Identification of human fecal pollution sources in a coastal area: a case study at Oostende (Belgium)

Sylvie Seurinck, Martin Verdievel, Willy Verstraete and Steven D. Siciliano

ABSTRACT

From April to June 2001, a monitoring study at Oostende (Belgium) was conducted to obtain an insight into fecal pollution impairing water quality at this coastal area. Eight sampling sites were selected based on the historically low water quality at these sites compared to the remainder of the area. Indicator organisms such as fecal coliforms, *Escherichia coli* and fecal streptococci were monitored by plating. A real-time PCR assay for quantification of the human-specific HF183 *Bacteroides* 16S rRNA genetic marker was used to detect human fecal pollution at the sampling sites. Human fecal pollution was detected at all sampling sites. However, the frequency of detection ranged from 30–100% and the amount of human-specific *Bacteroides* markers recorded varied between the sampling sites. Concentrations of 10^7 human-specific *Bacteroides* markers per l to levels below the detection limit of the real-time PCR assay were recorded. Our results indicate that human fecal pollution is a re-occurring problem in certain areas. Of all the environmental parameters monitored during the study, only rainfall was strongly related to the detection of the indicator organisms and the human-specific *Bacteroides* marker.

Key words | bacterial indicators, *Bacteroides*, microbial source tracking, real-time PCR

Sylvie Seurinck
Willy Verstraete (corresponding author)
Laboratory of Microbial Ecology and Technology (LabMET),
Ghent University,
Coupure Links 653, B-9000 Ghent Belgium
Present address: Ghent University,
Faculty of Agricultural and Applied Biological Sciences, Laboratory of Microbial Ecology and Technology (LabMET),
Coupure Links 653, B-9000 Ghent,
Belgium
Tel: +32 9264 5976
Fax: +32 9264 6248
E-mail: *Willy.Verstraete@ugent.be*

Martin Verdievel
Flemish Environment Agency,
A. Van De Maelestraat 96, B-9320, Erembodegem,
Belgium

Steven D. Siciliano
Department of Soil Science,
51 Campus Drive, University of Saskatchewan,
Saskatoon Saskatchewan, S7N 5A8,
Canada

INTRODUCTION

Recreational waters are susceptible to a variety of sources of microbial pollution, which can contain pathogenic microorganisms that cause gastrointestinal, upper respiratory tract, ears, eyes, nasal cavity and skin infections (WHO 2003). These microorganisms may come from point source discharges, such as raw sewage, stormwater, combined sewer overflows, effluents from wastewater treatment plants and industrial sources. Non-point source discharges, such as agriculture, forestry, wildlife and urban run-off, can also impair water quality (Griffin *et al.* 2001). Discriminating between these different sources of fecal pollution in order to develop remediation strategies has been a perennial challenge for water quality managers.

Member states of the European Union are required to implement the Bathing Water Directive 76/160/EEC. The European Commission proposed to revise this 25-year-old directive in October 2002 and the revised directive is now under international consultation (Council of the European Communities 2000). This revised draft directive will replace enumeration of total and fecal coliforms with *Escherichia coli* and intestinal enterococci enumeration. In addition to enumeration activities, a sanitary inspection of the recreational site to identify pollution sources and their significance will need to be carried out. Ideally this will result in the prediction of water quality linked to management systems and improved protection of public health (WHO 2003).

A variety of molecular-based methods has been proposed to identify fecal sources in water, including ribotyping (Parveen *et al.* 1999; Carson *et al.* 2001), repetitive extragenic palindromic-PCR (Dombek *et al.* 2000; Seurinck *et al.* 2003) and specific toxin marker detection (Khatib *et al.* 2002). In this study, the real-time PCR assay for quantification of the human-specific HF183 *Bacteroides* 16S rRNA genetic marker (Bernhard & Field 2000; Seurinck *et al.* 2005) was

doi: 10.2166/wh.2006.069

used. This human-specific *Bacteroides* marker seems to be widely distributed within the USA (Field 2002; Boehm *et al.* 2003) and was already detected in river waters in New Zealand (Gilpin *et al.* 2003) and in human feces from Belgian (Europe) individuals (Seurinck *et al.* 2005). This real-time assay can be used to detect and quantify human fecal pollution in water environments (Seurinck *et al.* 2005). So far, the conventional PCR assay for detection of the human-specific *Bacteroides* marker has been applied in the field with variable success due to inhibition or limiting detection levels (Bernhard *et al.* 2003; Boehm *et al.* 2003; Gilpin *et al.* 2003). Given the higher sensitivity of the real-time PCR assay for the detection and quantification of the human-specific *Bacteroides* marker (Seurinck *et al.* 2005), we decided to apply this method *in situ* at a coastal area and compare it to more traditional estimates of fecal pollution based on the traditional indicator organisms.

