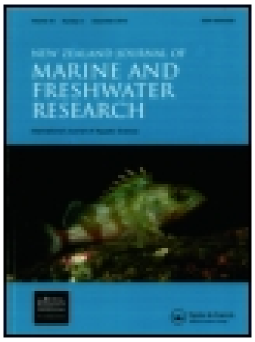

New Zealand Journal of Marine and Freshwater Research

ISSN: 0028-8330 (Print) 1175-8805 (Online) Journal homepage: https://www.tandfonline.com/loi/tnzm20

Faecal streptococci as faecal pollution indicators: A review. Part II: Sanitary significance, survival, and use

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New Zealand Journal of Marine and Freshwater Research, 1993: Vol. 27: 117–137
0028–8330/93/2701–0117 $2.50/0

Faecal streptococci as faecal pollution indicators: a review. Part II: Sanitary significance, survival, and use

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Abstract Some New Zealand regional councils are examining the use of faecal streptococci (or the subset, enterococci) to assist in identifying pollution sources, or as better indicators of disease risk in bathing waters than faecal coliforms. However, in spite of world-wide investigation, faecal streptococci have largely failed to fulfil their potential as pollution source (human versus animal) indicators in receiving waters. Many qualifications accompany the use of faecal coliform : faecal streptococci (FC : FS) ratios, and the species identification approach (using biochemical and DNA-based methods) has produced inconclusive results. Nevertheless, the FC : FS shift method (in which the ratio changes under sample storage) may warrant further investigation. Although reported results vary widely, most studies indicate that faecal streptococci outlive faecal coliforms in receiving waters and are more resistant to sunlight-induced inactivation. USEPA epidemiological studies showed that enterococcus concentrations were better correlated than faecal coliform concentrations with disease risk associated with bathing in sewage-polluted waters. These results, which implied that the enterococci better represented viral hazard, led the USEPA to recommend the use of enterococci (or *Escherichia coli* in freshwaters) as bathing water quality indicators. These recommendations have largely been followed in provisional New Zealand Department of Health guidelines. However, adoption of the USEPA criteria should be approached cautiously, because of doubts about their epidemiological applicability in New Zealand, and a lack of information about streptococcal concentrations and species profiles in local effluents and receiving waters.

M92025
Received 6 May 1992 ; accepted 18 January 1993

Keywords faecal streptococci; enterococci; sanitary significance; survival; review

INTRODUCTION

New Zealand receiving water microbiological standards have been based on either total coliform or faecal coliform bacteria for almost 30 years. The original standards were contained in eight water classes (four for inland waters and four for coastal waters) in the Waters Pollution Regulations 1963, issued under the Waters Pollution Act 1953. The 1953 Act and the 1963 Regulations were repealed and replaced by the Water and Soil Conservation Amendment (No. 2) Act 1971, which retained a similar set of nine water classes (one additional coastal class). The Water and Soil Conservation Act was repealed on 1 October 1991 with the introduction of the Resource Management Act (which contains 12 water classes), although water classifications made under the Water and Soil Conservation Act continue to stand as part of transitional regional management plans. A detailed history of microbiological standards for New Zealand receiving waters has been compiled by McBride (1990).

To date, faecal streptococci have not been included in New Zealand receiving water standards and they have not been widely used as faecal pollution indicators by local water managers. However, regional water quality surveys which include faecal streptococci (or the subset group, the enterococci) are becoming more common (e.g., Power 1991). Three factors have contributed to the increasing interest in these bacteria. First, some regional councils are considering the use of the faecal streptococci as a means of assessing the relative contributions of animal and human faecal sources to observed levels of microbiological contamination in natural water systems. Second, those sections of the Resource Management Act which replace the Water and Soil Conservation Act will allow regional councils to base numerical microbiological standards on whatever organisms they consider suitable to meet national narrative standards (there is also provision in the new Act for the setting of national numerical standards). Third, the adoption by the United States Environmental Protection Agency of enterococci as indicators in recreational waters (USEPA 1986), and the recommendation by the New Zealand Department of Health that the USEPA criteria should be applied in New Zealand (McBride et al. 1992), has led to a closer examination of this group in the context of local bathing water quality.

Part II of this paper reviews and discusses the literature on the occurrence of faecal streptococci, their sanitary significance, survival, value as pollution source indicators, and use (of the enterococci subset) as recreational water quality indicators. Their use as faecal pollution indicators in shellfish is briefly discussed. Particular attention is given to issues of relevance to New Zealand, such as their use to distinguish between human and animals sources, and the background to the adoption of the enterococcus guidelines by the USEPA. Possible problems associated with the introduction of the USEPA standards to New Zealand are outlined. The authors refer readers requiring further information on specific topics to the reviews by Geldreich & Kenner (1969), Clausen et al. (1977), Kenner (1978), Mundt (1982), Salas (1986), Cabelli (1988), and McBride (1990).

The nomenclature changes discussed in Part I of this review are likely to gain increasing recognition, particularly the transfer of some faecal streptococcal species to the genus *Enterococcus* (Schleifer & Kilpper-Bälz 1984; Collins et al. 1984). However, for the purposes of this review, the classification in *Bergey's Manual* (Sneath et al. 1986) will be adhered to, with all the faecal streptococci being placed in the genus *Streptococcus*.

OCCURRENCE OF FAECAL STREPTOCOCCI

To determine the usefulness of faecal streptococci as indicators of faecal pollution, it is important to ascertain whether they exist in nature only in association with faeces, or whether they can persist and multiply in the environment independent of faecal pollution. Some parts of this section are based on the excellent review of this topic by Clausen et al. (1977).

Occurrence in faeces and sewage

In animal faeces, the faecal streptococci generally outnumber faecal coliforms, although the overall concentration appears to differ markedly between species. For example, sheep faeces contain approximately 3.8×10^7 faecal streptococci per g compared to 1.6×10^7 faecal coliforms per g. Cow faeces contain 1.3×10^6 faecal streptococci per g and 2.3×10^5 faecal coliforms per g. In contrast, streptococcal concentrations in human faeces—which are typically around 3.0×10^6 g^{-1}—are generally less than those for faecal coliforms, which are typically around 1.3×10^7 g^{-1} (Mara 1974). The significance of the different faecal coliform : faecal streptococci ratios between humans and animal faeces is discussed below.

The ratio of enterococci to other faecal streptococci occurring in faeces is also known to differ among vertebrate species (see below). Overall, it appears that the enterococci (which dominate the faecal streptococci in human effluents) are present in municipal sewage in concentrations 10–100 times less than *Escherichia coli* (e.g., Miescier & Cabelli 1982).

Occurrence on plants

Numerous studies have reported the occurrence of streptococci, including enterococci, on plants (e.g., Mundt et al. 1958; Mundt 1961, 1963; Geldreich et al. 1964). Geldreich & Kenner (1969) found that 43% of plant faecal streptococci were enterococci, 9% were *S. bovis* or *S. equinus*, 34.9% were atypical *S. faecalis*, and 13% were *S. faecalis* var. *liquefaciens*.

Clausen et al. (1977) noted that there is some confusion as to the significance of faecal streptococci on plants. They outlined three possibilities:

Faecal streptococci may:

(1) be present as a result of direct contamination from warm-blooded animals and/or insects. This idea is supported by a study of plants from a wild environment (Mundt 1963), where low incidence of enterococci and species similarity with isolates from animal faeces suggested that their presence on plants resulted from chance contamination.

(2) be temporary residents on plants, where they are capable of limited reproduction. Work by Mundt (1961), which demonstrated simultaneous occurrence or absence of enterococci on plants and in the surrounding soil, suggested that soil counts may result from spreading of the plant population, by means of gravity, wind, rain, and insects.

(3) exist on plants in a truly epiphytic relationship, spread by seeds and able to reproduce on the growing plant. Mundt et al. (1962) demonstrated this phenomenon for a strain of *S. faecalis* var. *liquefaciens* on bean, rye, corn, and cabbage plants.

