Decay Rates of Faecal Indicator Organisms and Pathogens: Use of Microcosm and *In Situ* Studies For the Estimation of Exposure Risk In Recreational Waters

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ABSTRACT

This three year study compared the decay of the commonly used faecal indicator organisms *Escherichia coli*, enterococci and coliphage in coastal water and sediment using laboratory based microcosms with that for the pathogens *Salmonella typhimurium* and *S. derby*. Results from the laboratory study were validated with in-situ decay rates of faecal coliforms observed at a recreational coastal site following a significant stormwater event. Results demonstrated that for both indicators and pathogens, greater decay was observed in the overlying water compared with the surface sediment layer. In general, the decay rates of *Salmonella* spp. were greater than either enterococci or coliphage in overlying water and sediment. Decay rates of *E. coli* were similar to *Salmonella* spp. in overlying water, although greater in sediment. Increased temperature resulted in an increased decay rate for all organisms in the overlying water (and to a lesser extent in the surface sediment layer). Results from a 12 month investigation into faecal coliform concentrations at recreational coastal sites also demonstrated higher concentrations of faecal coliforms in sediment compared with overlying water. Sediments were therefore illustrated to act as a reservoir for both faecal indicator and pathogenic microorganisms and may represent an increased exposure risk if these organisms are resuspended back into the water column during recreational activities. Using measured decay rates and available dose-response data, a quantitative microbial risk assessment (QMRA) utilising Monte Carlo simulation was undertaken to estimate the risk of infection to *Salmonella* spp. following exposure to recreational coastal water subject to a range of faecal contamination levels. For exposure to recreational water of extremely poor quality (10^6 CFU 100 mL^{-1}) the maximum risk of infection (95% CI) on the day of the contamination event was above 2.0×10^{-1} and remained above 1×10^{-3} for three days following the initial high concentration.

KEY WORDS

Recreational water, sediment, faecal indicators, *Salmonella*, risk assessment

INTRODUCTION

Bathing in recreational coastal waters subject to high levels of faecal contamination is known to increase the risk of disease, in particular gastroenteritis, but also non-enteric diseases caused by respiratory, eye, ear and skin infections (Kay *et al*. 1994; Fleisher *et al*. 1996). In Australia, the level of faecal contamination in recreational coastal waters is estimated by the enumeration of faecal coliforms and *Escherichia coli* from the water column (NHRMC, 1990). These indicator organisms were traditionally selected, as they

were believed to behave similarly to pathogenic organisms of faecal origin when released into the environment. The ability of faecal coliforms to act as indicators of enteric viral and protozoan pathogens has, however, been questioned (Koh *et al*. 1994; Ferguson *et al*., 1996).

Studies have demonstrated that greater numbers of organisms are associated with suspended particles and sediment than are present in the water column under many in-situ conditions (Davies *et al*. 1995; Goulder, 1977; Obiri-Danso and Jones, 2000; Shiaris *et al*. 1987). The interaction of faecal coliforms with sediment particles may enhance survival by reducing exposure to various stressors such as light and predation, as well as providing an increased availability of nutrients. Pathogenic microorganisms associated with sediment particles have the possibility of being resuspended into the water column due to natural turbulence or human recreational activity (Irvine & Pettibone, 1993; Hood & Ness, 1982; Obiri-Danso & Jones, 2000). Enumeration of faecal coliforms from the water column only may therefore underestimate the total bacterial load and potentially the risk of recreational exposure.

This 3-year study compared the survival of commonly used faecal indicator organisms (faecal coliforms, *E. coli* and enterococci as well as somatic coliphage) with survival of two pathogens (*Salmonella typhimurium* and *Salmonella derby*) to determine the effectiveness of the indicator organisms at predicting the risk of survival of bacterial and viral pathogens. The project also investigated the persistence of a range of indicator and pathogenic organisms in both water and sediments, and examined the role of sediment type and temperature in survival.

