball area on 16 July (see Figures 2b and 2c). Bathers were exposed to one of these settings in teams of six children. On 25 June all teams were exposed simultaneously, and on 16 July teams were exposed in two time-series. The supervisor of each team accompanied the children in the water. A second person on the beach recorded place and duration of exposure and number of times the head was immersed for each child in the team of six. In the relay race each child was given a number. Number one of each team started with the race, number two started when number one was back in the team etc. So the person on the beach had to record the activities only for one child at a time. Recording was done by supervisors (25 June) or researchers (16 July). Children who participated in hit ball were given numbers on their cheeks and arms. Recording was done by teachers who knew the children, facilitating recognition. They also recorded place and duration of exposure and number of times the head was immersed. After leaving the water each bather was asked whether he/she had swallowed water. Bathers did not enter the water again on the trial day but participated in games on the beach.

An ambulance and a medical doctor were available on the beach. All children were taken back to school at 4.00 p.m.

2.10 Water quality monitoring and analysis

An exploratory analysis of the bathing site was carried out on 11 June 1996. Objectives were: 1) to determine the location and lay-out of the exposure settings, 2) to determine variation in water quality over time, between tracks and within tracks and 3) to test the sampling procedures. On this day three relay races were laid out, approximately 20m offshore at 0m, 20m and 100m. Each track consisted of a 6x4 metres area at a water depth of 1-1.2m where head immersions would occur (see Figure 2a).

For sampling, the 6x4 metres area was divided in 12 (imaginary) areas of equal size. A composite sample was obtained by taking in each area a sample of approx. 250 ml with a sterile beaker. The sample was immediately transferred to a 4L sterile container.

Samples were taken at time 0 (12h30), 10, 30, 60, 70 and 90 minutes. At t=30 only individual samples were taken at the twelve areas of the "100m" track. Between t=60 and t=70 the samplers disturbed the sediment to determine the extend of this on the water quality.

Samples were transported to the laboratory at 2-8°C and analysed for *E.coli* by the method of Havelaar and During (25).

On 25 June (first trial day) samples were taken as indicated in Figure 2b. They were taken 5 min. before the start of the race ("before" - 3L) to determine the baseline water quality, after the first swimmer ("first" - 3L), after the third swimmer (" middle" - 3L) and after the last swimmer ("last" - 10L). The 3L samples were composite samples of 12x approx. 250 ml, the 10L samples of 12x approx. 900 ml.

On 16 July (second trial day) samples at the relay races were taken as indicated in Figure 2c: 5 min. before the start of the first series ("before" - 3L), following the first swimmer on his/her first round ("first" - 3L), following the first swimmer on his/her second round ("middle" - 3L) and following the last swimmer on his/her second round ("last" - 10L). During the second series, 3L samples were taken only during the race (not before) and at the same moments as in the first series. All samples were composite samples as on 25 June.

Samples from the hit ball tracks were taken at the same time as on the relay race tracks and as indicated in Figure 2c; the hit ball game was paused during sampling.

On both trial days, samples of 200L were taken just outside track 1 for the analysis of enteroviruses according to van Olphen *et al* (26) and samples of 100L were taken for *Cryptosporidium* and *Giardia* analysis according to Kruidenier and Medema (27). On these sites pH and water temperature were also determined. Virus filters were eluted on-site.

The 3 and 10L samples were analysed for thermotolerant coliforms (200, 50, 12, 3 ml in duplicate), *E.coli* (same volumes) and faecal enterococci (400, 100, 25, 6 ml in duplicate) by Dutch standard methods. The 10L samples were additionally examined for *Aeromonas* (16, 4, 1, 0.25 ml in duplicate), *Pseudomonas aeruginosa* (100, 25, 6, 1 ml in duplicate) by Dutch standard membrane filtration methods, *Staphylococcus aureus* (100, 10, 1 ml in duplicate) by the method of Havelaar and During (28), *Salmonella* (3 x 1000, 3 x 100, 3 x 10 ml), *Campylobacter* (3 x 1000, 3 x 100, 3 x 10 ml) by Dutch standard MPN methods. A 1 l sample was sent to Aquasense, Amsterdam, to determine the concentration of algae and cyanobacteria microscopically.

Virus-sample eluates, protozoa sample filters and water samples were transported to the laboratory at 2-8°C. Virus-samples eluates were concentrated by ultrafiltration (26), starting on the sampling day. Bacteriological analyses were performed within 24 h. Of each sample, approx. 900 ml was mixed with glycerol and frozen at-70°C and stored at -20°C for approx. 8-10 weeks for F-specific RNA phages by ISO DIS 10705 (29). Phage recovery of this method was determined to be 80-100%. The protozoa-sample filters were stored cold for 2 days and processed and analysed according to Kruidenier and Medema (27).

2.11 Data handling and data analysis

Questionnaire data were entered twice using Epi-info (30). Check for inconsistencies and analysis of the data was carried out with the SAS programme (31). The significance of differences in medians of baseline characteristics between grades, between bathers and non-bathers and between consenting and non-consenting children were tested with a median test, and of differences in proportions with a Chi-square test or a Fisher's exact test, when appropriate. Sample size calculations were carried out in Epi-info (30). Water quality data were analysed in Excel (32).

3. RESULTS

Part I - Research results

In Part I (3.1 - 3.6), we describe the response rates in the study, population characteristics, exposure assessment, water quality data, and disease attack rates within one week of follow up. Results with respect to evaluation of the study design are presented in Part II (3.7 - 3.12), and those concerning sample size for future studies in Part III (3.13).

3.1 Response rate (see also Figure 4)

Overall, 84% (n=182) of children in grade 6, 7 and 8 participated in the study (Table 1). Rate of participation was substantially higher at school A than at school B and in grade 6 and 7 than in grade 8. It was remarkable that at school B, despite the low overall participation rate, almost all children in grade 7 participated, possibly because the father of one of the pupils worked at the Municipal Health Service and had signed the letter with information about the study.

Twenty-four children (13%) were excluded from randomisation: eleven were not permitted to swim and 13 had pre-existent ear disease or sinusitis. The remaining 158 children were randomised to the bather (n=77) or non-bather group (n=81). Randomisation was successful (i.e. those who were randomised into the bather (or non-bather) group one week before the trial day actually bathed (or played) on the trial day) for 91% of children (n=143) and this was higher at school B than at school A. Unsuccessful randomisation occurred for children who were randomised into the bather or non-bather group one week before the trial day but who were absent from school on the trial day, or who were ill on the trial day and were therefore not permitted to swim anymore. Unsuccessful randomisation was mainly due to (1) delay of the second trial day (school A) because of bad weather conditions and, consequently (2) absence because of the start of the summer holiday.

Complete information on classroom health data was obtained from 75% (n=107) of successfully randomised children and this was substantially lower at school A (63%) than at school B (100%). Complete information on classroom health data at school A was obtained from bathers and non-bathers equally, but was highest in grade 6, followed by grade 7 and grade 8. The start of the summer holiday at school A probably accounted for incomplete information on classroom health data. A disability questionnaire was sent to the parents of fourteen children and it was returned by 71% at both schools. A follow-up questionnaire was returned by 56% of parents of successfully randomised children. Complete response to both the health data questionnaires and the follow-up questionnaire was obtained from 48% of successfully randomised children.

A non-response interview was taken from the parents of 85% (29/34) of children who did not participate in the study. This concerned 26 parents (three parents had two children who did not participate) and they mentioned 42 reasons for refusal (Table 2). 'To be used as a guinea pig' was most frequently mentioned and this was reported by half of the parents. Pre-existent health complaints in the child that restrained it from bathing was the second major reason for non-participation.

Regarding the total response rate (including both informed consent to participate on the trial day and complete follow-up after randomisation), 50% of all children in grade 6, 7 and 8 (107/216), at both schools a similar percentage, participated successfully on the trial day and also completed all health data questionnaires up to one week after the trial day. Rates fell to ~ 30% if also completion of the questionnaire three weeks after the trial day was included. A maximum response rate of 82% can be maximally reached at one week of follow-up if all conditions are favourable. This is based on the

observed maximum response rates in the pilot study, i.e. 0.96 for informed consent at school A*0.87 for eligibility for randomisation at both schools*0.97 for successful randomisation at school B*1.0 for complete response at one week of follow-up at school B.

3.2 Population characteristics

Except for educational level of the parents (lower at school A) and country of birth of the father (less often The Netherlands at school A), baseline characteristics of children at school A were comparable to those of children at school B (data not shown). Baseline characteristics of children in grade 6, 7 and 8 (both schools combined) are presented in Table 3. Except for the frequency of swimming in pools in the summer of 1996 (lowest in grade 6), there were no substantial differences between the children in the three grades.

A comparison between bathers and non-bathers is given in Table 4. Despite randomisation, bathers and non-bathers were not completely comparable, but this can be expected if the numbers are small.

Children who did not participate in the study were more often boys and reported less often diarrhoea, ear inflammations and common cold than those who participated (Table 5). They were also less likely to swim in the summer (in either sea or fresh waters or swimming pools) than respondents.

3.3 Exposure assessment

Exposure of bathers to the water was assessed by the number of times heads were immersed and the proportion of bathers reporting to have swallowed water. This was done for all three exposure settings (short relay race, long relay race and hit ball). The results are presented in Table 6a. On 25 June (short relay race) the water temperature was only 16.5 °C and therefore the relay race was shortened. Bathers were exposed for a mean duration of one minute. Heads were immersed frequently but this may have been overestimated as supervisors counted the number of times lines were crossed correctly (over or under) and not the number of times heads were immersed. A quarter of the children swallowed water. On 16 July mean time of exposure was about 20 minutes at both settings (relay race and hit ball). On average, heads were more frequently immersed in the relay race than during hit ball, and the percentage of children who reported to have swallowed water was higher at the relay race. Younger children immersed heads less frequently than older children (Table 6b) and boys swallowed water more often than girls, but this was mainly observed for the hit ball exposure (50% vs 15%).

