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The effect of the time of sampling on the compliance of bathing water in NW England with the EU Directive on bathing water quality

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Abstract. The ability of Morecambe's three designated bathing waters to pass the EU Directive on Bathing Water Quality depends on the time of day when the sample is taken, the indicator organism tested for and whether the test uses the most strict (Guideline) or the least strict (Imperative) criteria. Morning and afternoon sampling for faecal coliforms, faecal streptococci and the pathogen *Campylobacter* was carried out monthly over the 1996 and 1997 bathing seasons. In the afternoons average faecal coliforms declined by 77% in 1996 and 87 % in 1997 compared with the mornings, faecal streptococci by 79% and 83 % and campylobacters by 66 % and 86 %. This decline in bacterial numbers between morning and afternoon was related to variations in water temperature and levels of ultraviolet radiation. All three bathing waters failed the Guideline criteria of the EU Directive on Bathing Water Quality. Using the Imperative criteria, no bathing waters passed in the mornings of either year, some passed in the afternoons of 1996 and all passed in the afternoons of 1997. The increased pass rate in 1997 coincided with improved sewage treatment, high temperatures and increased levels of sunshine. In 1997 sampling by the Environment Agency produced fewer failures than our morning sampling but more than our afternoon sampling. Their sampling was done around midday. It is suggested that where possible all sampling of EU designated bathing waters should be carried out in the early morning.

Keywords: Bathing water; *Campylobacter*; EU guideline criteria; EU imperative criteria; Faecal indicator; Sewage.

Introduction

Public concern over the microbiological quality of UK coastal waters continues unabated (Kay & Wyer 1997). Morecambe Bay, with three of Lancashire's 11 designated bathing waters, has consistently failed the EU Directive on Bathing Waters Quality (Anon. 1976) since 1990 (Marine Conservation Society 1996). In Europe, bathing water quality is regulated under the EU Directive 76/160/EEC (Anon. 1976). The Directives allow for certain waters to be designated for recreational activities and also set limits for the amount of microbial contamination permitted in them. As part of a survey on the sources of faecal pollution of the coastal waters around Morecambe, it had been noticed that widely varying faecal indicator data were sometimes obtained at different times of the day even though the prevailing conditions were otherwise similar.

Sampling of bathing waters off Morecambe is, like bathing itself, constrained by the state of the tide and is restricted to high water. In order to show whether the time of sampling had any effect on numbers of organisms recovered, days were chosen when sampling could be done twice on the same day, that is, early in the morning and late in the afternoon. Sampling was carried out monthly, from May to September, over the 1996 and 1997 bathing seasons. In 1996 Morecambe's sewage was macerated, but otherwise untreated, and discharged directly into the sea off Morecambe at high water. By the beginning of the 1997 bathing season a new sewage treatment plant had been constructed and the secondary-treated sewage was discharged into the sea several miles south of Morecambe (Fig. 1).

This paper reports on the effects of the time of sampling on the numbers of the indicator bacteria, faecal coliforms and faecal streptococci, and the pathogenic bacterium *Campylobacter* in bathing waters off Morecambe and provides data on the efficacy of the new sewage treatment scheme.

Materials and Methods

Sampling

Morecambe Bay is shallow and has a high tidal range, from 7.5m to 10.2 m. This means that Morecambe's beaches can normally only be sampled at high water because at low-water the sea is several km from the shore and is inaccessible. On most days, only one high-tide can be conveniently sampled during a monitoring program; however, on days when high-water is before 9am it is possible to sample two high-tides on the same day. The offshore current is predominantly south-north (Fig. 1).