The Belgian North Sea is an active seaside tourist area. In that respect, fecal pollution sources need to be identified and models are required to predict fecal pollution into the North Sea. The first step in the model development would be the determination of factors that are correlated with poor water quality. From April to June 2001, we conducted a monitoring study at the coastal area of Oostende (Belgium). The first objective was to determine the water quality and the level of human fecal pollution at this coastal area. Therefore fecal coliforms, *E. coli* and fecal streptococci and the human-specific *Bacteroides* marker were quantified. The second objective was to determine the environmental factors related to poor water quality in this coastal area. To accomplish these objectives, we partnered with the Flemish Environment Agency (FEA) and selected eight different sites in the coastal area of Oostende (Belgium) with historically low water quality based on previous monitoring studies conducted by the FEA.

METHODS AND MATERIALS

Study area

The study area is shown in Figure 1. Oostende has approximately 4,500 m of sandy beach coastline. The city's primary source of revenue is tourism; on a typical summer day there are often 1,500 tourists present (VMM 2000).

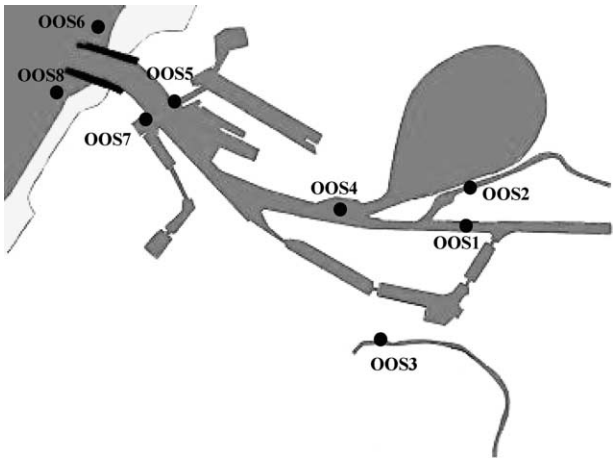

Figure 1 | The different sampling points (OOS1 to OOS8) at Oostende.

At the onset of this study, it was not known to what extent human sources impacted on water quality in Oostende. The sampling points were chosen based on their historically poor water quality. The canal Gent-Oostende (OOS1) receives effluent discharges from the wastewater treatment plant (WWTP) of Oostende (650,000 inhabitants equivalents), OOS2 lies behind the discharge point of a combined sewer overflow (CSO) on the river Noordede. At OOS3 two streams, namely the Dode Kreek and the Gauwelozekreek flow together. The Dode Kreek recieves intermittent wastewater discharges of three CSOs. The Gauwelozekreek recieves discharges of an emergency overflow of the WWTP in Oostende. The Voorhaven (OOS4) receives intermittent discharges of a stormwater overflow. The Visserijdok (OOS5) is a dock next to the fish market on the wharf. The Montgomerydok (OOS7) is a marina. OOS6 and OOS8 are two beach water sampling points, located on either side of the channel leading to the dock area of the harbour.

Sample collection

Samples were taken according to the procedure of the Flemish Environment Agency VMM/AMO/GP2/3.104. In brief, water samples were collected at the water surface. All samples were collected at low tide, except on 20 April 2001, 18 June 2001 and 20 June 2001. Samples (2 liters) were collected in sterile recipients, stored at 4°C and analyzed within 24 h after collection. The indicator organisms were enumerated twice a week, whereas the concentration of

human-specific *Bacteroides* marker was determined once a week. Weather data (wind direction, wind speed, rainfall and sunlight intensity) were obtained from the meteorological survey of Zeebrugge. Conductivity, salinity, pH, O_2 concentration in the water and water temperature were recorded in the field with hand-held meters (VWR Internationaal, Leuven, Belgium), according to the procedures of the Flemish Environment Agency VMM/AMO/GBM/2.002-2.003-2.004.

Microbial enumeration

Microbial enumeration was carried out by Chemiphar b.v.b.a (Brugge, Belgium). Fecal coliforms (FC) and *Escherichia coli* were quantified by ISO 9308-1 (membrane filtration) with mFC agar. Isolates that produced indole from tryptophan were confirmed as being *E. coli*. Fecal streptococci (FS) were enumerated according to ISO 7899-2 (membrane filtration) with KF Streptococcus agar. Isolates were confirmed as being FS by spreading on KAA medium and testing for katalase production.

DNA extraction and purification

Water samples (1 liter) were filtered through a 0.22 μm filter (Type GS, Millipore, Brussels, Belgium) prior to DNA extraction. Total DNA was extracted directly from the filters by a method based on the protocols described previously (el Fantroussi *et al.* 1999; Boon *et al.* 2000). This protocol was modified as follows. The filter was added to a 10 ml polypropylene round-bottom tube (Greiner bio-one, Kremsmuenster, Austria). To this, 4 ml of 10 mM Tris-HCl (pH 9) and 3 g of beads (0.10 – 0.11 mm diameter) (B. Braun Biotech International, Melsungen, Germany) were added. The mixture was beaten three times for 90 s using a bead beater (B. Braun Biotech International) at 2,000 rpm. Then, 160 μl of 50 mg of lysozyme per ml in 10 mM Tris-HCl (pH 9) was added, followed by incubation of the samples for 15 min at 28°C on a rotary shaker. Subsequently, 300 μl of 20% sodium dodecyl sulfate was added and samples were slowly mixed for 5 min. The supernatant was collected after centrifugation at 4,800 *g* for 15 min at 4°C. A chloroform – isoamyl alcohol (24:1) purification was done, followed by centrifugation at 4,800 *g* for 15 min at 4°C. The aqueous phase was transferred to a new tube and 0.8 volume of isopropanol was added for overnight precipitation. The pellet (crude extract) was obtained by centrifugation at 4,800 *g* for 25 min and was resuspended in 250 μl of DNase and RNase free filter sterile water (Sigma-Aldrich Chemie, Steinheim, Germany). A 100 μl aliquot of the crude extract was purified using Wizard PCR preps (Promega, Madison, WI) and the purified DNA was finally recovered in 50 μl of DNase and RNase free filter sterile water (Sigma-Aldrich Chemie).