3.4 Water quality

The exploratory analyses showed that the faecal pollution in the Byland on 11 June 1996 was low (Table 7). Before resuspension of the sediment, small differences were observed between the concentrations at the three tracks (max. factor 1.7). After resuspension, the variation was slightly higher (max. factor 2.5). At tracks "0m" and "20m", suspension of sediment caused a 1.5 - 2 fold higher *E.coli* concentration, at track "100m", the *E.coli* concentration was not affected. Overall, differences due to resuspension were larger than expected from random variation ($\chi^2 = 65.2$, $P < 0.001$). The 12 individual samples taken at t = 30 min at track "100m" showed little variation ($\chi^2 = 3.41$, $P = 0.98$).

On the first trial day (25 June) the water level was relatively low (lower than on 11 June), probably caused by a dry spring. On 16 July the level was substantially higher and rainfall in between the two trial days may have introduced pollutions from the river Rhine in the Byland.

On both trial days concentrations of thermotolerant coliforms met the European Guide level; on 25 June all concentrations were below 100 cfp/100 ml and on 16 July three of 28 samples had concentrations above this level (Table 8 and 9). *Salmonella* concentrations were < 0.3 cfu/l on both days. *Campylobacter* was present in concentrations of 0.4 - 0.9 cfu/l on 25 June and < 0.3/l on 16 July. *Giardia* and *Cryptosporidium* were found in concentrations of 0.1 - 0.3/l on both days.

Comparison of the samples taken on 25 June before and during exposure of the children to the water showed that water quality was not affected by swimmers. For F-specific RNA phage-counts a higher variation was observed, but it is not likely that these phages originated from swimmers. No substantial differences in concentrations of the indicators investigated were observed in the samples taken at the different tracks (Figure 3a).

Similar results were observed on the second trial day (Table 9 and Figure 3b): water quality was not likely to have been influenced by bathers and water quality varied only slightly between the exposure tracks. There was one exception to this: in the hit ball setting in the downstream track (track 1) concentrations of indicator organisms seemed to increase during the game. Resuspending of sediment and the downstream location might have caused these increases.

As observed in the athletes study (21), on 25 June concentrations of *Escherichia coli* were approximately three-fold higher than those of thermotolerant coliforms. This was not the case on 16 July. *E.coli* concentrations were higher than thermotolerant coliform concentrations, but only approximately 1.5-fold. Faecal enterococci concentrations were much higher on 16 July, the ratio between *E.coli* and faecal enterococci was 1:1 to 1:1.5. On 25 June this ratio was 1: 0.1 to 1: 0.15. F-specific RNA phage concentrations were relatively low at 16 July; the ratio with *E.coli* concentrations was 1:100 to 1:150. On 25 June this ratio was 1:30 to 1:40.

3.5 Microbiological analysis of refrigerated lunch

One lunch (all rolls and drinks) of 25 June was investigated for *E. coli*, *S. aureus*, *Salmonella* and *Campylobacter* by standard techniques. No pathogens were found. Of 16 July, one lunch was stored at -20 °C.

3.6 Disease attack rates

Occurrence of health complaints in the week after the trial day was calculated over subjects without similar complaints in the two days before the trial day. Several case definitions were studied and they produced varying attack rates.

Using similar case definitions as in the athletes study (21), it was suggested that non-bathing children reported higher attack rates of gastrointestinal, respiratory and skin/mucosal complaints than non-bathing endurance athletes (non-bathers are compared as the attack rates in bathers may have been influenced by water quality) (Table 10a). Applying more stringent case definitions also showed that, at least for gastroenteritis, attack rates in children may be higher than in endurance athletes (Table 10b).

Applying the most stringent case definitions, of all children (bathers and non-bathers) 4.7% reported gastroenteritis, 0.9% respiratory illness and 1.4% skin complaints. As water quality at both trial days was good in terms of risk levels observed in the athletes study (no swimming-associated risk of gastroenteritis after exposure to thermotolerant coliform concentrations below 100/100 ml) and as bathers on the first trial day were exposed for only one minute, we combined bathers and non-bathers to get an estimate of attack rates in an 'unexposed population'.

Of ten ill children who received a disability questionnaire, only one reported to have consulted a general practitioner, to have stayed away from school for one day, to have rested in bed and to have used drugs.

Part II - Evaluation of the study design

3.7 Approaching the schools

It was found difficult to enrol schools to participate in the study. Of nine schools who did not want to participate six mentioned ethical objections and three reported to be too busy in the period before the summer holidays.

It was found that oral communication and consultation at school was the best way to explain the objectives of the study and to refute ethical objections. It is therefore recommended to try and arrange a meeting at all eligible schools in order to explain the objectives of the study. The school director should further inform the representative advisory body, the school governors and the Inspectorate of Education. As such meetings take time and as meetings with representative advisory bodies are not arranged frequently, it is necessary to contact schools as early as possible, but at least three months before the first trial day. An additional possibility is to introduce the study at a regional consultative structure for primary education and to contact enthusiastic schools. A disadvantage of this approach might be that negative thoughts about the study at one school might easily influence other schools.

3.8 Approaching the parents

In accordance with the usual route of information given to parents, we chose to distribute information about the study in the classrooms. It might have been better if all parents were explained orally the objectives of the study and were given the opportunity to request additional information. We experienced this at school B, where the information was distributed before a school break. Parents could not contact school and many of them developed objections against the study. But among those who contacted the RIVM, ethical objections could be removed almost completely. From the non-response interview it became clear that negative thoughts have been mainly provoked by a few parents who influenced others. This shows that oral communication is important and that information should not be given before a school break or even a week-end. Oral information can be given at a parents' evening at school, although additional written information will also be necessary as not all parents attend such meetings. The information should be provided after the week-end (on a Monday) and a member of the research team should be available at school in the week thereafter.

It was experienced that written information should more explicitly stress the time schedule of the study, the number of questionnaires to be completed, under what conditions a trial day will be postponed and what activities will be organised on the trial day. In order to keep the information convenient, a small manual might be developed with all relevant information strictly organised.

3.9 Questionnaires

No major problems were reported for any of the questionnaires used in the study. There were, however, some minor problems with the baseline and follow-up questionnaire. Ten parents found one or more of the questions in the baseline questionnaire unclear (12 questions in total). This mainly concerned difficulties with classifying symptoms, and problems with the frequency coding of questions about yearly occurrence of skin irritations, ear and eye infections, common cold and headaches. The categories were coded as 'never', 'once or twice', 'three to eleven times' or 'twelve times or more' but they might be changed in 'once a week', 'once a month', 'once a quarter', 'once or twice a year' and 'never'. Questions about educational level and country of birth of the parents were found indelicate or unnecessary by 13 and four parents, respectively. Ten and two parents, respectively, refused to answer these questions. Although the questions seemed not a major reason for refusal, they were a cause of annoyance. As more direct information on health status can be obtained from other questions, it is recommended to remove both questions from future questionnaires. Furthermore, the follow-up questionnaire may better be distributed after the first week, as parents had problems remembering events retrospectively.

Completion of daily health forms in the classrooms proved feasible after giving carefully instructions to the children and in the presence of a member of the research team. Younger children appeared to be more motivated than children in grade 8, particularly near the end of the study period. This was also reflected by the lower response rate on the daily health forms that were given home at school A: they were returned most frequently by children in grade 6 and less frequently by children in grade 8 (table 1). It is therefore worth considering to enrol children in grade 5 (aged 8 or 9 years) in stead of children in grade 8. If future studies are carried out among larger numbers of children and time problems become apparent, questionnaires may be given home for the days after the trial day. In that case children should be trained to complete a form each day before going to school. At the end of the observation period all questionnaires should be collected at school.

All ten parents who returned a disability questionnaire confirmed the complaints reported by their children. Two of them reported that it concerned only mild complaints.

None of the questionnaires had missing values for more than 5% of the respondents, nor were any questions answered with 'don't know' for 5% or more of the respondents.

3.10 Randomisation

In future studies randomisation should be done as late as possible (e.g. one day before the trial day) and children should be informed about their randomisation status just before they arrive at the beach. In that case, postponement of a trial day will not disturb the randomisation and randomisation status will not influence food consumption or swimming behaviour before exposure.

3.11 Trial days

The pilot study has shown that the study design is strongly dependent on weather conditions. This is inconvenient, as much time and money is spent to organise a trial day, but it is inherent to the study design. In order to enhance the opportunity for progress of the trial day, at least two alternative dates should be scheduled each summer.

We have considered whether trial days can be organised after the summer holidays (in August or September). This has the advantage that higher water temperatures can be expected and that completion of questionnaires (both in classrooms as by parents at home) is not influenced by the holiday rush. For 1993-1996 it was found that the percentage of days on which the weather criteria as mentioned in 2.9 were met was lowest in May and September and highest in July and August (Table 11). This means that trial days can best be organised in July and August, although even in these months, the study criteria will on average be met on only 50% of all days. Information on water temperatures should therefore be collected, to investigate wether the criteria may be made less stringent if the water temperature is higher near the end of the summer. If trial days are organised in August or September it should be made sure that cyanobacteria or *P. aeruginosa* are not present at the study sites.

3.12 Non-water exposure

No major problems were encountered for the non-water related beach activities. The games and competitions were liked by the children. The help of supervisors from a sports institute is very useful. All children complied with their randomisation status, but more failures may be expected in warmer summers.

Part III - Sample size of future studies

3.13 Sample size

Future studies aim to investigate whether bathers have a higher risk of health complaints than non-bathers, and whether risk can be related to the microbiological quality of the water (i.e. if a dose-response relationship can be established). The number of children required to detect a significant difference in attack rates between bathers and non-bathers depends on the expected attack rate in the non-exposed group and the excess attack rate (or relative risk) in the exposed group. It further depends on the probability of concluding that there is a difference when in fact such a difference does not exist (type I error of size alpha) and the probability of concluding that there is no difference when in fact the attack rates differ (type II error of size beta).

Expected attack rates for exposed and non-exposed populations can be derived from several studies. They are presented in Table 12a, together with some hypothetical attack rates and relative risks. The calculations assume a type I error of 5%, a type II error of 10% and a ratio of non-bathers : bathers of 1 : 2. The table lists a large range of numbers and as definite estimates of attack rates and relative risks are not known, a definite number needed for future studies is difficult to give.