METHODOLOGY

Recreational Coastal Sites

The study sites were Henley Beach South, Onkaparinga River and the Port Adelaide River along the greater metropolitan Adelaide coastline, South Australia. Sediment type was classified by particle size analysis and organic carbon content (Sheldrick & Wang, 1993; Tiessen & Moir, 1993).

Survival Experiment

Stock bacterial suspensions of *E. coli* (ATCC 25922), *Enterococcus faecium* (ATTC 19434), *Salmonella typhimurium* (ATCC 14028) and a clinical strain of *S. derby* (isolated from a human stool sample; Institute of Medical and Veterinary Science, Adelaide) were prepared by inoculation into 10 mL nutrient broth (Oxoid) and incubated overnight at 37°C. Cells were harvested by centrifugation at 2,500 g for 10 minutes. The pelleted samples were resuspended in phosphate buffered saline (PBS), centrifuged and resuspended in PBS.

Coliphage suspension was prepared using host *E. coli* (FCC 84) cells inoculated into nutrient broth (Oxoid) and incubated overnight at 37°C. A single coliphage plaque previously isolated from raw human sewage was added to this suspension and incubated for a further 24 h at 37°C. To remove host bacterial cells, the suspension was centrifuged and the supernatant (containing coliphage) was filtered to further remove any bacterial cells present. All stock suspensions were maintained at 4°C until use.

Intact sediment core microcosms were inoculated by adding 500 µL of stock coliphage suspension and 50 µL of stock *E. coli*, *Enterococcus faecium*, *S. typhimurium* and *S. derby* suspension into the overlying water (500mL). Control water columns were similarly

inoculated. In the laboratory, the microcosms were maintained in a water bath at a constant temperature (10°C, 20°C or 30°C).

Enumeration of Microorganisms

Microorganisms were enumerated from both overlying water and sediment on days 0 (1 h after inoculation), 1, 2, 7, 14 and 28 following inoculation. Sediment samples were prepared by removing the top 10 mm of sediment from the intact core, of which 25 g was placed into 75 mL of 0.1% peptone in a sterile beaker. Sediment was sonicated for 10 min (x2), to separate bacteria from sediment particles (Craig *et al*. 1999).

E. coli, enterococci and coliphage were isolated from overlying water and sediment as described by the methods of Craig *et al*. (2001). Briefly, *E. coli* was enumerated by membrane filtration and incubation on membrane lauryl sulphate (MLS) agar and enterococci by the Enterolert™ defined substrate method using 97-well Quantitrays (IDEXX Laboratories, USA). Coliphage were isolated by a double-agar overlay method. *S. typhimurium* and *S. derby* were isolated by membrane filtration (47 mm diameter, 0.45 µm pore size; GN-6, Gellman) and incubation on xylose lysine desoxycholate (XLD) agar (Oxoid) at 37°C for 24 h. A representative number of presumptive colonies were confirmed as *Salmonella* spp. using a salmonella latex agglutination test kit (Serobact; Medvet Science, Adelaide).

The decay rate constant (k) for all organisms was calculated as the slope of the line when $\log_{10}(N_t/N_0)$ was regressed against time, where N_t is the number of bacteria at time t and N_0 is the number of bacteria at time 0 (Davies and Evison, 1991).

In-Situ Sampling

In conjunction with the laboratory-based microcosm experiment, a seasonal study was conducted at the same coastal sites to enumerate indicator organisms (faecal coliforms and *E. coli*) present in sediments and their respective overlying waters. Faecal coliforms and *E. coli* were enumerated and confirmed by the membrane filtration method described earlier (Australian Standard AS 4276.7, 1995).

In-situ decay rates were determined following a high rainfall event at Henley Beach South which resulted in a high level of faecal contamination due to the increased flow from the adjacent Torrens River outlet. Prior to this, there had been little or no flow of the Torrens River (a total of 1 mm for the previous 26 days; Bureau of Meteorology, Adelaide). Faecal coliforms were enumerated from overlying water and sediment daily for 10 days following the high rainfall event.