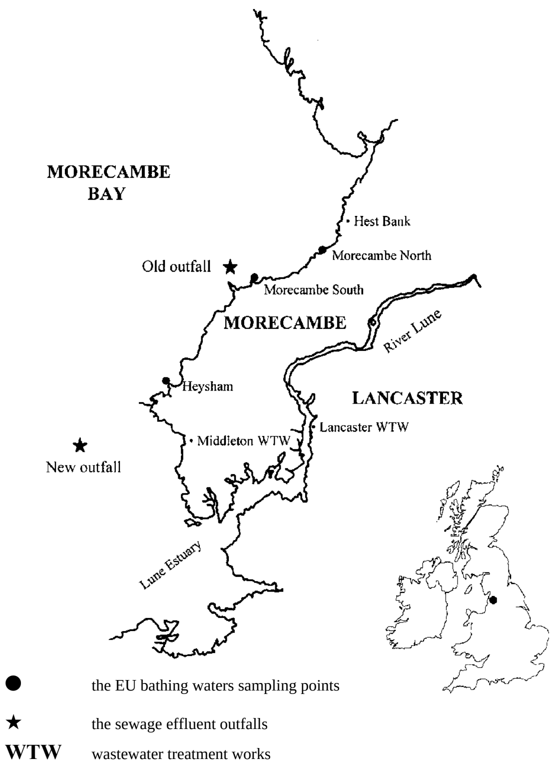


Fig. 1. The Morecambe Bay area and sampling sites with an insert of UK map showing the city of Lancaster .

Water samples were collected at the first and second high tide on the same day from May to September in 1996 and 1997 from three EU-designated bathing waters: Morecambe North, Morecambe South and Heysham (Fig. 1). Non-bathing season samples were taken in February and December to act as controls. It had been noted that samples taken in the winter months did not show the variation between same day morning and afternoon sampling, seen in the summer. Nine samples were collected in sterile 500ml Duran Schott glass bottles, using a 3.5 m telescopic sampler (E.M. Supplies, Dorset), transported to the laboratory in a cool box and normally analysed within the hour (Anon. 1992).

Enumeration of faecal coliforms

Faecal coliforms were estimated using a three-tube Most Probable Number method (MPN) according to standard procedures (Anon. 1992). Seawater dilutions of 10^{-1}-10^{-6} were prepared in 0.1% buffered peptone water (Oxoid CM509) and 1 ml of each dilution inoculated in triplicate into 5 ml of Minerals Modified Glutamate medium (Oxoid CM607). Tubes showing acid and gas production after incubation for 24 h at 44 °C were confirmed by plating on MacConkey No.3 agar (Oxoid CM115) and examined for typical colonies. Counts per 100 ml were calculated from MPN tables (Collins et al. 1989).