We have therefore assumed that a relative risk of bathing of two is of public health relevance and that the observed overall attack rate of gastroenteritis in the pilot study of about 4.5% is a valid estimate of the baseline attack rate in non-exposed children. In that case 1590 children (530 non-bathers and 1060 bathers) are needed to detect a relative risk of bathing of two with a type I error of 5% and a type II error of 10%. The attack rate of 4.5% may, however, be an overestimation of the true rate as only 75% of children completed the questionnaires in the week after the trial day and these might have been the ones with complaints. If we assume that all others did not have gastrointestinal complaints, the attack rate is estimated at 3.5% (5/143). In that case 2077 children are needed to detect a relative risk of two. If the real relative risk is 1.5 in stead of 2, at least 5211 children are needed to detect a significant difference in attack rates between bathers and non-bathers. Based on logistical considerations, a study among 2100 children seems feasible.

Although determining sample size requirements based on dose-response relations is almost impossible, as there is no prior knowledge about the dose-response relation, it is able to compare attack rates among children exposed to concentrations of thermotolerant coliforms < 100/100 ml (EU Guide level) and those exposed to higher concentrations. This is based on the observed threshold level in the triathletes study (21). If we assume that the attack rate among bathers exposed to concentrations < 100/100 ml is equal to that among non-bathers, and that for gastroenteritis the relative risk of exposure to higher concentrations is three to five (dependent on the case definition studied) (both assumptions are based on the results of the triathletes study), the required sample sizes to detect a significant effect (alpha=0.05 and beta=0.10) are given in Table 12b. This shows that studying 2100 children, of which 1400 bathers, makes it possible to detect a significant threshold effect if the relative risk is at least three and the attack rate among bathers exposed to concentrations < 100/100 ml is as low as 1.5%.

4. EVALUATION OF THE PILOT STUDY DURING AN EXPERT MEETING

On 13 November 1996, the study design and results from the pilot study were discussed with Prof. D. Kay from the Centre for Research into Environment and Health, University of Leeds and Prof. J.M. Fleisher, Department of Preventive Medicine and Community Health, State University of New York. They both have extensive experience in water microbiology and epidemiology of waterborne health risks and have been involved in the UK sea bathing studies between 1989 and 1992 (16, 18).

This meeting resulted in three main recommendations for the future study:

- Bathers should be exposed within a small area in the water.
- As far as possible, each individual should be ascribed a unique measure of 'exposure'. This might best be achieved by intensive water sampling, to characterise the microbiological quality of water encountered by each individual at the place and time of exposure.
- In the pilot study microbiological analysis have been carried out according to Dutch standard methods. To improve comparison of future results with those obtained in the UK studies, information should be available on:

1. The effects of storage of samples on Indicator Organism Densities (IOD).
2. The effects of differences in Dutch and UK microbiological analysis methods on IOD.

Based on these recommendations, we propose to study in advance:

- The effects of storage of water samples on the mean and standard deviation of IOD.
- The effects of the different analysis methods on the mean and standard deviation of IOD.

As the variability in IOD in fresh waters is probably smaller than in sea waters, exposure to IOD should be spread in time and place. We therefore propose to change the study design as follows:

- Expose bathers, two at a time, in an area of 5 x 5 metres for 10-15 minutes. Take one sample (1 L) during this time. The sample is a composite of smaller samples taken at different locations and times in the 5 x 5 area during the 10-15 minutes of exposure.
- Expose bathers at chest depth at four locations in the lake.
- Expose bathers of one school in the morning during eight separate exposure events, and of a second school in the afternoon during also eight separate events. As each event involves 2 * 4 children in parallel, this results in 128 bathers being exposed on one trial day. During each discrete exposure episode a water sample is taken (e.g. 64 samples per trial day, one for every two children being exposed to the same body of water).
- Samples are fully analysed for thermotolerant coliforms, *E. coli*, faecal streptococci, and F-specific RNA phages. Analysis for enteroviruses, protozoa and bacterial pathogens will be reduced if necessary, depending on a further analysis of workload.
- On each trial day there are 64 non-bathers (32 of each school).
- Four trial days are organised each year, two to three in June/July and one to two in August, depending on weather conditions and school holidays.
- One or two fresh water sites are studied each year.
- The study is spread over three years (i.e. 12 trial days) and about eight schools have to participate each year .
- This results in about 2300 children (1550 bathers and 750 non-bathers) being studied in three years.

Other points of concern should be:

- Efforts will concentrate on reaching complete follow-up after randomisation, in order to improve internal validity of the study, and less on reaching complete response before randomisation.
- Interviewing children is different from interviewing adults. Therefore more efforts will be made to improve the validity of the study outcomes, e.g. by also asking the parents about health complaints of their child according to defined case definitions, including dummy questions etc.
- Follow-up at three weeks may be too long, this might be changed in two weeks if this is supported by the literature on relevant incubation periods.

5. CONCLUSIONS

As fresh waters in the Netherlands are used extensively by millions of persons every summer, and as good quality bathing waters are important for tourism, health based water quality standards are needed to decide whether fresh waters are safe for bathing.

Because the health effects of freshwater bathing are generally limited, we need large epidemiological studies and intensive microbiological sampling and analysis to obtain useful results. Years of international experience have shown that health effects can best be studied by using randomised exposure trials and by determining for each bather individually the level of exposure to indicator organisms. Randomised trials are, however, time consuming, complex and largely dependent on weather conditions.

We carried out a pilot study in the summer of 1996 to determine whether a controlled randomised exposure design among primary school children can be used to assess the health risks of freshwater bathing in a recreative population. In general, exposing children to freshwater in an experimental setting seems feasible, although in a larger study some methods need modification or further consideration. Based on the results of the pilot study and on recommendations given by two international experts, we propose to design a future study as follows:

During three years twelve trial days are organised at three to six freshwater sites. The aim is to expose about 1500 bathers and 750 non-bathers, which allows for detecting a relative risk of bathing of two if the attack rate in the non-bathers is 3.5%. On each trial day, exposures to the water will take place during the entire day and at different locations in the water providing a range in water quality. Exposure for each individual bather will be localised in a small area. For every two bathers a water sample will be taken at the time and place of bathing to assess exposure to indicator organisms. About 24 schools should participate in the study and trial days will take place both before and after the summer holidays. The approaching of schools and parents, the choice of the grades for the study population, the approach of interviewing children and the length of the follow-up period need special attention. To improve comparison of the results with those obtained in the UK studies, the effects of storage of water samples and the use of different analysis methods on concentrations of indicator organisms will be studied in advance. It should be borne in mind that the success of the study is largely dependent on the ability to find volunteers to participate in the study and on weather conditions.

6. ACKNOWLEDGEMENTS

The authors want to thank all who helped to carry out this study: M. Storck-Loran for general assistance during the whole study and for attending the classroom interviews, W. Stoop and G. Natrop from the Local Health Authority West-Veluwe/Vallei for help in approaching and communication with the schools and parents, C. Collé, A. Hamer for providing information to select the study site, The van Dijckschool in Bilthoven for the opportunity to test the questionnaires, B. Halle, H. van Klaveren, T. van Kappele and students from CIOS Arnhem for help in organising the trial days, M. Bahar, R. Bakker, D. Branger, R. de Bruin, M. During, G. Engels, R. van der Heide, W. Lodder, K. Mooijman, R. Pot, T. Gorkom, W. Ritmeester, C. Schets and E. Speld from the Microbiological Laboratory for Health Protection of the RIVM for water sampling and analysis, Prof. D. Kay and Prof. J. Fleisher for critical discussions about the study design, and the schools, parents and children who participated in the study.

7. REFERENCES

1. Havelaar AH. Gezondheidsklachten in verband met recreatie rond oppervlaktewater in de zomer van 1993. Inf Bull 1994: 5 (11): 187-9 [In Dutch].
2. Havelaar AH. Gezondheidsklachten in verband met recreatie rond oppervlaktewater in de zomer van 1994. Inf Bull 1995; 6 (11): 240-4 [In Dutch].
3. Havelaar AH. Gezondheidsklachten in verband met recreatie in oppervlaktewater in de zomer van 1995. Inf Bull 1996; 7 (11): 223-6 [In Dutch].
4. Stevenson AH. Studies of bathing water quality and health. Am J Public Health 1953; 43: 529-38.
5. Cabelli V, Dufour AP, McCabe LJ, Levin MA. Swimming-associated gastro-enteritis and water quality. Am J Epidemiology 1982; 115: 606-16.
6. El Sharkawi F, Hassan M.N.E.R. The relation between the state of pollution in Alexandria swimming beaches and the occurrence of typhoid among bathers. Bulletin of the High Institute of Public Health Alexandria 1982; 12: 337-51.
7. Foulon G, Maurin J, Quoi N, Martin-Bouyer G. Relationship between the microbiological quality of bathing water and health effects. Revue Francaise des Sciences de L'Eau 1983; 2: 127-43.
8. Dufour AP. Health effects criteria for fresh recreational waters. Cincinnati, Ohio: US Environmental Protection Agency, 1984. EPA 600/1-84-004.
9. Seyfried PL, Tobin RS, Brown NE, Ness PF. A prospective study of swimming-related illness. II. Morbidity and the microbiological quality of water. Am J Publ Health 1985; 75: 1071-5.
10. Fattal B, Peleg-Olevsky E, Agursky T, Shuval HI. The association between seawater pollution as measured by bacterial indicators and morbidity among bathers at Mediterranean beaches of Israel. Chemosphere 1987; 16: 565-70.
11. Cheung WHS, Chang KCK, Hung RPS, Kleevens JWL. Health effects of beach water pollution in Hong Kong. Epidemiol Infect 1990; 105: 139-62.
12. Ferley JP, Zmirou D, Balducci F, Baleux B, Fera P, Larbaigt G. et. al. Epidemiological significance of microbiological pollution criteria for river recreational waters. Int J Epidemiol 1989; 18: 198-205.
13. Lightfoot NE. A prospective study of swimming related illness at six freshwater beaches in Southern Ontario [thesis]. University of Toronto, 1989.
14. Balarajan R, Soni Raleigh V, Yuen P, Wheeler D, Machin D, Cartwright R. Health risks associated with bathing in sea water. BMJ 1991; 303: 1444-5.
15. Corbett SJ, Rubin GL, Curry GK, Kleinbaum DG. The health effects of swimming at Sydney beaches. Am J Publ Health 1993; 83: 1701-6.
16. Kay D, Fleisher JM, Salmon RL, Jones F, Wyer MD, Godfree AF et al. Predicting likelihood of gastroenteritis from sea bathing: results from randomised exposure. Lancet 1994; 344: 905-9.
17. Asperen IA van, Rover CM, Colle C, Schijven JF, Bambang Oetomo S, Schellekens JFP et. al. Risk of otitis externa after swimming in recreational fresh water lakes containing Pseudomonas aeruginosa. BMJ 1995; 311: 1407-10.