Quantitative Microbial Risk Assessment

Exposure assessment was based on the initial concentration of *Salmonella* spp. in the recreational coastal water, the rate of decay at a specific temperature (in this case a mean water temperature of 20°C) and the volume of water consumed per exposure as described in the following equation:

$$n = C_0 \times 10^{kT} \times V_i \qquad \text{(Eq 1)}$$

where N in the number of organisms ingested, C_0 is the initial concentration of organisms in the overlying water (number of organisms mL^{-1}), k is the decay rate (day^{-1}) as determined by the microcosm experiments, T is time (d), and V_i is the volume of water (mL) ingested. The volume of water ingested per exposure (one hour) was assumed to be 20 – 50 mL with a uniform distribution (Ashbolt *et al*. 1997).

A pooled Beta-Poisson dose-response relationship for non-typhi *Salmonella* spp. was used to estimate risk of infection (Fazil 1996):

$$P_{inf} = 1 - (1 + [(d/N_{50}) \times (2^{1/\alpha} - 1)])^{-\alpha} \quad \text{(Eq 2)}$$

where P_{inf} is the probability of becoming infected after ingestion of *n Salmonella* cells with α and N_{50} being constants specific for the pathogen. For *Salmonella* spp., α was determined to be 0.3126 and N_{50} was 23600 (Fazil and Haas, 1996).

To take into account uncertainty within various model inputs Monte Carlo simulation was used, with 10,000 iterations per scenario (@Risk, version 4.0.5, Pallisade Corporation, 2000). The values and distributions for the various inputs were selected from both previously published data and results from this current study (Table 1).

Table 1 Summary of input variables and distributions.

Input Variable	Distribution	Value
[1]*Salmonella* spp:FC	Triangular	10^{-5} min 10^{-4} mode 10^{-3} max
[2]Decay rate (k)	Triangular	-0.94 min -0.91 mode -0.87 max
Volume of water ingested	Uniform	20 – 50 mL per exposure

[1]Range of reported Salmonella:FC ratios (Van Donsel and Geldreich, 1971; Baudart *et al*. 2000; Dionisio *et al*. 2000).
[2]Determined from laboratory based microcosm experiments for decay of *S. derby* in overlying water incubated at 20°C.

To describe a range of possible faecal contamination levels, three scenarios of high, medium and low levels of faecal coliforms were selected with lognormal distribution (parameters displayed in Table 2). A 'high' level of 1×10^6 CFU 100 mL^{-1} (poor water quality) was chosen to represent an incident of high faecal contamination due to stormwater pollution following a high rainfall event (as described in this study) or an accidental release of untreated wastewater. The low level of 150 CFU 100 mL^{-1} (good quality) is the current median guideline faecal coliform concentration for primary contact recreation in Australia (NHMRC 1990).

Table 2 Lognormal parameters for faecal coliform frequency.

Recreational Water Quality	Lognormal Distribution Parameters		
	μ	σ	Geometric mean (No. 100 mL^{-1})
Poor	6	0.8	1×10^6
Average	3	0.8	1,000
Good	2.18	0.5	150

μ= mean; σ = std. deviation

Statistical Analysis

Statistical analysis was undertaken using one-way ANOVA and significance expressed at $P \leq 0.05$ (SPSS version 10.0.5, 1999). Bivariate relationships between faecal indicator and pathogenic organism decay rates were examined using Pearson's correlation coefficient (r). All results were expressed as the mean ± standard deviation of three determinations.

RESULTS AND DISCUSSION

Table 3 shows the characterisation of sediment types at the three sites selected for the microcosm study, to compare indicators with pathogens in both the water column and sediment. Henley Beach consisted mainly of sand and Port Adelaide consisting of greater proportions of silt, clay and organic carbon. Onkaparinga sediment could described as intermediate.