Enumeration of faecal streptococci

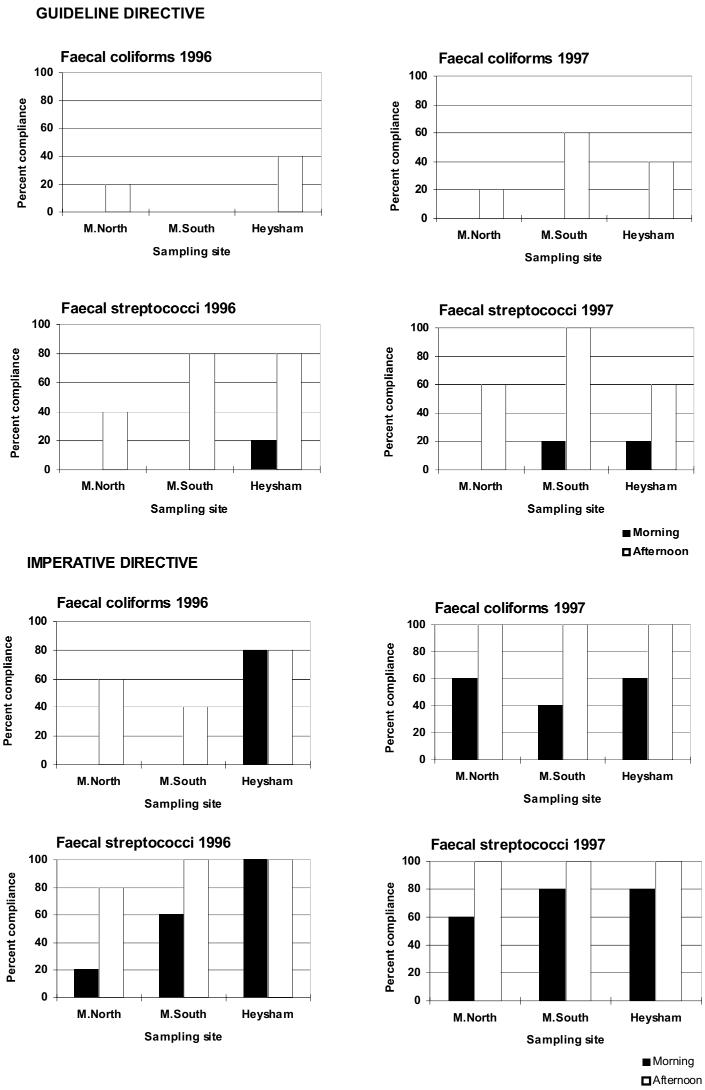
A membrane filtration technique was used in enumerating faecal streptococci (Anon. 1992). Triplicate 10 ml water samples were filtered through white, grid marked 47 mm diameter, Millipore HA-type cellulose filters with a pore size of 0.45 mm. Samples were filtered using a vacuum pump pressure of 65 kPa (500 mmHg) and a triple glass filtration unit (Millipore, Bedford). The filters were placed with the grid side upward on petri dishes of Slanetz and Bartley agar (Oxoid CM377) and grown for 4 h at 37 °C and for 44 h at 44 °C. Red, maroon, or pink colonies were counted as presumptive streptococci. Presumptive colonies were confirmed on MacConkey No. 2 agar (Oxoid CM109). All counts were expressed as cfu/100 ml.

Enumeration of Campylobacter

Thermophilic campylobacters were enumerated using the MPN method (Bolton et al. 1987). Presumptive *Campylobacter* colonies were confirmed by colony morphology, Gram stain with 0.85% carbolfuchsin as the counter stain, and catalase and oxidase activity. *Campylobacter* numbers were expressed as MPN/100 ml (Collins et al. 1989).

Meteorological data

Standard meteorological data for Morecambe was provided by Lancaster City Council's Environmental Health Services. Water temperatures were determined using a hand held thermometer (Orme, UK) at the time of sampling. Measurements of UVB radiation (290-320nm) were made at the campus of Lancaster University, ca. 5-7 km from the sampling sites, using BW100-UVB sensors (Vital Technologies, Bolton, Canada). As biological responses to UVB are highly dependent on wavelength (Mepsted et al. 1996), sensors were calibrated to Setlow's DNA action spectrum in daylight using a double monochromatic spectroradiometer (SR991-PC, Macam Photometrics, Livingston, UK). Calibrations were repeated every one to two weeks (Mepsted et al. 1996). The relationships between change in bacterial counts between morning and afternoon were quantified using linear regression. This approach highlighted important general relationships without making assumptions about the precise mathematical relationships.



EU Guideline 1976: < 100 faecal coliforms per 100 ml (80%)[a];
EU Guideline 1976: < 100 faecal streptococci per 100 ml (90%)[a];
EU Imperative 1976: < 2000 faecal coliforms per 100 ml (95%)[a];
EU Imperative 1994:< 400 faecal coliforms per 100 ml (95%)[a]

[a]Percentage of samples that must comply with the standard value.

Fig. 2. Differences in percentage compliance of Morecambe North, Morecambe South and Heysham bathing waters with EU Directive 76/160/EEC and 94/112/EEC between same day morning and afternoon sampling during the bathing seasons of 1996 and 1997.