18. Fleisher JM, Kay D, Salmon RL, Jones F, Wyer MD, Godfree AF. Marine waters contaminated with domestic sewage: nonenteric illnesses associated with bather exposure in the United Kingdom. Am J Public Health 1996; 86: 1228-34.
19. Nationaal Milieubeleidsplan (NMP). Tweede Kamer, 1988-89, 21137, 1-2, p.95 [In Dutch].
20. From proposal for revision of Directive 76/160/EEC. OJ N C 138, 17.5.1993, p1.
21. Asperen IA van, Medema GJ, Borgdorff MW, Sprenger MJW, Havelaar AH. Microbiologische zwemwaterkwaliteit in relatie tot gezondheidsklachten; een prospectief onderzoek bij triatleten. RIVM rapportnummer 289202009, Bilthoven, 1995 [In Dutch].
22. Medema GJ, Schijven JF, Mark van der HJ. Microbiologische kwaliteit van zwemwater. Milieubalans 1996. Rijksinstituut voor Volksgezondheid en Mileu, Bilthoven, in press [In Dutch].
23. Anon. Health criteria for the quality of recreational waters with special reference to coastal waters and beaches. Copenhagen: WHO, 1972.
24. Cabelli V, Dufour AP, Levin MA, McCabe LJ, haberman PW. relationship of microbial indicators to health effects at marine bathing beaches. Am J Public Health 1979; 69: 690-6.
25. Havelaar AH, During M. Evaluation of Anderson Baird-Parker direct plating method for enumerating *Escherichia coli* in water. J Appl Bact 1988;64:89-98.
26. Van Olphen M, de Bruin HAM, Schijven JF, Drost YC, Havelaar AH. Virologisch en bacteriologisch onderzoek van zwemwater op een aantal locaties in Nederland in de recreatieseizoenen van 1989 en 1990. RIVM-rapport 148704001, RIVM, Bilthoven; 1992.
27. Kruidenier L, Medema GJ. Belasting en inactivatie van de protozoa *Cryptosporidium* en *Giardia* van pompstation Weerseloseweg van NV Waterleidingsbedrijf Oost-Twente. RIVM rapport 289202012, Bilthoven; 1996.
28. Havelaar AH, During M. Model studies on a membrane filtration method for the enumeration of coagulase-positive staphylococci in swimming-pool water using rabbit plasma-bovine fibrinogen agar. Can J Microbiol 1985; 31: 331-4.
29. Anonymous. Water Quality - Detection and enumeration of F-specific RNA bacteriophages. ISO 10705-1. Geneva; International Organization for Standardization, 1995.
30. EPI Info 6.04. Centers for Disease Control and Prevention, Atlanta, Georgia, USA, 1995.
31. Statistical Analysis System. Cary, NC: SAS Institute, 1989.
32. Microsoft Excell Version 5.0c, 1994.

8. TABLES AND FIGURES

Table 1. Response rates in the pilot study (number and percentage).

	School A				School B				Total
	Grade 6	*Grade 7*	*Grade 8*	*Total*	*Grade 6*	*Grade 7*	*Grade 8*	*Total*	
Participation rate	51/51 (100)	40/40 (100)	36/41 (87.8)	127/132 (96.2)	13/25 (52.0)	28/30 (93.3)	14/29 (48.3)	55/84 (65.5)	182/216 (84.3)
Excluded from randomisation	9/51 (17.6)	1/40 (2.5)	6/41 (14.6)	16/127 (12.6)	3/25 (12.0)	5/30 (16.7)	0/29 (0.0)	8/55 (14.5)	24/182 (13.2)
Randomised bathers	21/51 (41.2)	19/40 (47.5)	14/41 (34.1)	54/127 (42.5)	5/25 (20.0)	11/30 (36.7)	7/29 (24.1)	23/55 (41.8)	77/182 (42.3)
Randomised non-bathers	21/51 (41.2)	20/40 (50.0)	16/41 (39.0)	57/127 (44.9)	5/25 (20.0)	12/30 (40.0)	7/29 (24.1)	24/55 (43.6)	81/182 (44.5)
Randomisation successful	36/42 (85.7)	36/39 (92.3)	25/30 (83.3)	97/111 (87.4)	10/10 (100)	22/23 (95.7)	14/14 (100)	46/47 (97.9)	143/158 (90.5)
Complete disability data	3/4 (75.0)	2/2 (100)	0/1 (0.0)	5/7 (71.4)	1/1 (100)	3/5 (60.0)	1/1 (100)	5/7 (71.4)	10/14 (71.4)
Complete health data-one week	26/36 (72.2)	22/36 (61.1)	13/25 (52.0)	61/97 (62.9)	10/10 (100)	22/22 (100)	14/14 (100)	46/46 (100)	107/143 (74.8)
Complete health data-three weeks	20/36 (55.6)	21/36 (58.3)	11/25 (44.0)	52/97 (53.6)	9/10 (90.0)	11/22 (50.0)	8/14 (57.1)	28/46 (60.9)	80/143 (55.9)
Complete health data-both periods	16/36 (44.4)	17/36 (47.2)	9/25 (36.0)	42/97 (42.3)	9/10 (90.0)	10/22 (45.5)	8/14 (57.1)	27/46 (58.7)	69/143 (48.3)
Complete non-response data	-	-	4/5 (80.0)	4/5 (80.0)	10/12 (83.3)	0/2 (0.0)	12/15 (80.0)	22/29 (75.9)	26/34 (76.5)

Table 2. Reasons for non-response in the study mentioned by 26 parents (number and percentage).

Reason	Parents
To be used as a guinea pig	12 (46.2)
Pre-existent health complaints in child	8 (30.8)
Child did not want to participate	5 (19.2)
Did not want to take a risk before summer holidays	3 (11.5)
Bathes never in surface waters	2 (7.7)
Child was not able to attend	2 (7.7)
Parent was crude at telephone	2 (7.7)
Water too cold	2 (7.7)
Not enough time to think it over	1 (3.8)
Not clear that participation was voluntary	1 (3.8)
Aims of study unclear	1 (3.8)
Child cannot swim	1 (3.8)
Indelicate questions in baseline questionnaire	1 (3.8)
Problems in family	1 (3.8)
Total	26 (100)

Table 3. Baseline characteristics of participating children according to grade (number and percentage).*

	Grade 6 (n = 64)	Grade 7 (n = 66)**	Grade 8 (n = 50)
Sex (boys)	26 (41)	31 (47)	17 (34)
Age (median and 5;95 perc)	10 (9;11)	11 (10;12)	12 (11;13)
Swimming certificate	64 (100)	66 (100)	50 (100)
Diarrhoea (never)	17 (27)	24 (36)	23 (46)
Diarrhoea (1-2 times/year)	38 (59)	37 (56)	25 (50)
Diarrhoea (3-11 times/year)	2 (3)	4 (6)	1 (2)
Skin rash (never)	42 (66)	48 (73)	37 (76)
Skin rash (1-2 times/year)	14 (22)	13 (18)	9 (18)
Skin rash (3-11 times/year)	6 (9)	1 (2)	3 (6)
Ear inflammation (never)	50 (78)	53 (80)	40 (80)
Ear inflammation (1-2 times/year)	10 (16)	11 (17)	9 (18)
Ear inflammation (3-11 times/year)	3 (5)	2 (3)	1 (2)
Cold (never)	4 (6)	4 (6)	5 (10)
Cold (1-2 times/year)	45 (71)	51 (77)	31 (62)
Cold (3-11 times/year)	13 (21)	11 (17)	13 (26)
Never bathed in sea in summer of 1996***	23 (79)	23 (72)	16 (89)
Never bathed in fresh water in summer of 1996***	18 (62)	19 (59)	12 (67)
Never bathed in pool in summer of 1996***	8 (28)	2 (9)	0 (0)†

* both schools combined

** two children have not completed a baseline questionnaire

*** except during trial day; information available from 80 children who completed a follow-up questionnaire

† $p < 0.05$

Table 4. Baseline characteristics of bathers and non-bathers (number and percentage).

	Bathers (n = 69)	Non-bathers (n = 74)
Sex (boys)	32 (46)	27 (36)
Age (median and 5;95 perc)	11 (10;12)	11 (10;12)
Swimming certificate	69 (100)	74 (100)
Country of birth of child (Nl)	64 (94)	73 (99)
Country of birth of father of child (Nl)	53 (78)	69 (93)‡
Country of birth of mother of child (Nl)	62 (91)	71 (96)
Educational level of parents (low)*	18 (27)	30 (43)
Educational level of parents (high)*	22 (33)	16 (23)
Diarrhoea (never)	28 (41)	31 (41)
Diarrhoea (1-2 times/year)	39 (57)	35 (47)
Diarrhoea (3-11 times/year)	0 (0)	5 (7)
Skin rash (never)	50 (72)	56 (77)
Skin rash (1-2 times/year)	14 (20)	13 (18)
Skin rash (3-11 times/year)	3 (4)	2 (3)
Ear inflammation (never)	63 (91)	59 (80)
Ear inflammation (1-2 times/year)	6 (9)	13 (18)
Ear inflammation (3-11 times/year)	0 (0)	2 (3)
Cold (never)	5 (7)	7 (10)
Cold (1-2 times/year)	53 (77)	50 (68)
Cold (3-11 times/year)	10 (14)	15 (21)
Never bathed in sea in summer of 1996**	25 (74)	37 (82)
Never bathed in fresh water in summer of 1996**	21 (62)	28 (62)
Never bathed in pool in summer of 1996**	7 (21)	4 (9)

* educational level; low: lower general secondary (MAVO) or vocational (LBO) education, middle: higher general secondary education (HAVO), pre-university education (VWO) or intermediate vocational education (MBO), high: higher vocational education (HBO) or university (WO)

** except during trial day; information available from 80 children who completed follow-up questionnaire

‡ $p < 0.01$

Table 5. Baseline characteristics of consenting and non-consenting children at school B. (number and percentage).