Many of the enterococci isolated from plants have been reported to differ from strains characteristic of warm-blooded animals. These differences have included biochemical reactions and DNA/DNA homology (Mundt 1975). Geldreich et al. (1964) detected starch hydrolysis in 37.7% of enterococci cultures isolated from plants, whereas this characteristic was uncommon in strains recovered from other sources. Facklam (1972) also failed to recover enterococci capable of starch hydrolysis from human Group D strains. Mundt (1973) reported consistent differences in litmus milk reactions between *S. faecalis* strains isolated from plant and human sources.

At least one recognised streptococcal strain, *S. faecium* var. *casseliflavus*—now named *S. faecium* subsp. *casseliflavus* in *Bergey's Manual* (Sneath et al. 1986)—appears to be strongly associated with plants (Mundt et al. 1967; Mundt & Graham 1968) although it has also been isolated from insects (Martin & Mundt 1972). The strain does not appear to be associated with humans (Facklam 1972). In addition, an atypical strain of *S. faecalis*—now named *S. faecium* subsp. *mobilis* in *Bergey's Manual* (Sneath et al. 1986)—has been reported as growing on fermenting or rotting vegetation (Langston & Bouma 1960).

Association with insects

Faecal streptococci have been recovered from a variety of insects (Geldreich et al. 1964; Martin & Mundt 1972). The species recovered include *S. faecalis*, *S. faecium*, and *S. faecalis* var. *casseliflavus* (Martin & Mundt 1972)—now named *S. faecium* subsp. *casseliflavus* in *Bergey's Manual* (Sneath et al., 1986)—and *S. faecalis* var. *liquefaciens* (Geldreich et al., 1964), but not *S. bovis* or *S. equinus*. The studies by Geldreich et al. (1964) and Eaves & Mundt (1960) suggest that faecal streptococci are not consistent residents of insects but are present as a result of chance contacts with streptococci in the environment. According to Clausen et al. (1977), similarities between plant and insect strains suggest frequent exchange of organisms whereas dissimilarities between these strains and human streptococci suggest little intermingling of these populations.

Occurrence in soil

The contribution of storm water run-off to the bacterial quality of surface waters means that streptococcal concentrations in water will be strongly related to their incidence in soil. Medreck & Litsky (1960) found enterococci in only 2.2% of unpolluted soil samples in a watershed compared to 71.8% for total coliforms and 1.1% for faecal coliforms. In a study involving identification of faecal streptococci species from agricultural soils, Geldreich & Kenner (1969) found that 35% were *S. faecalis* var. *liquefaciens*, 63% were other *S. faecalis* strains, and 2% were *S. bovis* and *S. equinus*.

The survival of faecal streptococci in soil is also relevant to the use of the organisms as biological signatures, particularly with respect to the use of the FC : FS ratio. To date, reports on relative survival in soils have been inconclusive or contradictory. Bergner-Rabinowitz (1955) found no difference between the survival of coliforms and *S. faecalis* in soils, both persisting throughout a 38-day experiment. However, Mallman & Litsky (1951) found that *S. faecalis* persisted for about 40 days in soil compared to 77 days for *E. coli* and 18 days for *Salmonella typhi*. In tests involving the periodic inoculation of soils, Van Donsel et al. (1967) found that faecal streptococci were outlived by faecal coliforms in summer (2.7 and 3.3 days, respectively), exhibited the same survival in autumn (13 days for both), but survived much longer than faecal coliforms in winter, persisting as long as 20 days.

Distribution of *S. faecalis* var. *liquefaciens*

There is some controversy surrounding the sanitary significance of *S. faecalis* var. *liquefaciens*. Geldreich & Kenner (1969) reported high numbers of this subspecies in two unpolluted wells, and high percentages in insects and soil. As a result, Geldreich (1976) advocated caution in interpreting the significance of levels of faecal streptococci below 100 per 100 ml of water, recommending that results should be evaluated in conjunction with faecal coliform data, since *S. faecalis* var. *liquefaciens* apparently predominates at these densities because of its ability to persist in the environment. Overall, *S. faecalis* var. *liquefaciens* does not appear to be a predominant species in water, however. For example, Bartley & Slanetz (1960) found that the organism constituted between 5 and 14% of the faecal streptococci isolated from sewage, sea water, rivers, ponds, and wells.

Some authors have queried the relevance of peptonisation tests in the differentiation of faecal streptococci from faecal and non-faecal sources. Clausen et al. (1977) doubted the ubiquitous nature of *S. faecalis* var. *liquefaciens*, maintaining that the significant concentrations of this subspecies in the faeces of certain animals may be the origin of the reported isolations from insects and soils. Kenner (1978) also demonstrated that a significant number of peptonising strains could be isolated from humans and animals and considered that earlier reports of small numbers of *S. faecalis* var. *liquefaciens* in the environment was not a constraint to the use of faecal streptococci as indicators of faecal pollution. In other taxonomic studies, Jones et al. (1972) and Bridge & Sneath (1983) did not find sufficient differences to warrant sub-species status and recommended all isolates be considered as *S. faecalis*. There are no sub-species of *S. faecalis* listed in *Bergey's Manual* (Sneath et al. 1986).

USE OF FAECAL STREPTOCOCCI TO DISTINGUISH BETWEEN HUMAN AND ANIMAL SOURCES

The idea of using streptococci to differentiate between human and animal sources appears to have been discussed first by Winslow & Palmer (1910). Since then, the faecal streptococci have been frequently investigated as a means of distinguishing between different types of faecal pollution in water, particularly between sources of human and animal origin. Unfortunately, the results have frequently been inconclusive, contradictory, or confusing.

There are two possible ways of using the faecal streptococci as biological signatures of different faecal sources: the first involves comparing their concentrations to those of faecal coliforms; the second entails identification of the constituent species in the different faecal sources and receiving waters.

Faecal coliform : faecal streptococci (FC : FS) ratio

Several authors (e.g., Geldreich & Kenner 1969; Mara 1974; Feachem 1975) have suggested that the ratio of faecal coliforms to faecal streptococci may be used to indicate the source of faecal pollution in water. In human faeces, faecal coliforms outnumber faecal streptococci. The faecal coliform to faecal streptococci (FC : FS) ratio is > 4 (Geldreich & Kenner 1969). Enterococci predominate in human faecal streptococci populations (Cooper & Ramadan 1955; Kenner et al. 1960; Mead 1965). In animal faeces, particularly those of cattle, the predominant faecal streptococci are members of the *S. bovis-equinus* group, which outnumber the faecal coliforms to give a FC : FS ratio of < 0.7 (Kjellander 1960; Raibaud et al. 1961).

It is therefore theoretically possible to ascribe a human or animal source to faecal pollution of water based on the evidence of FC : FS ratios of > 4 or < 0.7. Geldreich & Kenner (1969) recommended that the FC : FS ratio should only be considered indicative of a particular source if the sample is taken within 24 h of the faecal material entering the watercourse. However, this would not take into account the time between defaecation and discharge and, as noted by Burman et al. (1978), in practice it is usually difficult or impossible to determine the age of faecal pollution in water. In these circumstances, observation of the FC : FS ratio in stored samples may give some indication of the pollution source (Feachem 1976).

Although reported results differ, it appears that die-off rates of faecal coliforms and faecal streptococci in natural waters can be summarised as follows: *S. bovis-equinus* group > faecal coliforms > *S. faecalis-faecium* group (Kjellander 1960; Geldreich & Kenner 1969; McFeters et al. 1974). McFeters et al. (1974) concluded that these differences showed that the FC : FS ratio was "no longer of significance in determining the source of the contamination when considering bacteria that originate from domestic sewage". However, Feachem (1975) argued that the differential rates of die-off of faecal streptococcal species noted by McFeters et al. (1974) in fact enhanced the value of the FC : FS ratio in distinguishing between pollution sources, in the following manner: a predominantly human source (dominated by enterococci) should exhibit an initially high FC : FS ratio (> 4) which falls on storage whereas non-human sources (dominated by *S. bovis* and *S. equinus*) should exhibit an initially low FC : FS ratio (< 0.7) which subsequently rises.