Real-time PCR

The real-time PCR protocol based on Sybr® Green I detection, described in Seurinck *et al.* (2005) was used to quantify the human-specific HF183 *Bacteroides* 16S rRNA genetic marker in water samples. In brief, the human-specific HF183 forward primer developed by Bernhard & Field (2000) and the reverse primer developed by Seurinck *et al.* (2005) were used. PCR was carried out in a volume of 25 μl reaction mixture, which contained 2 μl of DNA. Amplification was performed by using buffers supplied with the qPCRTM core kit for Sybr® Green I (Eurogentec, Liège, Belgium). The reaction mixture contained 0.25 μM of each primer, 200 μM of each deoxynucleoside triphosphate with dUTP, 2.0 mM $MgCl_2$, 10 μl of real-time PCR 10X Buffer ($MgCl_2$-free), 2.5 U of Hot GoldStar DNA Polymerase, 3 μl Sybr® Green I 1/10, and DNase- and RNase-free filter sterile water (Sigma-Aldrich Chemie) to a final volume of 100 μl. The reactions were performed in MicroAmp Optical 96-well reaction plates with optical caps (Applied Biosystems, Foster City, CA). The PCR temperature program was initiated for 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 30 s at 95°C, 1 min at 53°C and 1 min at 60°C. Subsequently, during the melting curve analysis, the temperature increased from 60°C to 95°C at approximately 0.4°C per min. The template DNA in the reaction mixtures was amplified and monitored with an ABI Prism SDS 7,000 instrument (Applied Biosystems). Within each real-time PCR run all samples were analyzed in triplicate. A peak at melting temperature 78.4 ± 0.2°C was indicative for a positive and correct amplification as verified by sequencing of a human real-time PCR product (Seurinck *et al.* 2005). Concentrations of the human-specific *Bacteroides* marker below the upper limit of quantification (LOQ)

are considered as being detected, but not quantified. Concentrations of the human-specific *Bacteroides* marker above the upper limit LOQ are considered as being detected and quantified. The upper LOQ was defined as LOQ + SD. As determined previously (Seurinck *et al.* 2005), the $LOQ_{freshwater}$ of the real-time PCR assay is $(4.7 \pm 0.3) \times 10^5$ markers/l freshwater, resulting in the upper $LOQ_{freshwater}$ of 5.0×10^5 markers/l freshwater. The $LOQ_{seawater}$ was determined in the same way as the $LOQ_{freshwater}$. The $LOQ_{seawater}$ is $(2.6 \pm 0.7) \times 10^5$ markers/l seawater, resulting in the upper $LOQ_{seawater}$ of 3.3×10^5 markers/l seawater.

Statistical analyses

Statistical analyses were performed using SPSS version 11.5 (SPSS Inc., Chicago, IL). The Kruskal–Wallis test was used to determine if the sampling date or sampling site had an effect on the median values of the indicator organisms. The Steel–Dwass–Critchlow–Fligner *post hoc* tests were performed to determine which sampling sites differed in median of indicator organisms (Hollander & Wolfe 1999).

The chi square test of independence was used to assess which environmental factors were related to the detection of the indicator organisms and the human-specific *Bacteroides* marker in the water samples, and the non-compliance of the water samples with the *E. coli* standard. The hypothesis was tested at the 0.05 level of significance.

RESULTS

Fecal coliforms (FC), *E. coli*, fecal streptococci (FS) and the human-specific *Bacteroides* marker

Figure 2 presents the numerical data of FC, *E. coli*, FS and the human-specific *Bacteroides* marker for all sampling points. The canal Gent-Oostende (OOS1), the Dode Kreek and Gauwelozekreek (OOS3), the Voorhaven (OOS4), and the Montgommerydok (OOS7) contained high levels of the indicator bacteria. The European *E. coli* standard (5×10^2/100 ml) suggested in the revised draft Bathing Water Directive (Council of the European Communities 2000) was exceeded most of the time at these sites. The human-specific *Bacteroides* marker was detected in almost all water samples from these sites, which indicates that they are regularly contaminated with human fecal pollution. The river Noordede (OOS2), the Visserijdok (OOS5) and the beach water at OOS6 and OOS8 were only lightly contaminated based on the European *E. coli* standard. At these sampling sites the human-specific *Bacteroides* marker was less frequently detected and in lower amounts, except at OOS2 where high concentrations of 10^7 human-specific *Bacteroides* marker per l were recorded at the beginning of the sampling survey and at the end.