	Consenting (n = 53)	Non-consenting (n = 22)*
Sex (boys)	19 (36)	14 (56)
Swimming certificate	53 (100)	21 (95)
Diarrhoea (never)	17 (32)	11 (50)
Diarrhoea (1-2 times/year)	31 (58)	8 (36)
Diarrhoea (3-11 times/year)	4 (8)	1 (5)
Skin rash (never)	38 (72)	17 (77)
Skin rash (1-2 times/year)	11 (21)	3 (14)
Skin rash (3-11 times/year)	1 (2)	0 (0)
Ear inflammation (never)	39 (74)	19 (86)
Ear inflammation (1-2 times/year)	12 (23)	2 (9)
Ear inflammation (3-11 times/year)	2 (4)	1 (5)
Cold (never)	3 (6)	7 (32)
Cold (1-2 times/year)	39 (74)	11 (50)
Cold (3-11 times/year)	11 (21)	4 (18)†
Never bathed in sea in summer of 1996**	25 (86)	21 (95)
Never bathed in fresh water in summer of 1996**	22 (76)	21 (95)†
Never bathed in pool in summer of 1996**	3 (10)	7 (27)

* data on sex available for 25 children

** except during trial day ; information available from 29 consenting children who completed follow-up questionnaire

† $p < 0.05$

Table 6a. Assessment of exposure to the water at the three exposure settings

	Exposed (n)	Duration of exposure (mean and range in min.)	Frequency of head immersion (median and 5;95 perc)	Swallowed water (%)
Short relay race (25 June 1996)	23	1	13 (3;16)	26
Long relay race (16 July 1996)	25	23 (21-25)	10 (8;16)	52
Hit ball (16 July 1996)	23	21 (19-22)	6 (1;23)	30

Table 6b. Assessment of exposure to the water according to age and sex

	Exposed (n)	Frequency of head immersion (median and 5;95 perc)	Swallowed water (%)
Grade 6	28	8 (1;23)	39
Grade 7	28	12 (3;16)	36
Grade 8	15	13 (3;20)	33
Boys	31	13 (3;20)	45
Girls	40	9 (1;16)[†]	30

[†] $p < 0.05$ (comparing boys vs. girls)

Table 7. Water quality (Escherichia coli/100 mL) at 11 June 1996 (exploratory sampling)

Time (min)	Track "0m"	Track "20m"	Track "100m"
0	14	11	8
10	14	13	9
30	-	-	17**
60	16	25	15
70*	37	36	15
90	28	24	13

* after suspension of sediment by sampler

** average of *E.coli* concentration of 12 individual samples (18, 17, 15, 21, 20, 17, 14, 14, 18, 15, 19, 16 per 100ml)

*Table 8. Water quality on 25 June (averages and geometric means calculated over concentrations measured **during** exposure)*

	Thermotolerant coliforms/100 mL*					
Track	Before	First	Middle	Last	Average	Geomean
1	8	7.4	8.4	10	9	9
2	18	9.8	10	9.8	10	10
3	13	8.4	11	13	11	11
4	17	12	11	10	11	11

*corrected for overall confirmation rate (93%)

	Escherichia coli/100 mL*					
Track	Before	First	Middle	Last	Average	Geomean
1	26	34	24	44	34	33
2	48	36	40	32	36	36
3	42	38	38	28	35	34
4	46	40	40	36	39	39

*corrected for overall confirmation rate (100%)

	Faecal enterococci/100 mL*					
Track	Before	First	Middle	Last	Average	Geomean
1	2.4	3.1	6.1	6.3	5.2	4.9
2	3.4	2.8	1.8	3.3	2.6	2.6
3	2.2	3.5	1.8	2.0	2.4	2.3
4	2.6	2.4	2.0	2.6	2.3	2.3

*corrected for overall confirmation rate (100%)

	F+ RNA phages/100 mL					
Track	Before	First	Middle	Last	Average	Geomean
1	0.14	0.63	1.16	1.52	1.10	0.63
2	0.54	1.04	1.04	0.75	0.94	0.81
3	0.49	0.25	1.29	0.48	0.67	0.52
4	0.12	0.13	2.08	1.06	1.09	0.43

	Other parameters (first series)			
Track no	1	2	3	4
Aeromonas /ml	154	76	130	84
Ps. aeruginosa /100 ml	<1	<1	<1	<1
Campylobacter /l	0.9	0.4	0.7	0.7
Salmonella /l	<0.3	<0.3	<0.3	<0.3
St.aureus / 100 ml	13	0.1	10	0.1
Cryptoporidium / l	0.3			
Giardia / l	0.1			
Enteroviruses/200 l	0			

Table 9. *Water quality on 16 July (averages and geometric means are calculated over concentrations measured **during** exposure)*

		Thermotolerant coliforms/100 mL* (first series)				
Track	Before	First	Middle	Last	Average	Geomean
1	37	62	108	204	125	111
2	47	48	47	52	49	49
3	24	34	30	100	55	47
4	62	27	32	34	31	31

		Thermotolerant coliforms/100 mL* (second series)				
Track	Before	First	Middle	Last	Average	Geomean
1		79	48	68	65	64
2		91	51	60	67	65
3		24	54	42	40	38
4		36	58	38	44	43

*corrected for overall confirmation rate (96%)

		Escherichia coli/100 mL* (first series)				
Track	Before	First	Middle	Last	Average	Geomean
1	74	100	167	225	164	155
2	80	45	65	76	62	61
3	44	49	64	162	92	80
4	52	49	80	63	64	63

		Escherichia coli/100 mL* (second series)				
Track	Before	First	Middle	Last	Average	Geomean
1		122	83	96	100	99
2		158	85	58	100	92
3		51	60	59	57	57
4		55	74	64	64	64

*corrected for overall confirmation rate (100%)

		Faecal enterococci/100 mL* (first series)				
Track	Before	First	Middle	Last	Average	Geomean
1	112	146	204	408	253	230
2	112	108	96	154	119	117
3	52	36	66	40	47	46
4	58	53	95	118	89	84

		Faecal enterococci/100 mL* (second series)				
Track	Before	First	Middle	Last	Average	Geomean
1		218	136	220	191	187
2		220	132	116	156	150
3		29	80	74	61	56
4		76	168	104	116	110

*corrected for overall confirmation rate (100%)

Table 9 continued

F+ RNA phages/100 mL (first series)

Track	Before	First	Middle	Last	Average	Geomean
1	0.00	0.36	0.00	0.00	0.12	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.13	0.00	0.00	0.04	0.00
4	0.24	0.37	0.24	0.12	0.24	0.22

F+ RNA phages/100 mL (second series)

Track	Before	First	Middle	Last	Average	Geomean
1		0.24	0.12	0.00	0.12	0.01
2		0.12	0.35	0.12	0.20	0.17
3		0.36	0.12	0.00	0.16	0.02
4		0.48	0.24	0.35	0.36	0.34

Other parameters (first series)

Track no	1	2	3	4
Aeromonas /ml	204	102	74	69
Ps. aeruginosa /100 ml	<1	<1	<1	<1
Campylobacter /l	<0.3	<0.3	<0.3	<0.3
Salmonella /l	<0.3	<0.3	<0.3	<0.3
St.aureus / 100 ml	<1	<1	<1	1
Cryptoporidium / l	0.1			
Giardia / l	0.2			
Enteroviruses/200 l	0			

*Table 10a. Disease attack rates in bathers and non-bathers in the week after the trial day. Case definitions are comparable with those used in athletes study (21)**

Symptoms*	Bathers n/N (%)	Non-bathers n/N (%)	Attack rates in athletes study[21] Triathletes (swimmers)	Duathletes (non-swimmers)	AR duathletes vs. AR non-bathing children
Gastrointestinal	7/47 (15)	9/51 (18)	5.2	2.1	$p < 0.001$
Respiratory	12/35 (34)	9/36 (22)	8.4	4.0	$p < 0.001$
Skin/mucosal	4/39 (10)	3/38 (8)	3.1	1.5	$p < 0.05$

* complaint(s) present during at least **two** parts of a day (morning, noon, evening and night) among those without similar complaints in the two days before the trial day;
gastrointestinal: diarrhoea, nausea, vomiting or stomach ache,
respiratory: coughing, runny nose or sore throat,
skin/mucosal: itchy skin or skin rash, ear ache, itchy eye(s)
n numbers with complaints in week after trial day ; N numbers without complaints in the two days before trial day

Table 10b. Disease attack rates in bathers and non-bathers in the week after the trial day. Case definitions are more stringent, some of them have also been used in the athletes study[21]

Symptoms*	Bathers n/N (%)	Non-bathers n/N (%)	Attack rates in athletes study[21] Triathletes (swimmers)	Duathletes (non-swimmers)	AR duathletes vs. mean AR bathing+non-bathing children
Gastroenteritis (UK)	1/50 (2)	4/55 (7)	3.6	1.7	$p = 0.09$
Gastroenteritis (USA)	1/50 (2)	4/57 (7)	2.2	0.8	$p < 0.01$
Gastroenteritis (NL)	1/50 (2)	3/57 (5)	0.4	0.0	$p < 0.001$
Respiratory	8/41 (20)	9/49 (18)	-	-	
Respiratory (UK)	0/50 (0)	1/57 (2)	-	-	
Skin	2/50 (4)	0/54 (0)	-	-	
Ear	0/49 (0)	0/54 (0)	-	-	
Eye	0/50 (0)	0/55 (0)	-	-	