Subsequent studies of changes in FC : FS ratios have produced variable results. Burman et al. (1978) found that, apart from one sample of treated sewage effluent, stored samples of sewage effluents and receiving river waters all exhibited steady or increasing FC : FS ratios, in contrast to the predictions of Feachem (1975). However, Feachem's predictions were given some support by the work of Wheater et al. (1979), who studied ratios in raw sewage samples and in samples collected above and below a river outfall. In survival experiments, it was found that in the first 24 h, ratios increased in nearly all samples. This increase continued in the samples from upstream of the outfall, but in downstream samples and in the sewage effluent the initial increase was reversed after 24 or 48 h. Thereafter, the ratios decreased. *S. bovis* was the predominant organism in samples from above the outfall but was a smaller proportion of downstream samples. The species was not recorded in sewage. Storage for 72 h reduced the proportions of *S. bovis* in all samples and, correspondingly, increased the proportion of *S. faecalis* and *S. faecium*. Wheater et al. (1979) also noted that although sewage (human) effluent ratios were invariably above 1, they did not always exceed 4, and that ratios varied depending on whether the British or United States methodology (media and incubation temperatures) was used.

Wheater et al. (1979) also investigated the survival of streptococci in faecal specimens (emulsified in water) from humans, cattle, sheep, and pigs. In human samples, the FC : FS ratios increased in the first 24 h because of an increase in faecal coliforms but fell thereafter, as faecal coliform die-off exceeded faecal streptococci die-off. In faecal samples from cattle and sheep, the ratios increased over the total study period of 96 h. As in the water and effluent samples, these changes were associated with the species composition of the faecal streptococci populations. *S. bovis* was not isolated from any human specimens and only small changes occurred in the incidence of the predominant two species—*S. faecalis* and *S. faecium*—throughout the storage period. In the samples from cattle and sheep, *S. bovis* was initially the predominant species, accounting for up to 75% of the streptococci present. However, the proportion of *S. bovis* fell to between 10 and 30% after 72 h and between 0 and 8% after 96 h.

Wheater et al. (1979) recommended caution in the use of FC : FS ratios as a means of determining pollution sources in streams, and even greater caution when using these ratios in storm water, because of the many unquantifiable factors involved. The effects on FC : FS ratios of variable survival of faecal streptococci species, disinfection of waste water, and the method used to count faecal streptococci are cited in *Standard Methods* (APHA 1992) as reasons against recommending the use of such ratios to differentiate between human and animal pollution sources.

Species composition

The ratio of enterococci to other streptococci in faeces differs among vertebrate species. Human faeces are characterised by a predominance of enterococci, although the available information on species composition is confusing and contradictory. Some authors (e.g., Buttiaux 1958) have reported that *S. faecium* is the dominant human species. However, Cooper & Ramadan (1955) found *S. faecalis* was more prevalent, constituting about 40% of human streptococci, with *S. durans* about 4%. Geldreich & Kenner (1969) reported that *S. faecalis* var. *liquefaciens* constituted about 25% of the total human streptococcus population. Wheater et al. (1979) found *S. faecium* was more prevalent than *S. faecalis* in humans, but that this was also true for cattle, pigs, and poultry. They found that *S. faecalis* was the dominant organism in sheep, cats, dogs, and rodents.

Several faecal streptococci were earlier reported to be unique to human faeces, including *S. faecalis* (Bartley & Slanetz 1960) and *S. durans* (Cooper & Ramadan 1955) but this is no longer accepted (Clausen et al. 1977). Only the non-Group D faecal streptococci—*S. mitis* and *S. salivarius*, which are buccal streptococci—are regarded as reliably indicative of human sources. Although *S. salivarius* is generally considered to be rarely present in human faeces, Moore & Holdeman (1974) reported that the species ranked 38th on a list of human faecal bacteria: this makes it the most frequently encountered faecal streptococcus in human faeces, and placed it just behind *E. coli*, which ranked 37th. The most abundant species was *Bacteroides vulgatus*.

Although there is much conflicting information about the incidence of *S. faecium*, *S. faecalis*, and *S. durans* in both human and animal sources, there appears to be agreement that *S. bovis* and *S. equinus* are largely confined to animals. *S. bovis* can occasionally occur in large numbers in human faeces in association with large-bowel disease (Parker & Ball 1976; Sneath et al. 1986). Kenner et al. (1960) and Geldreich & Kenner (1969) reported combined percentages of *S. bovis* and *S. equinus* ranging from 18.9% for pigs to 66.2% for cattle with the remaining population in animals appearing to be mainly enterococci. Medrek & Barnes (1962) found that *S. bovis* was the predominant species in most cattle and sheep. *S. faecalis*, *S. faecium*, and *S. durans* were rare in cattle but formed a significant proportion of the population in sheep. Pourcher et al. (1991) found that *S. faecalis* predominated in human and poultry faeces, with *S. bovis* typical of bovine and pig faeces.

Some authors consider that *S. bovis* characterises ruminants (e.g., Middaugh et al. 1971). However, Wheater et al. (1979) also found *S. bovis* in horses, pigs, dogs, cats, hens, ducks, and seagulls. Nevertheless, the proportion was highest in the ruminant animals (cattle and sheep), where it formed almost 70% of the streptococcal flora. There was a lower incidence of *S. bovis* in horses but *S. equinus* was also present, and the two organisms accounted for between 80 and 90% of the total streptococci.

Ramadan & Sabir (1962) isolated 361 faecal streptococci strains from 11 species of farm animals. They found that 14.8% were *S. bovis*, 12.7% were *S. faecalis* var. *liquefaciens*, and the remaining 72.5% were atypical *S. faecalis* groups. They reported that none of the strains could survive heat resistance and heat-tellurite tolerance tests, which the authors considered to be important in distinguishing between human and animal sources.

There have been other attempts to identify streptococcal sources by simple biochemical methods. For example, Kjellander (1960) devised a sodium azide sorbitol agar to distinguish sorbitol-fermenting streptococci (considered to be *S. faecalis*) from non-sorbitol-fermenting strains (*S. faecium*). Kjellander considered the former species was mainly of human origin and the latter mainly of animal origin and devised a quotient to distinguish between the two sources. However, the value of *S. faecalis* : *S. faecium* ratios as a differentiation method is very doubtful. Kenner et al. (1961) pointed out that isolation techniques may not be equally selective for streptococcal populations in different environments. Wheater et al. (1979) noted that geographic and dietary differences may also affect species distributions and pointed out that sorbitol-fermenting types of *S. bovis* are readily recoverable. They suggested that sorbitol fermentation by different isolates explained the conflicting findings of Cooper & Ramadan (1955) who considered that *S. faecalis* was the dominant human species and Buttiaux (1958) who considered that *S. faecium* was more prevalent. In their own study, Wheater et al. (1979) found that *S. faecium* was more prevalent than *S. faecalis* in humans, cattle, pigs, and poultry. *S. faecalis* was the dominant organism in sheep, cats, dogs, and rodents.

Although many workers who examined faecal streptococci populations in human and animal faecal stools have commented on the implications of their findings with respect to pollution source identification, there have been relatively few studies designed to test this approach in aquatic environments. One of the difficulties associated with such field studies is the broad spectrum of streptococcal biotypes likely to be encountered. For example, biochemical classification of faecal streptococci from the Nile River showed that about 78% of the isolates were distributed amongst atypical or unclassifiable categories (Saleh 1980).

Bayne et al. (1983) examined Group D streptococci isolates in the vicinity of a sewer outfall. Above the outfall, *S. faecium* var. *casseliflavus*—a species which is associated with plants rather than animals (Sneath et al. 1986)—predominated, whereas *S. faecalis* var. *faecalis* predominated below the outfall. Wheater et al. (1979) found that *S. bovis* was the predominant organism in samples from above a sewer outfall but was a smaller proportion of downstream isolates. The species was not recorded in sewage samples.