Results

Compliance of bathing waters with the EU Directive on bathing water quality

EU Guideline compliance

The results for Guideline compliance of the EU Bathing Water Directives are shown in Fig. 2.

a. Morning samples. When faecal coliforms are used as the indicator, no samples taken in the morning from any of the bathing waters in either 1996 or 1997 passed the EU Bathing Water Guideline Directive of fewer than 100 bacteria per 100 ml of water. However, if faecal streptococci (100/100 ml) are used 20% of samples from Morecambe South passed in 1997 and 20% from Heysham passed in 1996 and 1997.

b. Afternoon samples. Guideline compliance was higher in samples taken in the afternoons. Faecal coliform data show that 20% of Morecambe North samples passed in both 1996 and 1997, Morecambe South increased from 0% passes in 1996 to 60% in 1997 and Heysham had a 40% pass rate in both years. Using faecal streptococci as the indicator, Morecambe North increased in passes from 40% in 1996 to 60% in 1997, Morecambe South increased from 80% in 1996 to 100% in 1997 but Heysham decreased in passes from 80% in 1996 to 60% in 1997.

EU Imperative compliance

The results for Imperative compliance of the EU Bathing Water Quality Directives are shown in Fig. 2.

a. Morning samples. When the EU Imperative (Anon. 1976) criterion for faecal coliforms (2000/100 ml) is applied more of the bathing waters passed. In 1996 neither Morecambe North nor Morecambe South passed on any sampling date but Heysham passed 80% of the time. In 1997 passes at Morecambe North increased to 60%, Morecambe South to 40% but Heysham decreased to 60%. Using the proposed 94/112/EEC criterion for faecal streptococci (400/100 ml) as the indicator, the pass rate at Morecambe North increased from 20% in 1996 to 60% in 1997 and at Morecambe South from 60% to 80%, but at Heysham it decreased from 100% to 80% (Anon. 1994a, b).

b. Afternoon samples. In 1996 60% of Morecambe North, 40% of Morecambe South and 80% of Heysham bathing waters passed the Imperative criteria for faecal coliforms in the afternoon. In 1997 all three bathing waters achieved 100% compliance. Even higher compliance levels were achieved when measurements were based on the proposed faecal streptococci (Anon. 1994a, b). Morecambe North increased its pass rate from 80% in 1996 to 100% in 1997. Morecambe South and Heysham passed 100% of the time in both years.

Counts of faecal indicators and thermophilic campylobacters

The results for the numbers of faecal coliforms, faecal streptococci and thermophilic campylobacters in Morecambe's EU designated bathing waters during the 1996 and 1997 bathing seasons is shown in Table 1. Data for the non-bathing season controls, taken in February and December of each year, showed no differences between morning and afternoon sampling for either the faecal indicators or *Campylobacter*. In contrast, during the bathing seasons the numbers of faecal indicator bacteria were almost always lower in the afternoon compared with the morning. *Campylobacter* was normally only detected in the morning.

The average percentage reductions in faecal indicator numbers between morning and afternoon sampling are shown in Table 1. Faecal coliforms decreased by between 65-92% and faecal streptococci by between 72-86%. Decreases were generally greater in 1997 than in 1996.

In 1996, morning faecal coliform numbers (expressed as the geometric means) were highest at Morecambe North and lowest at Heysham (Table 1). At Morecambe North and Morecambe South lower average counts were recorded in 1997 than in 1996, however, at Heysham they increased slightly between 1996 and 1997. Faecal streptococci numbers showed the same pattern.

Campylobacter was usually recovered from all three bathing waters in both bathing seasons, especially in the mornings. In 1996, the frequency of isolation varied between 80-100% in the morning compared to between 20-60% in the afternoon. Untransformed *Campylobacter* counts ranged from 1 to 70/100 ml in the morning to less than 3/100 ml in the afternoon. The highest count, 70/100 ml was recorded at Heysham. In 1997, recovery of *Campylobacter* was slightly lower than in 1996. In the mornings it ranged between 60-80% and in the afternoons between 0-20%. Counts varied between 1 and 264/100 ml in the morning to between 1 and 43/100 ml in the afternoon. The highest counts, 264/100 ml in the morning and 43 /100 ml in the afternoon, were both at Heysham.