*(UK): gastroenteritis according to case definition that is comparable to the definition used in UK bathing water studies (16): vomiting; or diarrhoea (three or more loose stools in 24 hours) or nausea accompanied by a fever,
(USA): gastroenteritis according to case definition that is comparable to the definition used in USA bathing water studies (5): vomiting; or diarrhoea accompanied by a fever or resulting in bed rest; or nausea or stomach ache accompanied by a fever,
(NL): diarrhoea (two or more loose stools in 24 hours) accompanied by at least two of the symptoms nausea, vomiting, stomach ache or fever other complaint(s) being present during at least **two** parts of a day (morning, noon, evening and night)
Respiratory: any two of the complaints coughing, sneezing, runny nose or sore throat, each being present during at least **two** parts of a day (morning, noon, evening and night)
Respiratory (UK): At least one of the symptoms in each of the following categories: 1). fever; 2). headache and/or bodyaches and/or unusual fatigue and/or anorexia; 3). sore throat and/or runny nose and/or dry or productive cough (18).
Skin: itchy skin and skin rash, both being present during at least **two** parts of a day (morning, noon, evening and night) in the two days after the trial day
Ear: ear ache or discharge, each being present during at least **two** parts of a day (morning, noon, evening and night)
Eye: ache or inflammation of the eye, each being present during at least **two** parts of a day (morning, noon, evening and night)
n numbers with complaints in week after trial day; N numbers without complaints in the two days before trial day

Table 11. Weather conditions during the bathing season of 1993-1996. Values are mean (sd) unless stated otherwise*

Month	N days	Sun (%)	Rain (%)	Air temperature (° C)	Compliance with study criteria-I**		Compliance with study criteria-II***	
					N	%	N	%
May	68	32.6 (15.0)	39.4 (21.6)	16.9 (3.7)	6	8.8	9	13.2
June	120	39.2 (19.8)	32.1 (22.6)	19.4 (4.0)	40	33.3	51	42.5
July	124	42.9 (19.5)	33.6 (22.2)	22.6 (4.1)	56	45.2	67	54.0
August	124	42.2 (19.7)	35.1 (24.2)	21.9 (3.4)	51	41.1	67	54.0
September	60	26.7 (12.2)	49.7 (26.3)	17.8 (1.5)	6	10.0	8	13.3

* Expected proportions or temperature, estimated one day before actual day (more than one day (max 5 days) in 13 occasions). Data provided by the Laboratory of Air Research (RIVM) and measured by the Roal Dutch Meteorological Institute.

** Days on which following criteria were met: % sun ≥ 40%, % rain ≤ 20%, air temperature ≥ 19 °C.

*** Days on which following criteria were met: % sun ≥ 30%, % rain ≤ 30%, air temperature ≥ 19 °C.

Table 12a. Estimation of sample size needed for future studies (assuming alpha=5%, beta=10%) comparing bathers and non-bathers

	RR = 1.5			RR = 2.0			RR = 2.5			RR = 3.0		
p_0	N_0	N_1	Total	N_0	N_1	Total	N_0	N_1	Total	N_0	N_1	Total
1	8176	16353	*24529*	2521	5042	*7563*	1330	2661	*3991*	865	1730	*2595*
1.5	5417	10834	*16251*	1668	3336	*5004*	878	1757	*2635*	570	1141	*1711*
2	4037	8074	*12111*	1241	2482	*3723*	653	1306	*1959*	424	847	*1271*
2.5	3209	6418	*9627*	985	1970	*2955*	518	1035	*1553*	335	670	*1005*
3	2657	5314	*7971*	814	1629	*2443*	427	854	*1281*	276	552	*828*
3.5	2262	4525	*6787*	692	1385	*2077*	362	725	*1087*	234	468	*702*
4	1967	3934	*5901*	601	1202	*1803*	314	629	*943*	202	405	*607*
4.5	1737	3474	*5211*	530	1060	*1590*	276	553	*828*	178	356	*534*
5	1553	3106	*4695*	473	946	*1419*	246	493	*739*	158	316	*474*

RR: relative risk
p_0: expected attack rate in unexposed population
N_0: sample size of unexposed population
N_1: sample size of exposed population

ATTACK RATES (AR) (PER 100 CHILDREN) FOUND IN PREVIOUS STUDIES:

Gastroenteritis AR in present study
non-bathers *7.0*
bathers *2.0*
both combined *4.5*

Gastroenteritis AR in athletes study (21)
bathers *0.4 to 3.6 (dependent on case-definition)*
non-bathers *0.0 to 1.7 (dependent on case-definition)*

Highly credible gastroenteritis AR in Cabelli study (24)
bathers 0-10 years *2.4*
non-bathers 0-10 yrs *< 0.5*

Highly credible gastroenteritis AR in Cheung study (10)
bathers 0-10 years *4.1*
non-bathers 0-10 yrs *0.0*

Gastroenteritis AR in Fattal study (11)
bathers 0-10 years *22.1*
non-bathers 0-10 yrs *13.3*

Table 12b. Estimation of sample size needed for future studies (assuming alpha=5%, beta=10%) comparing bathers exposed to concentrations < or > 100/100 ml*

p_0	RR=2	RR=3	RR=4	RR=5
1.5	2183	743	416	281
2.5	1290	436	243	163
3.5	908	305	169	112
4.5	695	232	127	84

RR: relative risk
p_0: expected attack rate in unexposed population (bathers exposed to conc. therm. coliforms < 100/100 ml)
* numbers for each group