Rutkowski & Sjogren (1987) investigated a media formulation (designated M2), which allows the growth of a wider range of streptococci than is normally associated with the faecal streptococci, and used it to characterise various pollution sources. In non-human sources, the Group D non-enterococci constituted 93.3% of the total streptococcal population, with the remainder being enterococci. No oral streptococci (*S. mitis*, *S. sanguis*, and *S. salivarius*) were found in samples from animal sources. In human sources (sewage treatment plants) the enterococci dominated at 61.5%, the Group D viridans enterococci followed at 29.4%, and the oral streptococci ranged from 2.0% (receiving streams) to 7.6% (treatment plants).

SURVIVAL OF FAECAL STREPTOCOCCI

Survival in sewage, river water, and ground water

Evison & Tosti (1980) noted that, compared to coliforms, faecal coliforms, and *E. coli*, relatively little work has been done on the survival of faecal streptococci in aquatic environments. Furthermore, as with the data on species composition and source differentiation, the available information on survival is often confusing and contradictory.

Some studies have shown that faecal streptococci outlive coliform indicators. For example, Cohen &

Shuval (1973) compared the survival of total coliforms, faecal coliforms, faecal streptococci (most of which were probably enterococci), and viruses in sewage. Samples were taken before and after primary settling and biological filtration. Removal of faecal streptococci was considerably less than that of coliforms and, thus, more closely paralleled virus survival. In an open sewage channel receiving effluent, viruses again showed the least die-off, followed by faecal streptococci.

However, the phenomenon of superior streptococcal survival has not been supported by other studies. For example, Hanes et al. (1964) reported that overall die-off rates of enterococci stored in sewage diluted with BOD water were similar to or greater than coliforms. Burman et al. (1978) also observed die-off rates that were similar to or greater than faecal coliforms (most of which were considered to be *E. coli*) in stored samples of settled sewage, diluted sewage, and river water. Stored treated sewage samples gave variable results with die-off rates being higher than, equal to, and less than those of faecal coliforms.

There are several possible explanations for the conflicting results in the literature. First, the survival rate of a faecal streptococci sample will depend on species composition, because the survival rates of different faecal streptococci species appear to differ significantly. For example, Geldreich & Kenner (1969) found that *S. faecalis* and *S. faecalis* var. *liquefaciens* persisted significantly longer than faecal coliforms or *Enterobacter aerogenes*. However, *S. bovis* died off rapidly in storm water samples and *S. equinus* exhibited a very short survival in pure cultures in the laboratory. Second, it appears that the comparative survival of coliforms (including faecal coliforms and *E. coli*) and faecal streptococci in the same water or effluent sample will differ according to the available nutrients. In laboratory experiments, Allen et al. (1952) recorded the multiplication of *E. coli* in as little as 0.28 ppm of organic matter in solution, whereas much higher concentrations were required for the growth of *S. faecalis*. Regrowth of coliforms in the absence of streptococcal regrowth may explain the similar or inferior streptococcal survival characteristic reported by Hanes et al. (1964). The phenomenon may also explain the settled and dilute sewage results reported by Burman et al. (1978), but is less useful in explaining their river water results. Although regrowth of faecal streptococci in the environment is evidently rare, it should be noted that it may occur in the nutrient-loaded waste water of vegetable processing plants (Mundt et al. 1966).

Streptococcal survival, like that of other indicator organisms and pathogens, is also likely to be considerably influenced by water temperature. Geldreich & Kenner (1969) found that all their test organisms, including the faecal streptococci, died off more rapidly at 20°C than at 10°C. Burman et al. (1978) reported that faecal streptococci died off faster than *E. coli* at both high (20–30°C) and low (5–8°C) temperatures.

The literature also contains conflicting reports on the resistance of faecal streptococci to chlorination. For example, Evans et al. (1968) reported that chlorination reduces numbers of total coliforms, faecal coliforms, and faecal streptococci with equal efficiency. However, Silvery et al. (1974) found that faecal streptococci were more resistant to chlorination than coliforms.

Data on the survival of faecal streptococci in soil water and ground water are rare. McFeters et al. (1974), using survival chambers immersed in a tank through which ground water was continually pumped, found that *S. faecium*, *S. faecalis*, and *S. durans* all exhibited superior survival characteristics to coliforms, but that coliform survival exceeded that of *S. bovis* and *S. equinus*. Using a similar experimental set-up, Keswick et al. (1982) also found that an unidenti-fied streptococcus from sewage exhibited superior survival to *E. coli* and to coliphage f2. Bitton et al. (1983), using ground water stored in glass flasks, found that *S. faecalis* survived better than *Salmon-ella typhimurium* and *E. coli* and had survival characteristics similar to those of coliphage f2.

In New Zealand, Guy & Small (1976) investigated the survival of faecal coliforms and faecal streptococci in soil and drainage water. In drainage water, coliform concentrations tended to increase over 48 h but faecal streptococci concentrations decreased. Faecal coliform concentrations appeared to decrease when stored at 5, 10, and 15°C over 4 days but increased at 20°C. Faecal streptococci concentrations decreased at all temperatures over 4 days. Guy & Visser (1979) found that *S. bovis* survived in sterilised slurries of New Zealand soils for periods ranging from 1 day to 1 month, compared to periods ranging from 25 days to 3 months for *E. coli*.

Survival in sea water

Slanetz & Bartley (1965) compared the survival of coliforms, faecal coliforms, and faecal streptococci in dialysis bags suspended in sea water. The bags contained both dilutions of untreated sewage effluent and pure cultures in sterile sea water. Bacterial analyses were conducted on the bag contents at 1–2-day intervals over periods of up to 12 days. Coliform and faecal coliform numbers in bags containing sewage increased 3–10 times over the first 1–2 days and remained at these levels for about 7 days before the onset of die-off. There was no increase in the numbers of faecal streptococci in the bags and a rapid decrease occurred within 4–6 days. The authors concluded that faecal streptococci would make the most accurate indicators in sea water containing organic material "... since they do not multiply in such water and they show appreciable die-off rates within a 2–3-day period". However, the spectral characteristics of the dialysis bags (i.e., which sunlight wavelengths would have been cut off) were not presented in this study.

Other workers have also noted regrowth of coliforms in nutrient-enriched sea water. For example, Hanes et al. (1964) and Hanes & Fragala (1967) documented the apparent multiplication of some coliforms and *E. coli* in both fresh and saline sewage-polluted waters. In contrast, unpolluted sea water appears to be toxic to other coliforms (Savage & Hanes 1971). Hanes & Fragala (1967) and Foxworthy & Kneeling (1978) reported that faecal streptococci (which were termed "enterococci") survived longer in sea water than coliforms and *E. coli*. Hanes & Fragala (1967) concluded that "the enterococcus organism may be the best indicator of recent pollution in sea water".

As noted in studies of faecal streptococcal survival in effluents and freshwaters, the absence of streptococcal regrowth in the marine studies by Slanetz & Bartley (1965) and Hanes & Fragala (1967) is probably attributable to the more demanding nutrient requirements of these organisms.

Studies carried out by Colwell and her associates (Xu et al. 1982; Grimes et al. 1986) have demonstrated the phenomenon of "non-culturability" of pathogenic bacteria in sea water. Using pure cultures of *E. coli*, it has also been demonstrated that non-culturability is more pronounced (by about 100 times) on selective than on non-selective media (Gray-Young et al. 1991), a finding which has been confirmed in both natural fresh and marine water samples (Green et al. 1991). Recent studies have demonstrated this effect in *Streptococcus faecalis* added to drinking water (Byrd et al. 1991). It is possible that, in addition to interferences from the accompanying microflora (Volterra et al. 1986), reported differences in faecal streptococci recovery rates on different media could be related to their ability to recover the "non-culturable" population.

Vasconcelos & Schwartz (1976) investigated bacterial survival in sea water using a stirred diffusion chamber. They found that *S. faecalis* survived longer than either *E. coli* or *Enterobacter aerogenes* but that all three species survived well (a 10-fold reduction or less) over a period of 7 days. However, the chamber did not appear to permit the unrestricted entry of sunlight, so the relevance of these data to exposed sea water conditions is doubtful.