Table 1. Bacterial numbers* in same-day water samples collected early morning (PM) and late afternoon (AM) from Morecambe North, Morecambe South and Heysham during the 1996 and 1997 bathing seasons. % ⇓ = % reduction

Sampling site	Faecal coliforms		Faecal streptococci		*Campylobacter*	
Time	Count	% ⇓	Count	% ⇓	Count	% ⇓
1996						
Morecambe North						
PM	6367	87	622	86	3	42
AM	812		89			
Morecambe South						
PM	5597	65	429	72	5	74
AM	1968		120		1	
Heysham						
PM	1479	78	131	79	7	82
AM	325		28		1	
1997						
Morecambe North						
PM	1995	86	269	83	12	90
AM	275		47		1	
Morecambe South						
PM	1413	92	120	84	5	77
AM	120		19		1	
Heysham						
PM	1622	83	191	82	24	92
AM	275		35		2	

*Faecal *coliforms* and *Campylobacter* results are average geometric means of MPN estimations (n = 45).
*Faecal *streptococci* result is average geometric mean of cfu (n=135).

Relationship between changes in bacterial counts and UVB or temperature

For all three bathing waters regression analysis shows a highly significant relationship between the reduction in the numbers of *Campylobacter* between morning and afternoon samples and between water temperature (Fig. 3) and UVB dose (Fig. 4). Reductions in counts of faecal coliforms and faecal streptococci during the day were less clearly related to temperature and UVB, although still statistically significant. It is evident from the slopes that *Campylobacter* had the greatest response to temperature and/or UVB, followed by faecal coliforms and then faecal streptococci.

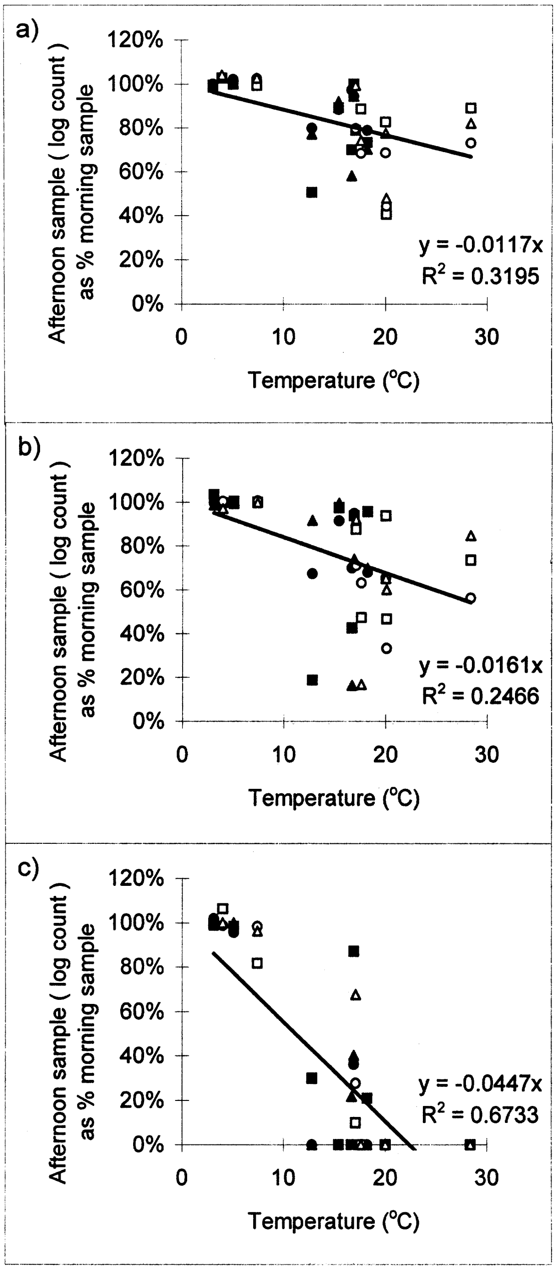


Fig. 3. The effect of afternoon compared to morning sampling on counts of **a.** faecal coliforms; **b.** faecal streptococci and **c.** *Campylobacter* at three sites in relation to sea temperature. Filled symbols: 1996; open symbols: 1997; squares: Morecambe North; circles: Morecambe South; triangles: Heysham. Lines are linear regressions of change in count against temperature, fitted using all data.