Figure 3 presents the median values of FC, *E. coli*, FS and the human-specific *Bacteroides* marker for each sampling point. The median values of all indicator organisms differed with sampling site according to the Kruskal–Wallis test ($P < 0.05$). *Post hoc* analysis showed that the median FC values were not significantly different with sampling site between OOS1 and OOS4, OOS1 and OOS7, OOS2 and OOS5, OOS2 and OOS8, OOS4 and OOS7, OOS5 and OOS6, OOS5 and OOS8, and OOS6 and OOS7. Almost identical results were observed for the median *E. coli* values. However, the median values between OOS1 and OOS4, and OOS6 and OOS8 were also significantly different, whereas the median values of OOS7 and OOS8 were not significantly different. The median FS values were only significantly different between OOS1 and OOS3, OOS2 and OOS6, OOS3 and OOS5, OOS3 and OOS6, OOS3 and OOS8, OOS5 and OOS7, OOS6 and OOS7, and OOS7 and OOS8. The highest median values of FC and EC were found for the Dode Kreek and the Gauwelozekreek (OOS3). Only the median EC value of the river Noordede (OOS2), the Visserijdok (OOS5) and the beach water (OOS6, OOS8) did not exceed the European *E. coli* standard.

Only the median FS values differed with sampling date according to the Kruskal–Wallis test ($P < 0.05$), which implies that there were no specific sampling days with overall poor water quality based on all the indicator organisms. Therefore, no *post hoc* tests were performed.

The human-specific *Bacteroides* marker was detected at all sampling sites. However, the frequency of detection ranged from 30–100%. The amount of human-specific *Bacteroides* markers varied between the sampling sites.