Evison & Tosti (1980) investigated the survival of faecal streptococci in Bay of Naples sea water. Field sampling suggested that natural attenuation of faecal streptococci was less than for *E. coli*. In plexiglass chambers immersed in sea water the results were variable: T_{90} values both longer and shorter than of *E. coli* were recorded. In laboratory studies with sea water, these authors found that survival of both *E. coli* and faecal streptococci was inversely related to temperature but that faecal streptococci consistently survived longer than *E. coli* at all temperatures. Survival of faecal streptococci in the dark was considerably better than of *E. coli*. These authors referred to preliminary laboratory data which showed salmonellae species were also more resistant to sunlight than *E. coli* and concluded that "... faecal streptococci must be seriously considered as a more suitable indicator than *E. coli* in sea water".

Why faecal streptococci should exhibit superior survival characteristics in sea water is not completely clear, although they are known to be tolerant of NaCl. Hanes & Fragala (1967) found that indicator bacteria die-off was related to the concentration of sea water but that enterococcal die-off was less affected than die-off of coliforms and *E. coli*. Mitchell & Chamberlin (1978) also showed coliforms to be sensitive to salinity, surviving more poorly in sea water (T_{90} of 2.2 h) compared to fresh water (T_{90} of 62.3 h). However, the phenomenon of superior survival of streptococci in sea water may, in part, be the result of superior resistance to sunlight (see below).

Effects of sunlight on faecal streptococci survival

Although the inactivating effects of sunlight on bacteria in receiving waters are well known, the relative contribution of different sunlight wavelengths to these effects is still not well understood. Gameson & Gould (1975) have reported that half of the lethal effect of sunlight is caused by wavelengths below 370 nm whereas the remainder results from light wavelengths of 370–400 nm. However, Fujioka et al. (1981) maintained that visible light was more important than UV light with respect to bacteriocidal effects on both coliforms and faecal streptococci in sea water.

Of the range of sunlight wavelengths reported to inactivate bacteria in receiving waters, ultraviolet (UV) light is probably the most often investigated. However, Harris et al. (1987) noted that because of the difficulty of accurately measuring UV dose, even under laboratory conditions, much of the data published on resistance of specific micro-organism to UV light has been inconsistent and contradictory. In addition, the sensitivity of micro-organisms to UV radiation can be influenced by many factors, including the growth medium, the stage of the culture, and the strain of the micro-organism (Morton & Haynes 1969; Chang et al. 1985).

There have been few studies specifically investigating the effects of UV light on faecal streptococci. However, Harris et al. (1987) found that *S. faecalis* was significantly more sensitive to UV inactivation than *E. coli*, although the overall differences between the two species became negligible when both were subjected to photoreactivation. The slopes of the UV dose/survival response curves for both bacteria levelled off at higher doses, indicating the existence of interfering factors at these levels. The authors suggested that these factors could include inactivated cells shielding the remaining active cells.

Fujioka & Narikawa (1982) found that both faecal coliforms and faecal streptococci in raw sewage stored in clear glass or translucent polyethylene containers were resistant to the effects of sunlight. However, under the same conditions of storage, exposure to sunlight of sewage diluted 1 : 100 in sea water resulted in the inactivation of 90% of faecal coliforms within 38 min whereas 90% of faecal streptococci were not inactivated after 2 h.

Why streptococci should be less susceptible than faecal coliforms to sunlight is not clear. Aggregation of streptococcal cells in chains or clumps may have some shielding effect. In addition, Kapuscinski & Mitchell (1981) demonstrated that, in *E. coli*, sunlight damages the catalase enzyme system which is necessary for the degradation of peroxide. Such an effect may not apply to faecal streptococci, which are catalase negative.

FAECAL STREPTOCOCCI AS INDICATORS IN RECREATIONAL WATERS

A satisfactory recreational water quality indicator organism is one that can be quantitatively related to the potential health hazards resulting from recreational use of that water. This implies that the indicator will have the same source specificity as pathogens, be easy to detect, and exhibit similar but superior survival characteristics (Elliot & Colwell 1985). Many micro-organisms have been suggested as potential indicators and the topic has been reviewed by several authors, including Cabelli (1978) and Stanfield (1985). Cabelli (1979) maintained that indicators can be satisfactory related to health hazard except where faecal matter is discharged from small populations or where there is an epidemic in the contributing population.

Information on the suitability of indicators such as faecal streptococci may be obtained from both epidemiological studies and laboratory and field studies of the comparative survival of the indicator and pathogens. However, guidelines and standards are ideally derived from epidemiological studies.

The development of microbiological standards in North America, Europe, and some other countries has been reviewed in USEPA (1986), McNeill (1985), and Salas (1986). A summary of the North American developments, based on the excellent review by Salas (1986), with particular regard to the faecal streptococci, is given below. The development of microbiological standards in New Zealand, which have closely followed those of the United States, has been reviewed by McBride (1990).

Epidemiological studies

Epidemiological studies are difficult and expensive to carry out. The most effective studies are "prospective", i.e., water quality surveys are coincident with the water use survey: thereafter the users (and a non-user control group) are followed up to ascertain whether an illness occurred. However, more often, epidemiological surveys take a "retrospective" form, i.e., they are carried out after the water use occurred and do not contain relevant water quality information. They are usually not tied to particular locations and periods of use. Since they rely on readily available medical records, only notifiable diseases are examined, even though most illnesses contracted during bathing are not notifiable.

Some early epidemiological studies in the United States (Stevenson 1953) and the United Kingdom (PHLS 1959) were deficient in design (Cabelli et al. 1983) and, in the United Kingdom investigation, this may have contributed to the failure to show a link between bathing and notifiable disease incidence. However, since then a significant amount of epidemiological evidence has accumulated on the relationship between bathing in polluted waters and illness. Many of these studies have been reviewed by

Shuval (1986). Cabelli (1988, 1989), Jones & Kay (1989), and Jones et al. (1990) have also listed reports of prospective epidemiological studies of marine beaches in England (Brown et al. 1987), France (Foulon et al. 1983), Spain (Mujeriego et al. 1982), other European beaches (WHO 1986), Egypt (Cabelli 1980; El Sharkawi & Hassan 1982), Israel (Fattal et al. 1986), Hong Kong (Cheung et al. 1988; Holmes 1989) and the United States (NJDH 1988). Similar studies have been conducted in freshwaters in Canada by Seyfried et al. (1985a, 1985b), Connecticut (Cabelli 1988), and France (Ferley et al. 1989). These studies have all demonstrated an excess rate of minor disease incidence amongst bathers when compared to non-bather control groups. The diseases observed in these epidemiological studies have generally been associated with infections of the gastro-intestinal tract; ear, nose, and throat (ENT); and the skin. The "Cabelli protocol" (i.e., the USEPA prospective epidemi-ological study design) is currently being tested at New Jersey beaches (Jones & Kay 1989).

Several problems have been identified with the "Cabelli protocol". The identification of illness by personal perception (based on one telephone interview) has been found to be prone to error (Jones et al. 1990). In addition, Fleisher (1991) seriously questioned the validity of water quality criteria based on pooled data from dissimilar areas and noted that the statistical analysis of the USEPA data was not carried out using techniques found to be robust for infectivity studies.

Development of faecal streptococci-based indices

The events leading to the recent emergence of faecal streptococci (specifically, the enterococci) as recreational water quality indicators began with the first scientific investigations into the relationship between contact recreation and the incidence of disease. These were conducted by the American Public Health Association in the early 1920s and were based on swimming pools and other bathing places (Simons et al. 1922).