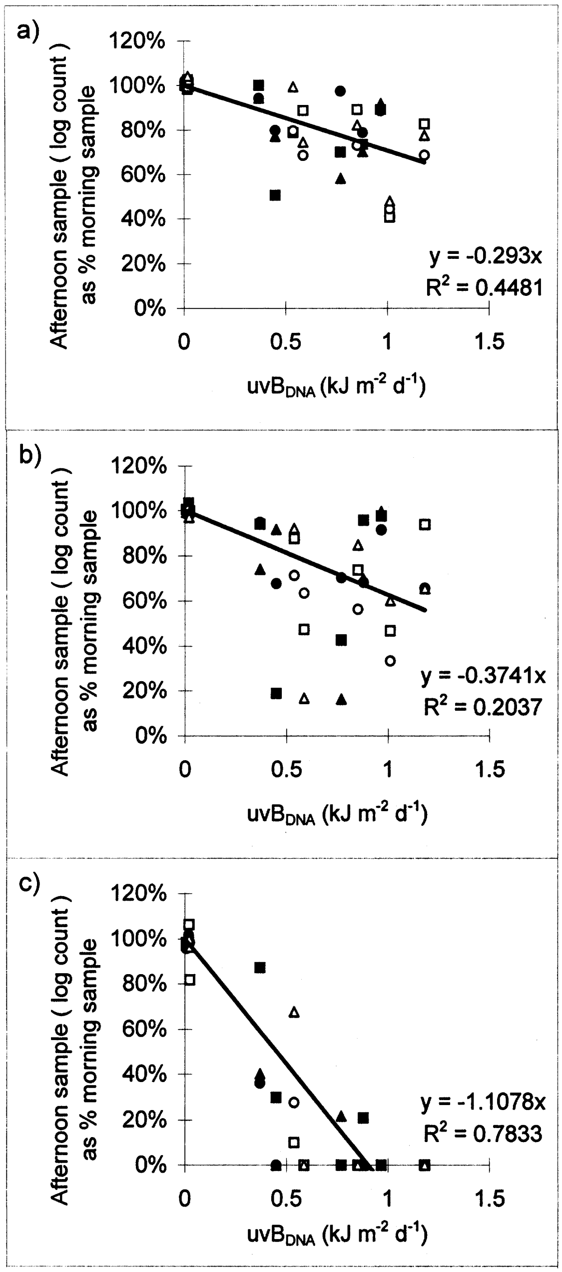


Fig. 4. The effect of afternoon compared to morning sampling on counts of **a.** Faecal coliforms; **b.** Faecal streptococci; **c.** *Campylobacter* in relation to UVB radiation. Explanation symbols see Fig. 3.

Discussion

When a bathing water complies with the EU Guideline criteria for bathing waters the bathing water is considered to be of very good quality (Anon. 1976). When it complies with the EU Imperative criteria it is considered to be of a lesser quality but is not thought to be a risk to health. In a two year monitoring program of Morecambe's three EU designated bathing waters in which sampling was carried out only in the morning none of the beaches passed the Guideline criteria and only occasionally passed the Imperative criteria (Obiri-Danso & Jones 1999).

The data presented in the current paper show that the ability of Morecambe's bathing waters to pass the EU Directive on Bathing Water Quality depends on the time of day when the sample is taken, the indicator organism tested for and whether the test uses the most strict (Guideline) or the least strict (Imperative) criteria.

When faecal coliforms are used as the test organism none of the bathing waters passed the Guideline criteria, regardless of the time of sampling or the year. On individual sampling dates more bathing waters passed the Directive in the afternoon than in the morning. If the Imperative criteria are used no bathing waters passed in 1996 or in the mornings of 1997, but all passed in the afternoons in 1997.