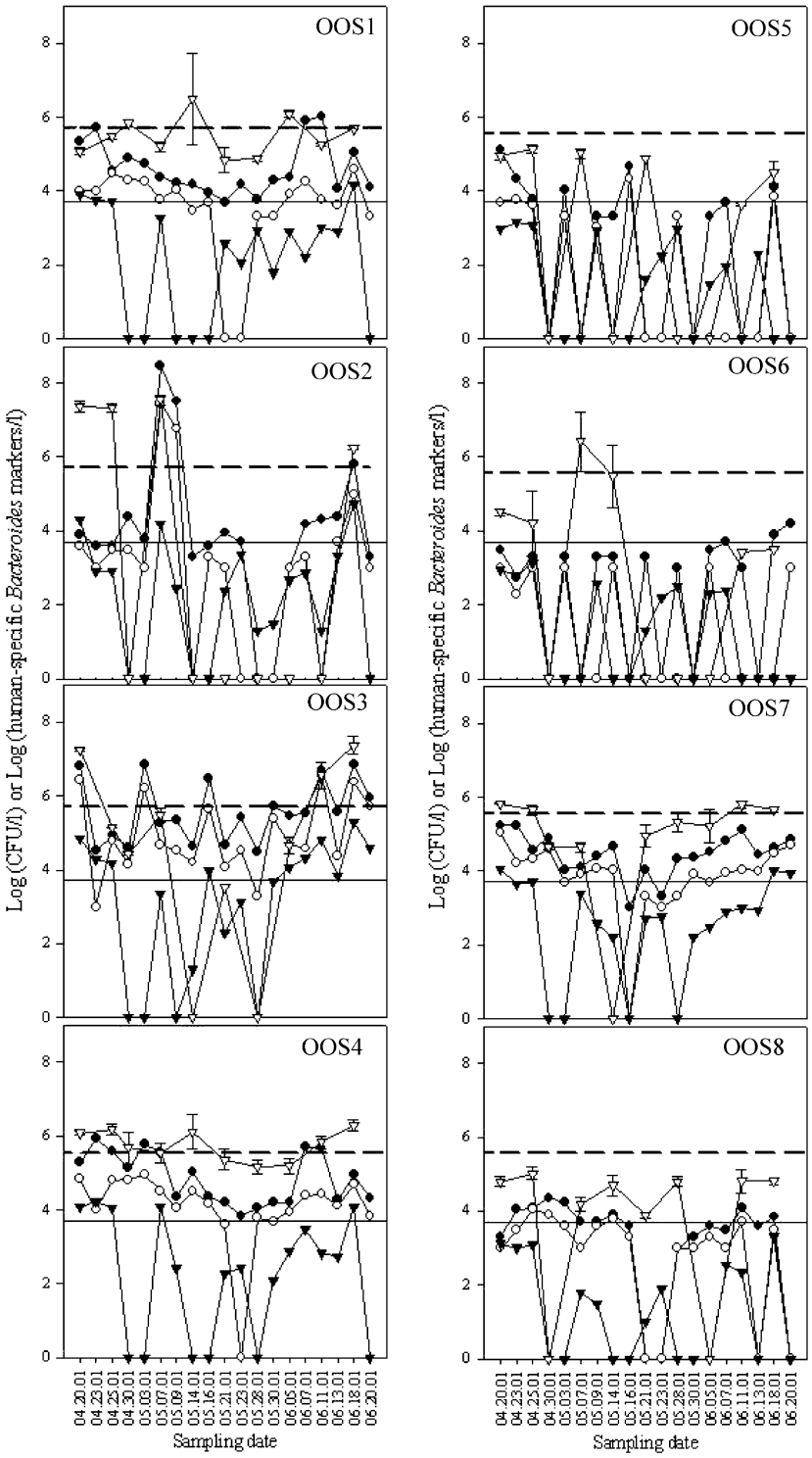

Figure 2 | Amount of fecal coliforms (●), *E. coli* (○), fecal streptococci (▼) and human-specific *Bacteroides* marker (▽) detected at all sampling points. The European standard for *E. coli* numbers (5×10^2/100 ml) is indicated by the solid black line. For OOS1, OOS2, and OOS3 the upper $LOQ_{freshwater}$ (5.0×10^5 markers/l freshwater) of the real-time PCR assay for the human-specific *Bacteroides* marker is represented by the dashed black line. For OOS4, OOS5, OOS6, OOS7 and OOS8 the upper $LOQ_{seawater}$ (3.3×10^5 markers/l seawater) of the real-time PCR assay for the human-specific *Bacteroides* marker is represented by the dashed black line.

Concentrations of 10^7 human-specific *Bacteroides* markers per l to levels below the detection limit of the real-time PCR assay were recorded.

Effect of environmental factors on water quality at Oostende

Rainfall (24 h prior to sampling, and on sampling day), water temperatures below 10°C and a north-west wind direction were the environmental factors to be significantly associated with the detection of the human-specific *Bacteroides* marker with a chi squared probability of $P < 0.05$ (Figure 4), whereas wind speed, oxygen levels, light intensity, salinity, conductivity and pH of the water were not associated with the detection of the human-specific *Bacteroides* marker (data not shown).

Rainfall was also significantly associated with the detection of the indicator organisms FC, *E. coli* and FS but was not linked to exceeding the European *E. coli* norm. North-west wind direction and water temperatures below 10°C were only significantly associated with the detection of FC and FS, respectively.

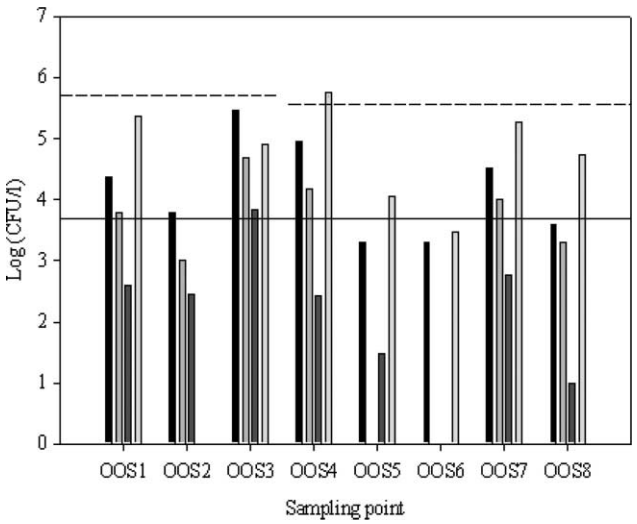

Figure 3 | The median values of fecal coliforms (■), *E. coli* (▩), fecal streptococci (■) and human-specific *Bacteroides* marker (□) for each sampling point over the course of the survey. The European standard for *E. coli* numbers (5×10^2/100 ml) is indicated by the solid black line. For OOS1, OOS2, and OOS3 the upper $LOQ_{freshwater}$ (5.0×10^5 markers/l freshwater) and for OOS4, OOS5, OOS6, OOS7, and OOS8 the upper $LOQ_{seawater}$ (3.3×10^5 markers/l seawater) of the real-time PCR assay for the human-specific *Bacteroides* marker is represented by the dashed black line.

DISCUSSION

We were able to identify the sites impacted with human fecal pollution at Oostende. Human fecal pollution was detected at all sampling sites. However, the frequency of detection and the amount of human-specific *Bacteroides* markers recorded varied between the sampling sites. The highest frequency of detection (100%) was found at the canal Gent-Oostende (OOS1) and the Voorhaven (OOS4). The highest amounts of human-specific *Bacteroides* marker (10^7 human-specific *Bacteroides* marker per l) were found at the Dode Kreek and Gauwelozekreek (OOS3) and the river Noordede (OOS2).

The canal Gent-Oostende (OOS1), the Dode Kreek and Gauwelozekreek(OOS3), the Voorhaven (OOS4) and the Montgommerydok (OOS7) were highly polluted since the European *E. coli* standard was exceeded most of the time. The human-specific *Bacteroides* marker was detected in almost all water samples from these sites, which indicates that they are regularly contaminated with human fecal matter. At these sites high concentrations of 10^6 human-specific *Bacteroides* markers per l were regularly found. Both the Dode Kreek and Gauwelozekreek (OOS3), and the Voorhaven (OOS4), receive discharges of overflows. At the Voorhaven (OOS4) dredging activities took place from 25 April to 14 May. Grimes (1980) recorded higher levels of indicator organisms during dredging activities in the Mississipi River due to resuspension of the river sediment. It could be that the high levels in the Voorhaven (OOS4) during 25 April to 14 May are not caused only by overflow discharges but also by the dredging activities. The canal Gent-Oostende (OOS1) receives the effluent of the WWTP of Oostende. The Montgomerydok (OOS7) does not receive discharges from overflows: at this marina, the human fecal pollution could originate from fecal waste discharges from chemical boat toilets. The river Noordede (OOS2), the Visserijdok (OOS5) and the beach water at OOS6 and OOS8 were only lightly polluted based on the European *E. coli* standard. At these sampling sites the human-specific *Bacteroides* marker was less frequently detected and in lower amounts, except at the river Noordede (OOS2) where high concentrations of 10^7 human-specific *Bacteroides* marker per l were recorded at the beginning of the sampling survey and at the end.