Moore (1975) attributed the early application of bacterial guidelines to sea water to Winslow & Moxon (1928): in a study of New Haven Harbour, United States, where typhoid fever was attributed to swimming in grossly polluted water, they cautiously suggested that the total coliform count of bathing waters should not exceed 100/100 ml. A much higher maximum total coliform count of 10 000/100 ml was suggested by Coburn (1930), who referred to a bathing area where counts exceeded this concentration without evident ill health in bathers. Ludwig (1983) noted that the California standard of 1000/100 ml was developed during the 1940s when studies showed that, when 80% of coliform counts remained below this concentration, beaches remained aesthetically satisfactory with no visual evidence of sewage pollution.

Cabelli et al. (1983) stated that the United States (total) coliform limit of 1000/100 ml "apparently developed from two sources: the predicted risk of salmonellosis as obtained from calculations made by Streeter (1951) on the incidence of *Salmonella* species in bathing waters, and attainability as determined by Scott (1951) from microbiological surveys conducted at Connecticut bathing beaches". According to Salas (1986), this Connecticut standard was then adopted by many other United States agencies.

The absence of a consistent rationale pertaining to recreational water standards led the United States Public Health Service (USPHS) to conduct a major series of epidemiological studies between 1948 and 1950. These were aimed at directly assessing the health risk of bathing in polluted waters and were conducted at bathing beaches on Lake Michigan, the Ohio River, and Long Island Sound. The findings (Stevenson 1953) were that statistically significant epidemiologically detectable health effects at levels of 2300 and 2700 coliforms/100 ml were demonstrated by the 1948 Lake Michigan and 1949 Ohio River studies. The 1950 Long Island Sound showed no coliform-disease relationship.

Subsequent work at the Ohio River site showed that about 18% of the coliforms were faecal coliforms (Cabelli et al. 1983) and would therefore indicate that detectable health effects would occur at a faecal coliform level of about 400/100 ml. To add a safety factor, the National Technical Advisory Committee to the United States Water Pollution Control Administration (NTAC 1968) halved this figure and developed a national faecal coliform guideline of 200/100 ml.

Various criticisms of this particular guideline were made (e.g., Henderson 1968; Cabelli et al. 1975; Moore 1975), and the Committee on Water Quality Criteria (CWQC 1972) concluded that they were not prepared to make a specific recommendation on "the presence or concentration of micro-organisms in bathing water because of the paucity of valid epidemiological data".

Subsequently, the USEPA (1976) presented faecal coliform guidelines essentially the same as those prepared in the NTAC (1968) document. However, Salas (1986) noted that the rationale for the USEPA guideline was not the studies reported by Stevenson

(1953) but the relationship between faecal coliform densities and the frequency of *Salmonella* sp. isolations in surface waters.

In an effort to correct the perceived deficiencies in the earlier epidemiological studies, the USEPA carried out an extensive series of studies during the 1970s at marine and freshwater bathing beaches. These studies have been summarised well in USEPA (1986) but, because they are central to the current interest in enterococci as indicators in recreational waters, they are briefly outlined below.

Prospective epidemiological studies of swimmers and non-swimmers at several paired (non-polluted and "barely acceptable") United States beaches were used. The marine studies were conducted in New York, Boston, Massachusetts, and at Lake Pontchartrain (near New Orleans). In the New York and Boston studies, the "barely acceptable" beaches were contaminated from multiple point sources, usually of treated and disinfected effluents. The freshwater studies were conducted on Lake Erie and Keystone Lake (near Tulsa, Oklahoma). Some 26 700 respondents were used in the marine studies and 45 500 in the freshwater studies.

Two types of symptom were defined: (i) Gastroenteritis (GI), which entailed vomiting, diarrhoea, stomach ache or nausea, and (ii) highly credible gastroenteritis (HCGI), in which the victim reported vomiting, diarrhoea (with a fever or symptoms severe enough for him/her to remain home, remain in bed or seek medical advice) or stomach ache or nausea with a fever.

The principal finding was that a significant "Swimming-Associated Gastroenteritis Rate" was always observed at the more polluted beaches but not at the less polluted beaches. It was concluded that "... there is a measurable and significant risk of acute gastroenteritis associated with swimming in marine waters which are contaminated with human faecal wastes to levels less than those which would be aesthetically unacceptable" (Cabelli 1988). Skin, ear, eye, and respiratory symptoms were attributed to cross-contamination between bathers in conditions of heavy use and poor water exchange.

The results of the marine bathing beach studies were reported by Cabelli (1982) and Cabelli et al. (1983) and those of the freshwater studies by Dufour (1984). In the early marine studies, on two New York City beaches, several indicators were measured to determine which ones were best correlated with gastroenteritis in swimmers. This work indicated that, for marine beaches, enterococci showed the strongest relationship to gastroenteritis, *E. coli* was a very poor second and there was little, if any, correlation between swimming-associated GI symptoms and mean total coliform or faecal coliform densities. At freshwater beaches, illness was best correlated with *E. coli* densities, but the correlation with enterococci was still almost as high as at marine beaches. The correlation coefficients of all the indicator organisms tested are shown in Table 1. Thereafter, only enterococci, *E. coli*, and faecal coliforms were used in the subsequent marine and freshwater beach studies.

It was estimated from the results of the USEPA research that use of the existing faecal coliform standard (200/100 ml) would result in 8 illnesses per 1000 swimmers at freshwater beaches and 19 illnesses per 1000 swimmers at marine beaches. New bacteriological criteria, which approximated this currently "accepted" level of risk were calculated (USEPA 1986). The outcome was the recommendation (USEPA 1986) that "states should begin the transition process to the new indicators", which are *E. coli* or enterococci for freshwater bathing beaches, and enterococci for marine water beaches. A summary of the USEPA criteria for bathing beaches is presented in Table 2.

Table 1 Correlation coefficients for swimming associated gastroenteritis rates against mean indicator densities at marine and fresh water bathing beaches. Marine data from trials conducted at New York City Beaches 1973–75 (Cabelli 1976); freshwater data from Cabelli (1982). (Modified from: USEPA 1986.)

		Correlation coefficients	
Type of water	Indicator	Data by summers	Data by grouped trials*
Marine	Enterococci	0.75	0.96
	E. coli	0.52	0.56
	Klebsiella	0.32	0.61
	Enterobacter/Citrobacter	0.26	0.64
	Total coliforms	0.19	0.65
	Clostridium perfringens	0.19	0.01
	Pseudomonas aeruginosa	0.19	0.59
	Faecal coliforms	–0.01	0.51
	Aeromonas hydrophila	–0.09	0.60
	Vibrio parahaemolyticus	–0.20	0.42
	Staphylococci	–0.23	0.60
Fresh	Enterococci	0.74	
	E. coli	0.80	
	Faecal coliforms	–0.08	

*Groups of trials (days) with similar mean indicator densities during a given summer

Relationship of faecal streptococci counts to viral hazard

Although the symptoms of the illnesses recorded in the USEPA study were reported, no attempt was made to ascribe particular aetiological agents to these symptoms. Cabelli (1980) suggested that the most likely causes of illness in swimmers at the New York (marine) bathing beaches were rotaviruses and parvo-like viruses. There is now strong epidemiological evidence to suggest that, as yet, poorly characterised small round viruses (SRVs) are associated with waterborne outbreaks of gastroenteritis (IAWPRC 1991). The suggestion by Cabelli (1980) implies that the survival of viruses in marine environments is more closely paralleled by that of the enterococci than that of other indicators, such as total coliform and faecal coliform bacteria.

As yet, there do not appear to have been any studies reliably linking disease incidence with virus density. This has, in part, been attributed to the low efficiency of methods for the isolation and enumeration of viruses (IAWPRC 1991). For example, in an examination of coastal water samples using an electron microscope, Børsheim et al. (1990) found that virus concentrations exceeded bacterial counts by an order of magnitude, suggesting that current virus recovery techniques may not allow accurate estimation of virus concentration. In addition, the viruses most strongly associated with faecal-oral transmission by water, such as Small Round Viruses (SRVs), hepatitis A, and non-A, non-B hepatitis viruses, are particularly difficult to recover (IAWPRC 1991).