When faecal streptococci are used as the test no bathing waters passed the Guideline criteria in 1996 or in the mornings of 1997, but Morecambe South passed in the afternoons in 1997. Studies in both the USA and the UK have suggested that faecal streptococci are the preferred indicators for recreational water quality in marine waters as there is a better relationship linking their concentration with gastro-enteritis reporting by bathers (Kay et al. 1994; Cabelli et al. 1982). For this reason an Imperative criterion for faecal streptococci (less than 400/100ml) was included in the proposed amendment to the 1976 Directive, submitted to the European Commission in 1994 (Anon. 1994a, b). If the proposed Imperative criteria for faecal streptococci are used for Morecambe's bathing waters only Heysham passed in the mornings, all three passed in the afternoons in 1997 with only Morecambe North failing in 1996. These results, which would have meant that the bathing waters failed the EU Directive on Bathing Water Quality, differed from those obtained by the Environment Agency who showed that all three of Morecambe's bathing waters failed the Imperative criteria in 1996, that Morecambe North and Morecambe South passed and Heysham failed the Imperative criteria in 1997 (Anon. 1997).

In the routine monitoring of Morecambe Bay bathing waters, carried out by the Environment Agency, recorded geometric means for faecal coliforms were 805, 356, 638 in 1996 and 393, 354 and 458 in 1997 for Morecambe North, Morecambe South and Heysham, respectively. These counts are lower than those of our morning samples but higher than our afternoon samples. However, geometric means for faecal streptococci in afternoon samples in our study were between 10-80% lower in 1996 and 70-90% lower in 1997 than those of the Environment Agency. The differences in results may be due to a variety of reasons. Firstly, sampling was done on different dates; secondly, the MPN methodology was used for estimating faecal coliforms in our study and this generally gives higher counts than the membrane filtration methods used by the Environment Agency (Koch 1994); and thirdly, most of the Environment Agency's samples were collected around midday (www.environment-agency.gov.uk/), which is several hours after our morning samples were taken and enough time for the numbers to decline, especially in warm and sunny conditions.

The average decline in indicator numbers between the morning and afternoon sampling over the whole of the bathing season was 77% in 1996 and 87% in 1997 for faecal coliforms, 79% in 1996 and 83% in 1997 for faecal streptococci, and 66% and 86% for *Campylobacter* (Table 1). Such reductions between morning and afternoon counts were clearly a function of the prevailing weather, being greatest under conditions of higher water temperature and/or high UVB radiation (Figs. 3 and 4). Since these two factors were highly correlated over our sample dates, it is not possible to distinguish their relative importance. However, it is known that solar radiation is the major factor associated with the decline of enteric bacteria in aquatic environments (Davies-Colley et al. 1994; Solic & Krstulovic 1992; Davies & Evison 1991; Chamberlin & Mitchell 1978), although the specific roles of different wavelengths is less clear. Ultimately, both temperature and ultraviolet radiation may need to be considered. For example, Wegelin et al. (1994) have reported that synergism between water temperature and sunlight with temperatures above 55 °C enhances the solar fluence germicidal effect by a factor of approximately 2 for *Streptococcus faecalis* and *E. coli.* It has also been proposed that faecal streptococci are more resistant to sunlight (u.v. radiation) than *E.coli* although they are also much more sensitive compared to pathogens of concern (Alkan et al. 1995; Davies-Colley et al. 1994; Fujioka et al. 1981). This may explain the smaller percentage reductions obtained for faecal streptococci than faecal coliforms, especially in 1997, although faecal coliforms were 10-fold more in numbers than faecal streptococci.

The increased compliance in 1997 could be due to changes in the treatment of Morecambe's waste-water (sewage). In 1996 Morecambe's sewage was macerated, but otherwise untreated, prior to discharge directly into the sea at ca. 500 m south of the Morecambe South bathing beaches. Since March 1997 Morecambe's sewage has been subject to secondary treatment and is discharged into the sea at ca. 1.5 km south of Heysham through a much longer outfall pipe (2.5 km). This may also be the reason that Heysham's level of compliance was lower in 1997 than in 1996. UVB radiation and temperatures had significant impacts on all the different micro-organisms from all beaches in 1997 compared to 1996 possibly because of the 'cleaner' sewage effluent.