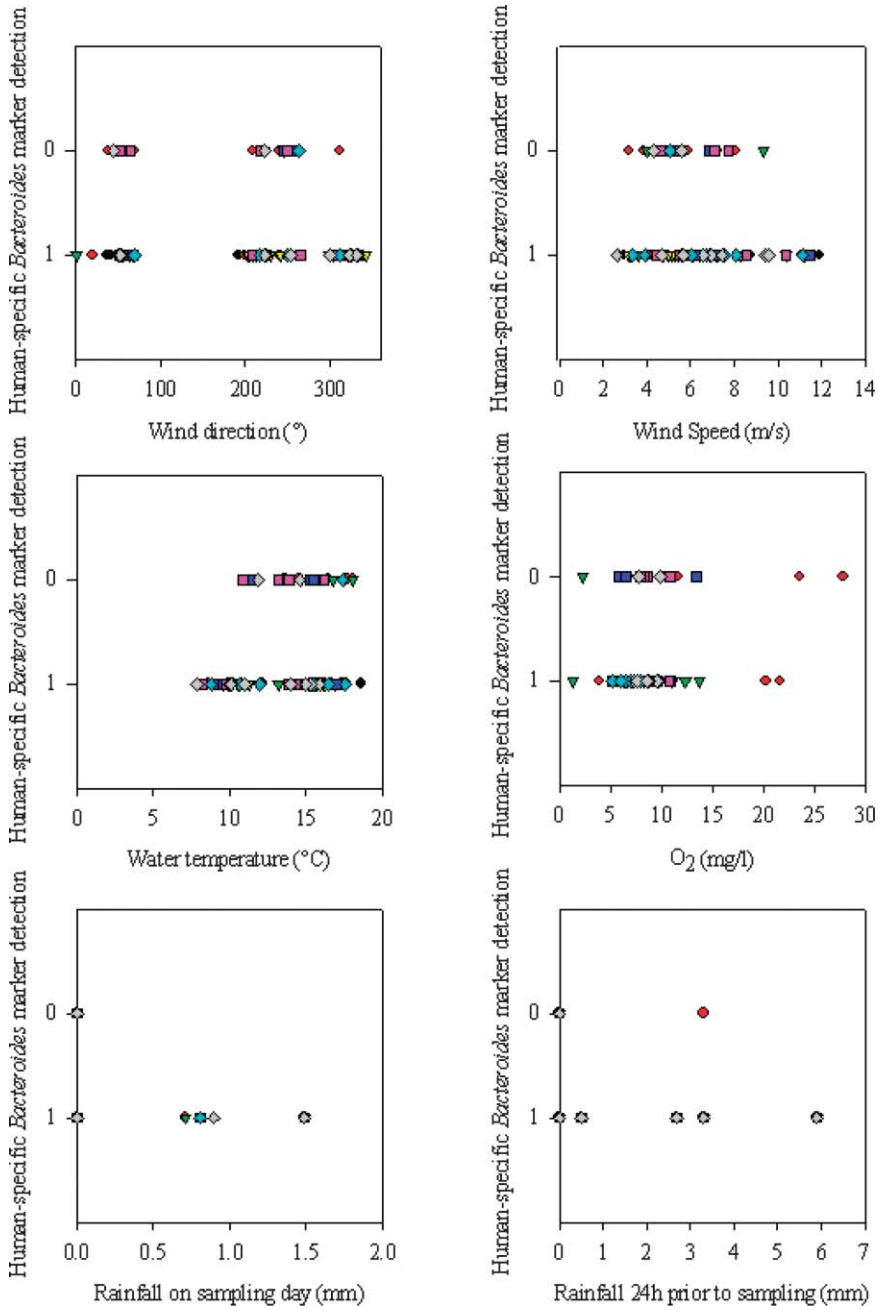

Figure 4 | Human-specific *Bacteroides* marker detection (0: not detected; 1: detected) as a function of different environmental factors (wind direction, wind speed, water temperature, O_2, rainfall on sampling day, and rainfall 24 h prior to sampling) for OOS1 (●), OOS2 (●), OOS3 (▼), OOS4 (▽), OOS5 (■), OOS6 (■), OOS7 (◆), and OOS8 (◇) over the course of the survey.