Some workers (e.g., Edmond et al. 1978; Lucena et al. 1982; Payment et al. 1982) have reported no correlation between virus incidence and indicator bacteria numbers, whereas Fattal et al. (1983) found significant correlations between viruses and coliform, faecal coliform, and faecal streptococci concentrations. As with other types of pathogen, which tend to be present in receiving waters intermittently, or at least in widely variable concentrations, it may not be realistic to expect a consistent relationship between enteric viruses and any one indicator organism. Many of these studies used polioviruses, because there are satisfactory techniques available for their enumeration and they are present in large numbers in sewage due to vaccination programmes. However, in a study of virus survival in sea water, Gironés et al. (1989) found that polioviruses did not survive as well as rotoviruses, which are known to cause gastro-intestinal disease.

There have been very few studies specifically comparing the survival of enteric viruses and bacterial indicators in marine waters. However, Fattal et al. (1983) investigated the relative "die-away" of bacteria and enteric viruses by comparing the concentrations of each at increasing distances from a marine sewage outfall. They found that total and faecal coliform bacteria disappeared more rapidly than the viruses, whereas the reduction in faecal streptococci concentrations was similar to viral "die-away". Other workers have reported the isolation of viruses from marine water when indicator bacteria have been present in very low numbers (Gerba et al. 1979). However, as with the studies comparing viral incidence and bacterial indicator counts, uncertainty over both the pathogenicity of the viruses investigated and the efficiency of virus recovery techniques means that these results are difficult to interpret.

Alternatives to faecal streptococci

Although the USEPA investigation indicated the superiority of enterococci (and *E. coli* for fresh waters) as disease risk indicators, alternatives continue to be identified. For example, staphylococci were found to be the best overall health risk indicator in the studies in Israel (Fattal et al. 1986), Hong Kong (Cheung et al. 1988), and in the study by Calderon et al. (1991). In the Hong Kong study, *E. coli* was also a reasonable indicator, but there was a relatively low correlation between total symptom rates and enterococci or faecal streptococci. In the Canadian study (Seyfried et al. 1985a, 1985b), the Cabelli protocol was followed at freshwater bathing sites in Ontario. Although there were significant statistical relationships between disease incidence and both faecal coliforms and faecal streptococci, total staphylococci were the best indicator of disease risk. However, some of the studies where staphylococci were the best indicator of disease risk were conducted at heavily used and/or poorly

Table 2 Geometric mean densities for bathing waters recommended by USEPA (a full description of the criteria, including confidence limits for different levels of use, is given in USEPA 1986). Based on not less than five samples equally spaced over a 30-day period, the geometric mean of the indicator bacteria densities should not exceed the following:

Freshwater	*E. coli*	126 per 100 ml
or:*	Enterococci	33 per 100 ml
Marine water	Enterococci	35 per 100 ml

*USEPA recommends that only one indicator should be selected for use flushed beaches, where cross-infections amongst bathers were a strong possibility. The statistical approach used by Calderon et al. (1991) has also been questioned (McBride pers. comm.). The relationship between bather density and staphylococci was confirmed in an extensive study of Hong Kong beaches (Cheung et al. 1991), where *Escherichia coli* was used to monitor faecal pollution. Thus, streptococci may still be a superior indicator of sewage contamination.

Bacteriophages, because of their potential for indicating viral hazard, are also the subject of investigation as alternative faecal pollution indicators, particularly in chlorinated effluents. Cabelli (1988) referred to unpublished data showing that F male-specific bacteriophages are more resistant to waste water chlorination and survive in marine waters longer than coliforms or enterococci, and suggested that, where a beach is polluted by a chlorinated effluent, the gastroenteritis health risk may be underestimated by the enterococcus count. Havelaar & Nieuwstad (1985) have also demonstrated that very high doses of chlorine are required to reduce concentrations of F male-specific phages. Phages of the anaerobic bacterium *Bacteroides fragilis* also show promise as indicators, because they appear to be specific to human faeces and to be more closely correlated than coliphages with counts of both enteroviruses and rotaviruses (Jofre et al. 1986). These and other studies of bacteriophages as faecal indicators have been reviewed by IAWPRC (1991).

FAECAL STREPTOCOCCI AS INDICATORS IN SHELLFISH

Compared to the available data on the accumulation of coliforms, there has been little investigation into the accumulation of faecal streptococci in shellfish tissues. However, Slanetz et al. (1964) correlated the results of coliform, faecal coliform, and faecal streptococci concentrations in shellfish with the presence of *Salmonella* sp. and some enteric viruses. In a further study, Slanetz et al. (1968) found that concentrations of coliforms, faecal coliforms, and faecal streptococci per 100 g of oyster tissue were 10–20 fold higher than the equivalent numbers per 100 ml of the surrounding sea water. The relative concentrations of the different indicator bacteria were similar in the shellfish and the sea water.

Slanetz et al. (1965) found that the ratio of coliforms to faecal streptococci in seawater samples was approximately 10 : 1 and the ratio of *E. coli* to faecal streptococci was 2.4 : 1. The equivalent ratios in oysters were 20 : 1 and 3 : 1, respectively. The faecal streptococci isolated included *Streptococcus faecalis*, *S. faecalis* var. *liquefaciens*, *S. faecium*, and various biotypes. The species of coliforms and faecal streptococci isolated from sea water above the oyster beds were similar to those isolated from the oysters.

The United States regulations pertaining to shellfish harvesting waters were not affected by the USEPA epidemiological investigations. They remain based on coliform bacteria, although in "Quality Criteria for Water" (USEPA 1976) it was recommended that the standards should be amended to become based on faecal coliforms.

At the time that the USEPA "Ambient Water Quality Criteria for Bacteria" report (USEPA 1986) was published, the USEPA was co-sponsoring (with the National Oceanic and Atmospheric Administration) research into the effectiveness of the enterococci and *E. coli* as indicators of the quality of shellfish harvesting waters. The United States Food and Drug Administration (USFDA) was also reviewing these studies. If a correlation is demonstrated between gastro-intestinal disease and the consumption of raw shellfish from waters with measured densities of enterococci and *E. coli*, then it is likely that a change to the new indicators for shellfish harvesting waters will be recommended. Preliminary findings have been reported which suggest that there may be a correlation between illness and enterococcus counts in shellfish flesh at harvest (Sobsey 1989).

Recently, the USFDA National Advisory Committee on Microbiological Standards for Raw Molluscan Shellfish recommended the abandonment of faecal coliforms in growing waters and shellfish tissues, although these do not appear to have been replaced by enterococci.

IMPLICATIONS FOR NEW ZEALAND BATHING WATER STANDARDS

Recently, a document entitled *Provisional Microbiological Water Quality Guidelines for Recreational and Shellfish-Gathering Waters in New Zealand* (McBride et al. 1992) has been circulated under the auspices of the New Zealand Department of Health. This document maintains that the specific numerical standards from USEPA (1986)—based on enterococci (or *Escherichia coli* in fresh waters)—are, with some statistical modifications, "the most suitable criteria" for New Zealand. By implication, these numerical criteria are intended to meet the national narrative standards for bathing waters outlined in the Resource Management Act 1991. However, there are several potential problems associated with the introduction of the United States standards to New Zealand.

First, the USEPA marine criteria are based on a specified "acceptable level of risk"—19 cases of illness per 1000 bathers. However, there is no information available on "acceptable risk perception" among New Zealand bathers, health authorities, or water managers. Such information would be an important prerequisite to either adopt overseas criteria or to implement any local epidemiological investigation(s).

Probable differences in immunity levels between United States and New Zealand bathers (acknowledged in the *Provisional Guidelines*) could also invalidate the USEPA numerical criteria in New Zealand waters. For example, in an Egyptian study (Cabelli 1980), it was found that enterococcus counts of 620 per 100 ml and 3400 per 100 ml were associated with 19 cases of gastro-enteritis per 1000 bathers in tourists and local residents, respectively (Cabelli 1989). Thus, enterococcus counts of nearly 18 and 100 times the counts calculated for United States beaches were required to produce the same health effects among tourists and local bathers, respectively. The tourists were apparently from other areas in Egypt and, although 5 times more susceptible to the sewage pollution than local bathers, evidently still had a far higher immunity level than equivalent United States bathers.