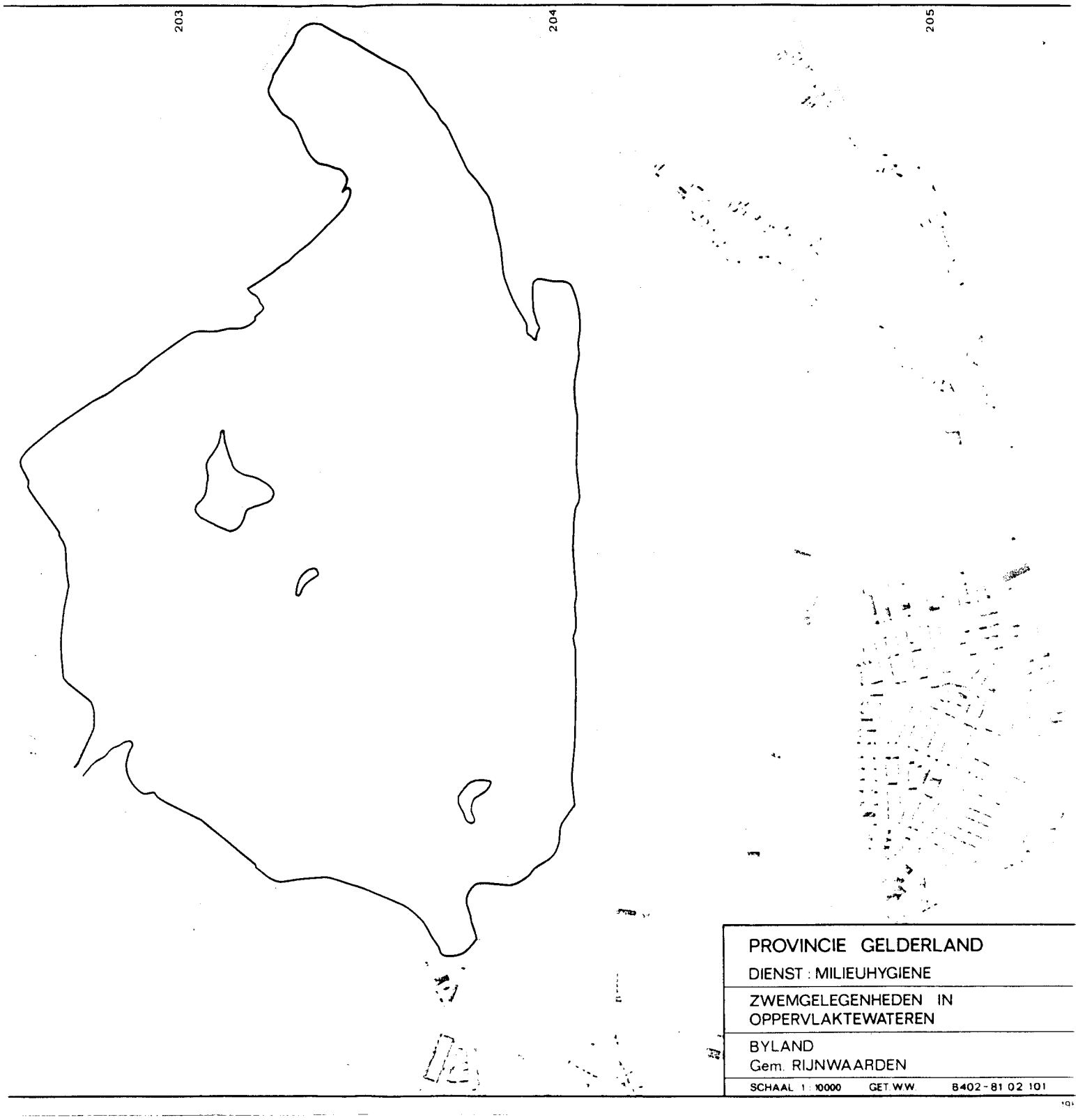


Figure 1. Fresh water study site of the pilot study

1) Pretest tracks, 11 June 1996

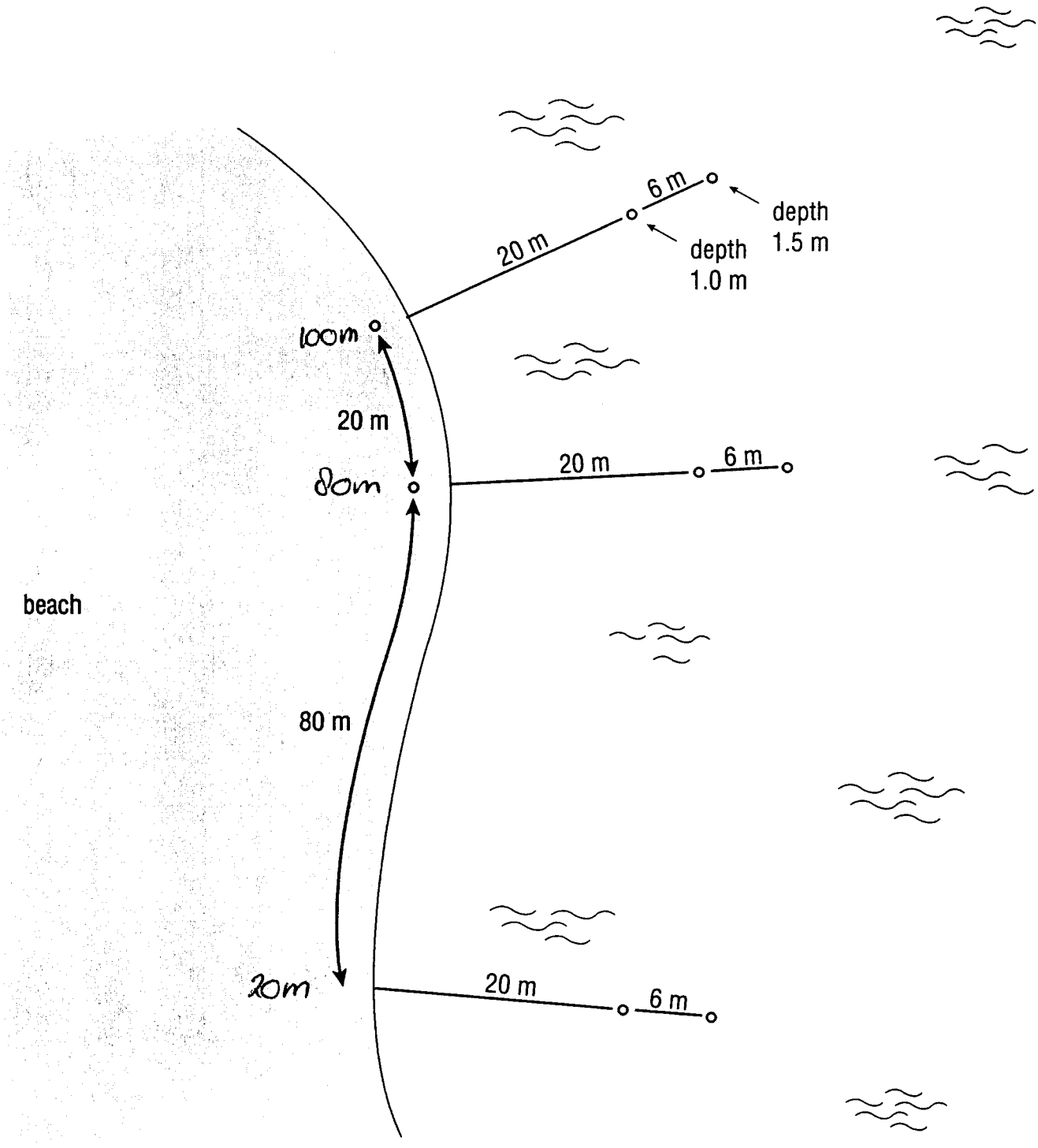
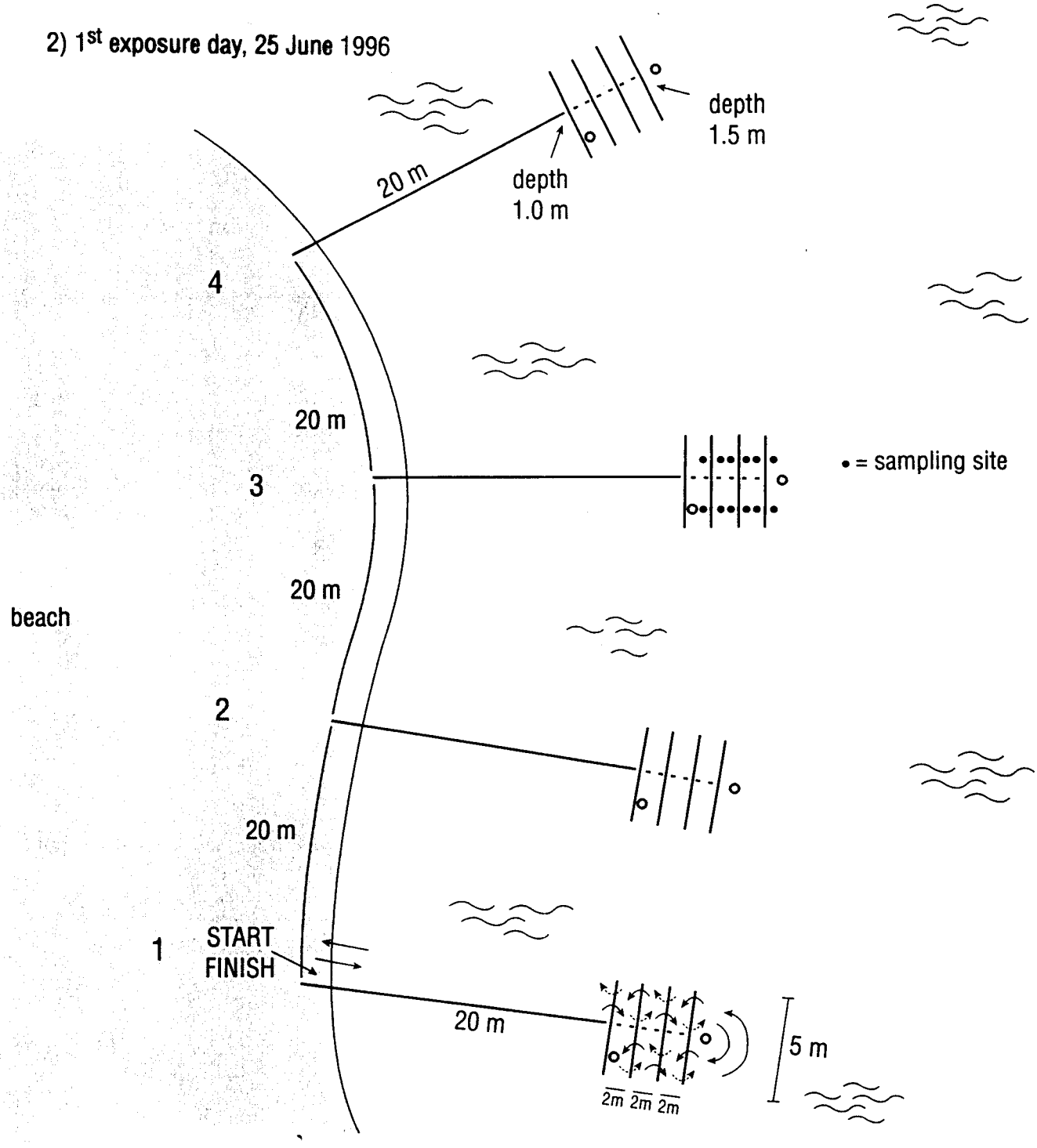


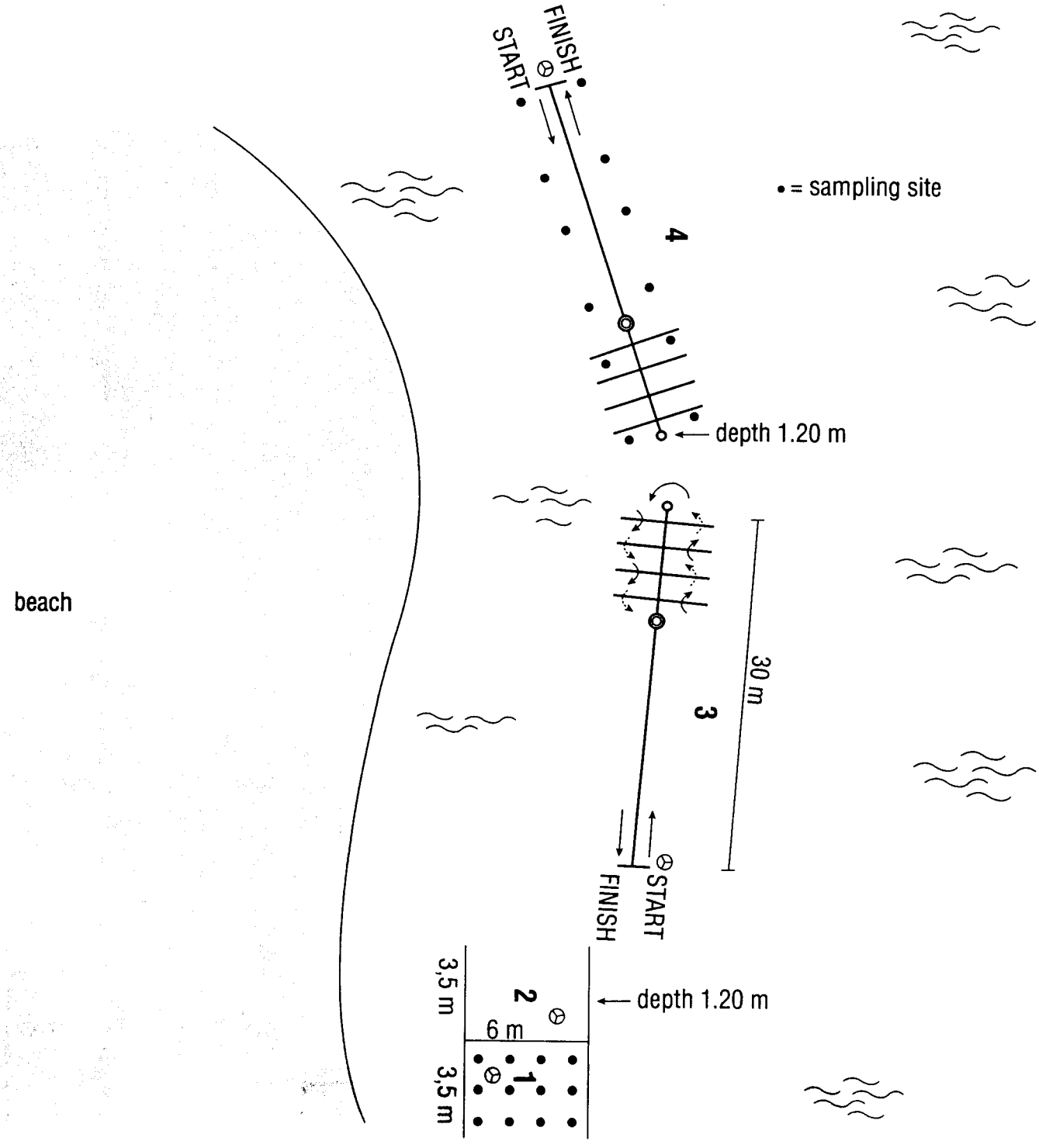
Figure 2a. Exposure setting of preparatory analyses



At each relay race track swimmers had to swim over the first line, swim under the second, swim over the third and under the fourth line.Then swim swim round the buoy and swim the same way back to the first line, touch the second buoy and return by the same course as they came. When the first swimmer returned on the shore, the second swimmer started with the race etc.