Since discharge of the human-specific *Bacteroides* marker is always accompanied by indicator organisms, it can be assumed that the detection of the human-specific *Bacteroides* marker in the absence of indicator organisms means that the human-specific *Bacteroides* marker persists longer in the water than the indicator organisms. This could be expected since we are comparing two different assays: only the indicator organisms which are still culturable are detected by means of cultivation whereas the real-time PCR also detects the human-specific

Bacteroides marker in dead cells. This was observed for the lightly polluted Visserijdok (OOS4) and for the beach water at OOS6. This longer persistence of the human-specific *Bacteroides* marker implies that recent and old human fecal pollution cannot be distinguished. However, the longer persistence of the human-specific *Bacteroides* marker has the benefit that human fecal pollution will be detected for a long period after the discharge event, whereas indicator organisms will no longer be culturable or will have died off.

At all sampling sites, except the canal Gent-Oostende (OOS1) and the Voorhaven (OOS4), there were indications that animal fecal pollution was also present, since high levels of indicator organisms were present whereas no human-specific *Bacteroides* marker could be detected. The use of animal-specific markers should elucidate this in the near future. To our knowledge, only ruminant-specific (Bernhard & Field 2000; Khatib *et al.* 2002) and pig-specific (Khatib *et al.* 2003) markers exist so far.

Despite the low number of rainy days, we observed that rainfall was not related to non-compliance with the European *E. coli* norm, but both 24 h rainfall prior to sampling and rainfall during sampling were related to the detection of the indicator organisms and the human-specific *Bacteroides* marker. Various studies (Atherholt *et al.* 1998; Curriero *et al.* 2001; Morrison *et al.* 2003) showed that rainfall events are the major cause of poor water quality, since raw sewage is discharged by overflows, and the rainfall can cause a resuspension of sediment that can act as a sink for indicator organisms (Crabill *et al.* 1999). Water temperatures below 10°C were shown to be significantly associated with the detection of the human-specific *Bacteroides* marker and FS. Surprisingly, we could not conclude that light intensity, pH, O_2 and salinity were clearly correlated with the presence of fecal pollution in the water environment. Light intensity, O_2 and salinity influence the survival of microorganisms in aquatic environments (Mezrioui *et al.* 1995; Sinton *et al.* 1999; Muela *et al.* 2000; Wait & Sobsey 2001). The environmental factors, wind direction and wind speed influence the transport of the bacteria (Canale *et al.* 1993). Only the north-west wind direction factor seemed to be significantly associated with the detection of the human-specific *Bacteroides* marker and FC. Should a model be designed to predict water quality at Oostende, the rainfall factor must be included in the model. The results of the other factors should be interpreted with care since this study was a short term study.

CONCLUSION

We were able to determine the sampling points with high levels of human fecal pollution, namely the canal Gent-Oostende, the Dode Kreek and the Gauwelozekreek, the Voorhaven and the Montgommerydok by means of a new methodology based on the human-specific *Bacteroides* marker. There was an indication of animal fecal pollution, especially in the river Noordede since fecal indicator organisms were detected for a prolonged period without the detection of the human-specific *Bacteroides* marker. The detection of indicator organisms and the human-specific *Bacteroides* marker was strongly related to rainfall for this coastal area. Since the sites contaminated with human fecal matter are now located, future monitoring studies in this coastal area should include water samples at intermittent sampling points between these polluted sites, downstream as well as upstream, in order to situate the geographical location of the fecal pollution sources.

ACKNOWLEDGEMENTS

This research was supported by the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT-GRANT no 001386). We are indebted to the Flemisch Environment Agency, including Gaby Verhaegen and Wim Van-den-burg-Zethoven, for their cooperation and assistance with water sampling and transportation. We thank Ellen Deschepper for assistance with statistical analysis and Wim De Windt, Nico Boon, Tom Defoirdt and Roeland Grommen for critically reading the manuscript.

REFERENCES

Atherholt, T. B., LeChevallier, M. W., Norton, W. D. & Rosen, J. S. 1998 Effect of rainfall on *Giardia* and crypto. *J. AWWA* **90**, 66–80.

Bernhard, A. E. & Field, K. G. 2000 A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. *Appl. Environ. Microbiol.* **66**, 4571–4574.

Bernhard, A. E., Goyard, T., Simonich, M. T. & Field, K. G. 2003 Application of a rapid method for identifying fecal pollution sources in a multi-use estuary. *Wat. Res.* **37**, 909–913.

Boehm, A. B., Fuhrman, J. A., Mrse, R. D. & Grant, S. B. 2003 Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California. *Environ. Sci. Technol.* **37**, 673–680.

Boon, N., Goris, J., De Vos, P., Verstraete, W. & Top, E. M. 2000 Bioaugmentation of activated sludge by an indigenous 3-chloroaniline-degrading *Comamonas testosteroni* strain, I2gfp. *Appl. Environ. Microbiol.* **66**, 2906–2913.

Canale, R. P., Auer, M. T., Owens, E. M., Heidtke, T. M. & Effler, S. W. 1993 Modeling fecal coliform bacteria –II– Model development and application. *Wat. Res.* **27**, 703–714.

Carson, C. A., Shear, B. L., Ellersieck, M. R. & Asfaw, A. 2001 Identification of fecal *Escherichia coli* from humans and animals by ribotyping. *Appl. Environ. Microbiol.* **67**, 1503–1507.