Cabelli (1980) suggested that the main reason why enterococci were the best indicators in the United States study was that, compared, for example, to faecal coliforms, their survival rate more closely approximated that of enteric viruses, which were assumed to be the principal cause of disease amongst the bathers. However, the beaches were largely polluted by chlorinated effluents. In contrast, most New Zealand effluents are non-chlorinated, which means that pathogen : indicator ratios, particularly virus : indicator ratios, are likely to differ from those in the United States studies.

Animal inputs are also a potential threat to the valid application of the United States guidelines to New Zealand bathing waters (this is also acknowledged in the *Provisional Guidelines*). The relatively high ratio of farm animals to humans in New Zealand, together with significant inputs from animal processing effluents, suggest that local waters are likely to contain very different ratios of streptococcus species. Noonan et al. (1988) commented on problems associated with the application of the new indicators to New Zealand meatworks effluents. Although the public health significance of animal effluent pollution sources is, as yet, poorly understood, they probably constitute less of hazard than human sources. Thus, depending on sewage treatment levels, proportions of animal inputs and environmental factors such as sunlight and water clarity, the public health significance of specified enterococcus levels is likely to differ between New Zealand beaches. Furthermore, in a recent critical evaluation of the USEPA studies, Fleisher (1991) questioned the grouping of different types of beaches in the USEPA studies and the statistical basis of the enterococcus standards, and recommended the retention of standards based on faecal coliforms.

Some studies conducted since the USEPA investigations have supported the use of enterococci, but have not necessarily found the numerical criteria appropriate. These include the Egyptian study (Cabelli 1980), discussed above, and a French investigation (Ferley et al. 1989), both of which involved untreated sewage discharges. Although the latter study involved river waters and retrospective interviews with bathers to establish disease incidence, faecal streptococci (the authors maintained that their methodology, in effect, isolated enterococci) were more strongly correlated with disease risk than faecal coliforms. The final result was a suggested faecal streptococcus criterion of 20 per 100 ml.

Other studies have not supported enterococcal criteria. For example, a large-scale study conducted at Hong Kong Beaches (Cheung et al. 1988) showed that the best enteric infection predictor was *E. coli*. Others have shown health risk to be best indicated by staphylococci (e.g., Seyfried et al. 1985a, 1985b; Calderon et al. 1991), although levels of these bacteria may also be related to bather density in semi-enclosed waters. However, in the studies by Seyfried et al. (1985a; 1985b) faecal streptococci were counted, rather than enterococci.

There also appears to be doubt within the United States about the widespread applicability of the USEPA criteria. In a recent survey of bathing water standards in United States coastal states, Chasis et al. (1992) reported than nine out of 22 states were continuing to use faecal coliforms as indicators, five were using total coliforms and faecal coliforms, two had adopted enterococci while retaining total/faecal coliforms, and only six had exclusively adopted enterococci. Of the eight states that had partially or exclusively adopted enterococci, only three had applied the USEPA numerical criterion for marine waters (35 per 100 ml). The others had applied modified enterococci criteria ranging from 7 to 156 per 100 ml. The survey did not include freshwater bathing areas. Standards for the remaining 28 states are currently being collated (Geldreich pers. comm.).

The conflicting findings from various countries, and the apparent reluctance of many North American states to follow the USEPA recommendations, suggest that any changes in recreational water quality indicators in New Zealand should be approached cautiously. An Auckland-based pilot epidemiological study was scheduled for the 1992-93 summer (McBride pers. comm.), but investigations which would produce nationally applicable results are likely to be beyond the resources of individual New Zealand public health or water management agencies. The results of studies currently being carried out in the United Kingdom (Jones et al. 1990), where effluents tend to have limited treatment and, in coastal areas, are usually discharged via ocean outfalls, may be relevant to the New Zealand situation. The British studies hope to avoid some of the perceived deficiencies in the "Cabelli protocol" and identify the health risk which actually occurs at beaches which either do or do not meet the standards set by the European Community.

There would also be a range of minor problems associated with a transfer to enterococcus standards in New Zealand. Local ocean outfall design criteria are currently based on faecal coliform die-off rates (to ensure adequate time/distance separation from bathing beaches). If future marine bathing water standards are to be expressed in terms of enterococci, reliable information on enterococcal survival under conditions (particularly ambient sunlight regimes) in New Zealand marine and estuarine waters would be required. In addition, most microbiological records of bathing waters held by regional councils are based on faecal coliforms. To validate trends, a change to enterococci (or *E. coli*) would be best preceded by a period in which both faecal coliforms and the new indicator were enumerated, in order to establish the relationship between them.

The USEPA (1986) recommendations have the advantage of being linked to a specified methodology, in contrast to the wide range of methods currently employed by New Zealand laboratories for the enumeration of coliform and faecal coliform bacteria (Pyle 1981). However, membrane filtration enumeration of enterococci and *E. coli* is somewhat more time consuming than for faecal coliforms. In addition, as noted by Wood (1988), if the USEPA enterococcal standards are to be effectively implemented, a set of standard protocols for New Zealand laboratories may be needed.

SUMMARY AND CONCLUSIONS

1. In spite of extensive investigation, the faecal streptococci have so far largely failed to fulfil their potential as faecal pollution source (i.e., human versus animal) indicators. Many qualifications accompany the use of the faecal coliform to faecal streptococci (FC : FS) ratio. Although reported results have been variable, the FC : FS shift method (in which the ratio changes under sample storage) may warrant further investigation. The streptococcal species identification approach is frequently inconclusive, mainly because *S. faecium* and *S. faecalis* are found in both human and animal faeces, and *S. bovis*, which characterises animal sources, is a poor survivor in the environment. Standard biochemical streptococcal species identification methods tend to be time-consuming and unreliable. To date, DNA-based methods have not been useful in distinguishing human from animal sources.
2. Reports of streptococcal survival in the environment compared to other indicators are often inconclusive or contradictory. However, most studies have shown that faecal streptococci outlive coliforms and faecal coliforms in effluents and aquatic environments.
3. Epidemiological studies conducted by the USEPA have shown that enterococcus concentrations are better correlated than faecal coliform concentrations with the disease risk associated with bathing in sewage-polluted waters. These results, which implied that the enterococci better represented viral hazard, led to the current USEPA recommendations that the enterococci (or *E. coli* in freshwaters) should become the basis of bathing water standards in the United States These recommendations have been promoted as suitable for New Zealand in a set of provisional guidelines circulated under the auspices of the Department of Health.
4. Any move to introduce the USEPA recommended bathing water standards to New Zealand should be approached cautiously. Recent investigations elsewhere in the world have suggested that the USEPA results may not be universally applicable. There is also lack of information on streptococcal concentrations and species profiles in local effluents and natural waters. With New Zealand's high animal to human ratios and few chlorinated effluents, the epidemiological applicability of the USEPA recommendations (which was largely based on waters receiving chlorinated effluents

from metropolitan areas) is doubtful. Current epidemiological studies in the United Kingdom may provide more data of greater relevance to metropolitan areas in New Zealand.

5. If microbiological inputs to marine outfall design criteria are to change from coliforms and faecal coliforms to enterococci, there will be a need for better information on their relative concentrations in New Zealand effluents and receiving waters, the local epidemiological relevance of the enterococci, and survival rates under ambient light and water clarity conditions.

ACKNOWLEDGMENTS

The contribution of L. Sinton to this review was funded by the New Zealand Foundation for Research, Science and Technology (FoRST), as part of the DSIR Marine and Freshwater Ocean Outfalls programme, and the DSIR Geology and Geophysics Groundwater programme. A. Donnison was supported by a grant from FoRST, and C. Hastie was supported by Lincoln University (as a Master's student) and by FoRST. Dr M. J. Noonan (Lincoln University), Mr K. J. W. Taylor (Canterbury Regional Council), and the journal's referees provided valuable critical comment.

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