Rainfall, which affects the amount of agricultural runoff, and the large bird populations roosting close to the bathing waters also have an influence on indicator numbers, especially in the winter months (Jones & Obiri-Danso in press; Godfree et al. 1997; Wyer et al. 1996, 1995, 1994; White & Godfree 1985). They are unlikely to impact on our morning and afternoon samples, which were collected on dry and sunny days during the bathing season. It is also unlikely that these, and other factors such as land use and runoff into the catchment of the river Lune, have contributed to the differences in the results obtained between 1996 and 1997.

The results for faecal indicators are mirrored by those for the pathogen *Campylobacter*, which was recovered more in the mornings than in the afternoons and was more numerous in 1996 than in 1997. *Campylobacter* is known to survive in surface waters especially at low temperatures (Blaser et al. 1980). However, they are particularly sensitive to solar radiation (Wallace et al. 1993; Stelzer et al. 1991; Jones et al. 1990) and are more likely to be isolated in the morning when it is cooler and radiation levels are low. As *Campylobacter* survive for less time than the usual bacteria indicators, their presence in water is thought to be an indication of a recent faecal contamination.

This study has highlighted a number of management issues in the application and interpretation of the EU Directive, which are likely to be encountered in all EU member states. Most approaches regarding the safety of bathing or recreational waters involve defining an individual bathing water as passing or failing a defined microbiological standard. Since a single bathing or recreational water may vary widely in relation to microbiological measures of health risk within relatively short periods of time, such an approach has inherent limitations and may condemn bathing waters which are in fact safe for much of the time; or imply the safety of a bathing water which is, in reality unsafe on many occasions. This variability is both temporal and spatial and may be due to a number of factors. It may mean that different orders of magnitude in the degree of faecal pollution may be encountered within a few metres of a single bathing water, or within a few hours at the same sampling point. In such circumstances, the value of classifying a bathing water as intrinsically safe/unsafe which is based on a limited number of indicator bacteria estimated from a water sample, or group of samples taken at a specific sampling point in time is questionable.

Water quality assessments, to date, have relied heavily on the use of microbiological enumeration (Anon. 1992). This has a series of associated limitations: such data are considered expensive to collect in many countries; they are of questionable value given the local spatial and temporal variability in bathing water quality (i.e. there are fundamental sampling protocol problems); recent studies have also indicated that comparability of monitoring data generated, even by well-run laboratories may by extremely poor (i.e. there are problems of analytical reproducibility and inter-laboratory comparability) (Figueras et al. 1997). Because of the time delay before microbiological analytical results are available and the frequency of analysis undertaken, approaches which rely primarily on microbiological water quality analysis are always retrospective in nature. It is, in part, for this reason that bathing water 'safety' has often been assessed in terms of compliance with a standard across a retrospective period (usually the previous years bathing season). Such approaches may therefore fail to provide information useful for the issuance of public advisories prior to the onset of risk.

Adverse health effects appear common in people who immerse themselves in surface waters for recreation. As many as one third of people will suffer some adverse health effect after entering water that complies with current EU standards. Symptoms increase with increasing microbiological evidence of faecal pollution. However, most illness is mild and a proper clinical diagnosis is seldom made in prospective studies (Kay & Rees 1997; Kay et al. 1994).

The results in this paper show that one of the main factors determining whether a beach passes or fails the EU Bathing Water Quality Directive is the time of sampling. Samples taken early in the morning are likely to fail and those in the afternoon more likely to pass. If we take the view that the EU Directives are directly related to the risk to health then sampling ought to be carried out in the early morning, that is, taking the worst case scenario because percentage compliance is always lower in the morning than in the afternoon. This would not only apply to Morecambe but to all EU designated beaches throughout the European Union.

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