Figure 2b. Exposure setting of first trial day

3) 2nd exposure day, 16 July 1996



Swimmers at track 3 and 4 had to start in the water, swim approximately 20 metres pushing a ball ahead, stock the ball and swim over and under the 4 perpendicular lines, touch the buoy and return over and under the four lines again. Then pick up the ball and swim 20 metres back to the start/finish site. Then the next swimmer started. All swimmers had to finish the track twice.
At the hit ball track "swimmers" played hit ball for the same lenght of time as the swimming at track 3 and 4 took.

*Figure 2c. **Exposure setting of second trial day***

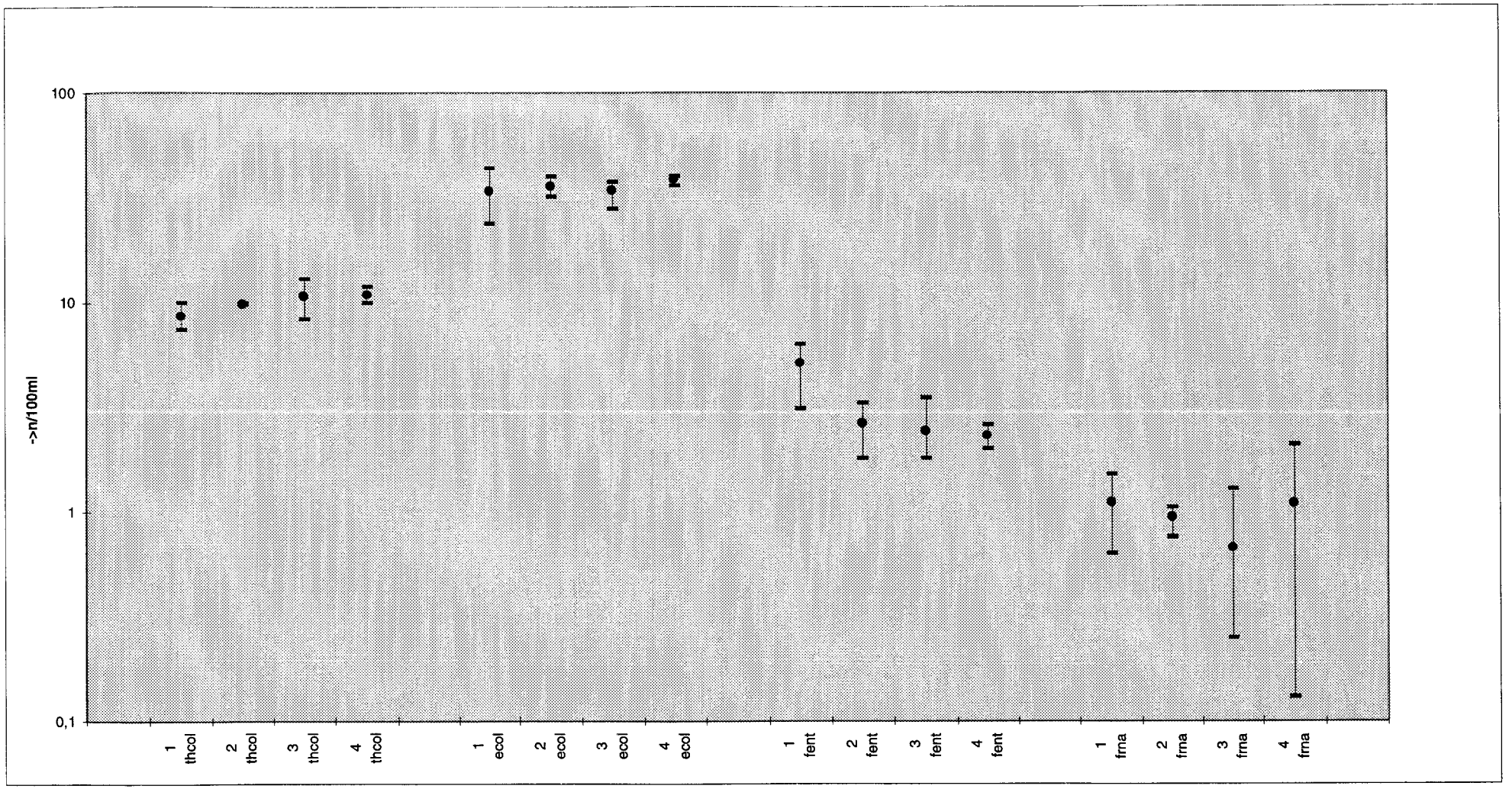


Figure 3a. Microbiological water quality at the Byland swimming tracks on 25 June 1996 (min, max and average value)
Thol=thermotolerant coliforms; ecol=E. coli; fent=faecal streptococci; frna=F+RNA phages

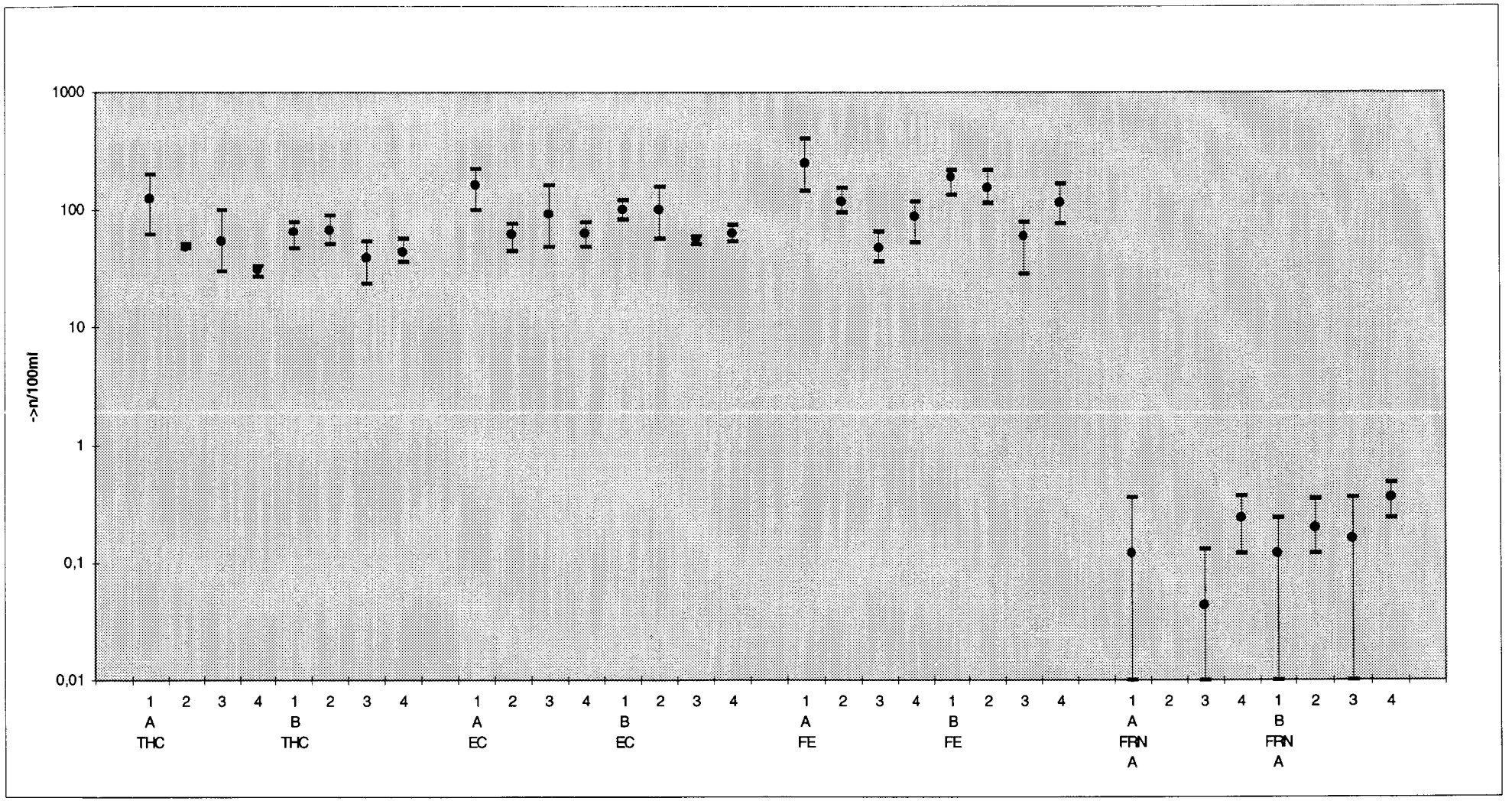


Figure 3b. Microbiological water quality at the Byland swimming tracks on 16 July 1996 (min, max and average value)
Thc=thermotolerant coliforms; ec=E. coli; fe=faecal streptococci; frna=F+RNA phages; A=first series; B=second series

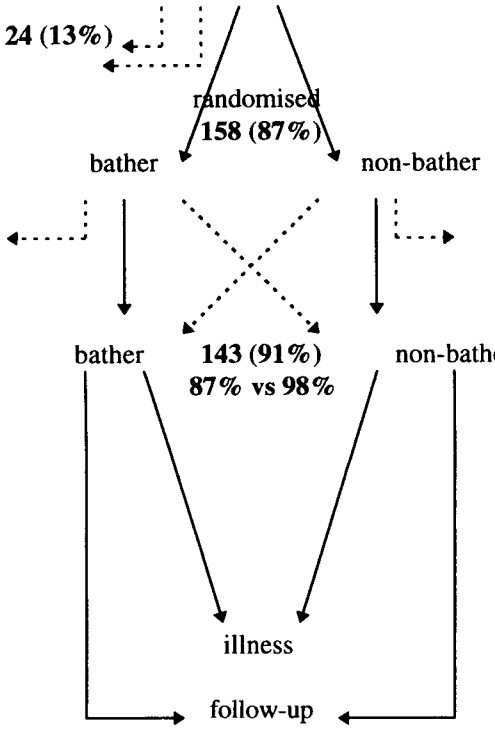


Figure 4. Response rates during the study