Council of the European Communities (CEC) 2000 *Proposal of the European Parliament and of the Council Concerning the Quality of Bathing Waters*, COM (2002)581 final 2002/0254 (COD), 24.10.2002.

Crabill, C., Donald, R., Snelling, J., Foust, R. & Southam, G. 1999 The impact of sediment fecal coliform reservoirs on seasonal water quality in Oak Creek, Arizona. *Wat. Res.* **33**, 2163–2171.

Curriero, F. C., Patz, J. A., Rose, J. B. & Lele, S. 2001 The association between extreme precipitation and waterborne disease outbreaks in the United States, 1948–1994. *Am. J. Public Health* **91**, 1194–1199.

Dombek, P. E., Johnson, L. K., Zimmerley, S. T. & Sadowsky, M. J. 2000 Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. *Appl. Environ. Microbiol.* **66**, 2572–2577.

el Fantroussi, S., Verschuere, L., Verstraete, W. & Top, E. M. 1999 Effect of phenylurea herbicides on soil microbial communities estimated by analysis of 16S rRNA gene fingerprints and community-level physiological profiles. *Appl. Environ. Microbiol.* **65**, 982–988.

Field, K. G. 2002 Fecal source tracking with *Bacteroides*. In: *Proceedings of Microbial Source Tracking Workshop, US EPA, Irving, CA*, (online: http://www.sccwrp.org/tools/workshops/source_tracking_agenda.html).

Gilpin, B., James, T., Nourozi, F., Saunders, D., Scholes, P. & Savill, M. 2003 The use of chemical and molecular microbial indicators for faecal source identification. *Wat. Sci. Technol.* **47**(3), 39–43.

Griffin, D. W., Lipp, E. K., McLaughlin, M. R. & Rose, J. B. 2001 Marine recreation and public health microbiology: quest for the ideal indicator. *BioScience* **51**, 817–825.

Grimes, J. D. 1980 Bacteriological water quality effects of hydraulically dredging contaminated upper Mississippi river bottom sediment. *Appl. Environ. Microbiol.* **39**, 782–789.

Hollander, M. & Wolfe, A. D. 1999 *Nonparametric Statistical Methods*. John Wiley & Sons, New York.

Khatib, L. A., Tsai, Y. L. & Olson, B. H. 2002 A biomarker for the identification of cattle fecal pollution in water using the LTIIa toxin gene from enterotoxigenic *Escherichia coli*. *Appl. Microbiol. Biotechnol.* **59**, 97–104.

Khatib, L. A., Tsai, Y. L. & Olson, B. H. 2003 A biomarker for the identification of swine fecal pollution in water using the STII toxin gene from enterotoxigenic *Escherichia coli*. *Appl. Microbiol. Biotechnol.* **63**, 231–238.

Mezrioui, N., Baleux, B. & Troussellier, M. 1995 A microcosm study of the survival of *Escherichia coli* and *Salmonella typhimurium* in brackish water. *Wat. Res.* **29**, 459–465.

Morrison, A. M., Coughlin, K., Shine, J. P., Coull, B. A. & Rex, A. C. 2003 Receiver operating characteristic curve analysis of beach water quality indicator variables. *Appl. Environ. Microbiol.* **69**, 6405–6411.

Muela, A., Garcia-Bringas, J. M., Arana, I. & Barcina, I. 2000 The effect of simulated solar radiation on *Escherichia coli*: the relative roles of UV-B, UV-A and photosynthetically active radiation. *Microbial Ecol.* **39**, 65–71.

Parveen, S., Portier, K. M., Robinson, K., Edmiston, L. & Tamplin, M. L. 1999 Discriminant analysis of ribotype profiles of *Escherichia coli* for differentiating human and nonhuman sources of fecal pollution. *Appl. Environ. Microbiol.* **65**, 3142–3147.

Seurinck, S., Verstraete, W. & Siciliano, S. 2003 The use of 16S-23S rRNA intergenic spacer region-PCR and repetitive extragenic palindromic-PCR analyses of *Escherichia coli* isolates to identify non-point fecal sources. *Appl. Environ. Microbiol.* **69**, 4942–4950.

Seurinck, S., Defoirdt, T., Verstraete, W. & Siciliano, S. 2005 Detection and quantification of the human-specific HF183 *Bacteroides* 16S rRNA genetic marker with real-time PCR for assessment of human fecal pollution in freshwater. *Environ. Microbiol.* **7**(2), 249–259.

Sinton, L. W., Finlay, R. K. & Lynch, P. A. 1999 Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. *Appl. Environ. Microbiol.* **65**, 3605–3613.

VMM 2000 *Trial for the Revised Bathing Water Directive Bathing Season 2000 Flanders*. Vlaamse Milieumaatschappij, Brussels, Belgium.

Wait, D. A. & Sobsey, M. D. 2001 Comparative survival of enteric viruses and bacteria in Atlantic Ocean Seawater. *Water Sci. Technol.* **43**(12), 139–142.

WHO 2003 *Guidelines for Safe Recreational-water Environments, Coastal and Fresh-waters*, Vol. 1. World Health Organization, Geneva, Switzerland.