Table 3. Particle size analysis. Particles sizes for sand 2 – 0.2 mm; silt 0.2 mm – 2 μm; clay < 2 μm. All results normalised to 100%.

SITE	%SAND	%SILT	%CLAY	%ORGANIC C
Henley Beach	98.47	0.08	1.41	0.05
Onkaparinga	95.48	1.26	2.91	0.35
Port Adelaide	83.05	4.24	10.33	2.38

Results of a 12 month sampling period from March 1999-2000 at Port Adelaide (site with the greatest proportions of silt and clay) are represented in Figure 1. These data clearly demonstrated the protective effect of sediment on survival of faecal coliforms, which were found to be well above the recreational guideline value for overlying water on all sampling occasions (150 CFU 100 mL^{-1}; ANZECC, 1992). The median concentration of faecal coliforms over the sampling period in the sediment was 2.1×10^4 cfu 100 g^{-1} (range 2.3×10^3 to 2.2×10^5 cfu 100 g^{-1}) compared with 61 cfu 100 ml^{-1} (range < 1 to 695 cfu 100 ml^{-1}) in the overlying water. No correlation was identified between the number of faecal coliforms in the sediment with that in the overlying water for any site ($P > 0.05$). In Australia, there are currently no guideline values for sediments in recreational waters and so this research is attempting to identify the potential risk to recreational users from re-suspension of sediments during recreational activity.

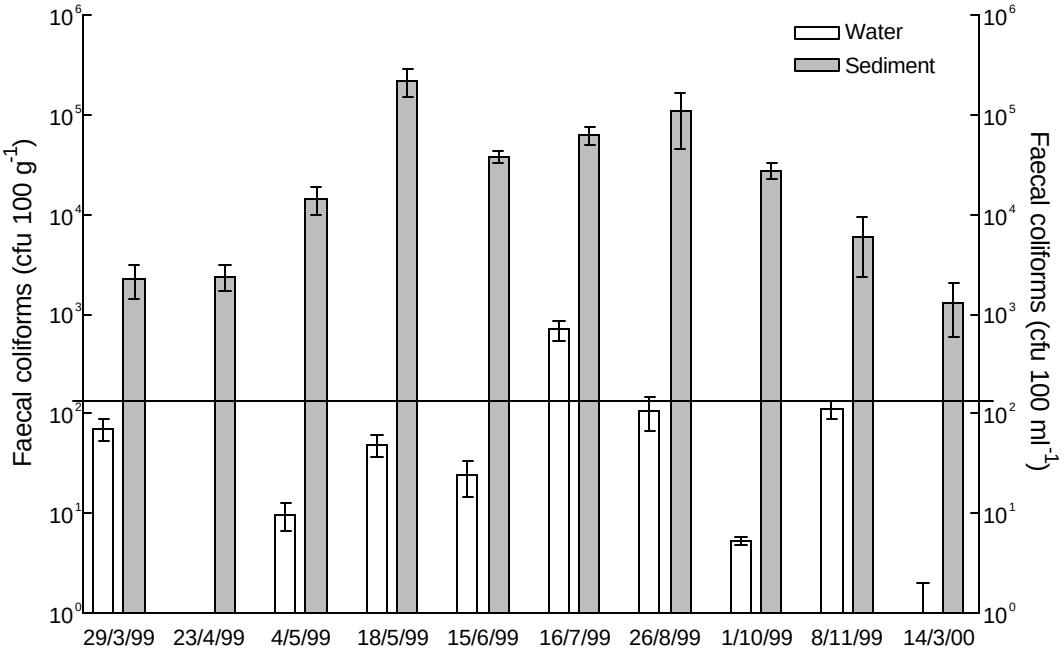

Fig. 1 Concentration of faecal coliforms (mean ± standard deviation) in overlying water and sediment from Pt Adelaide River (n=3). Line represents current NHMRC median faecal coliform guideline concentration for primary contact recreation (from 5 samples).

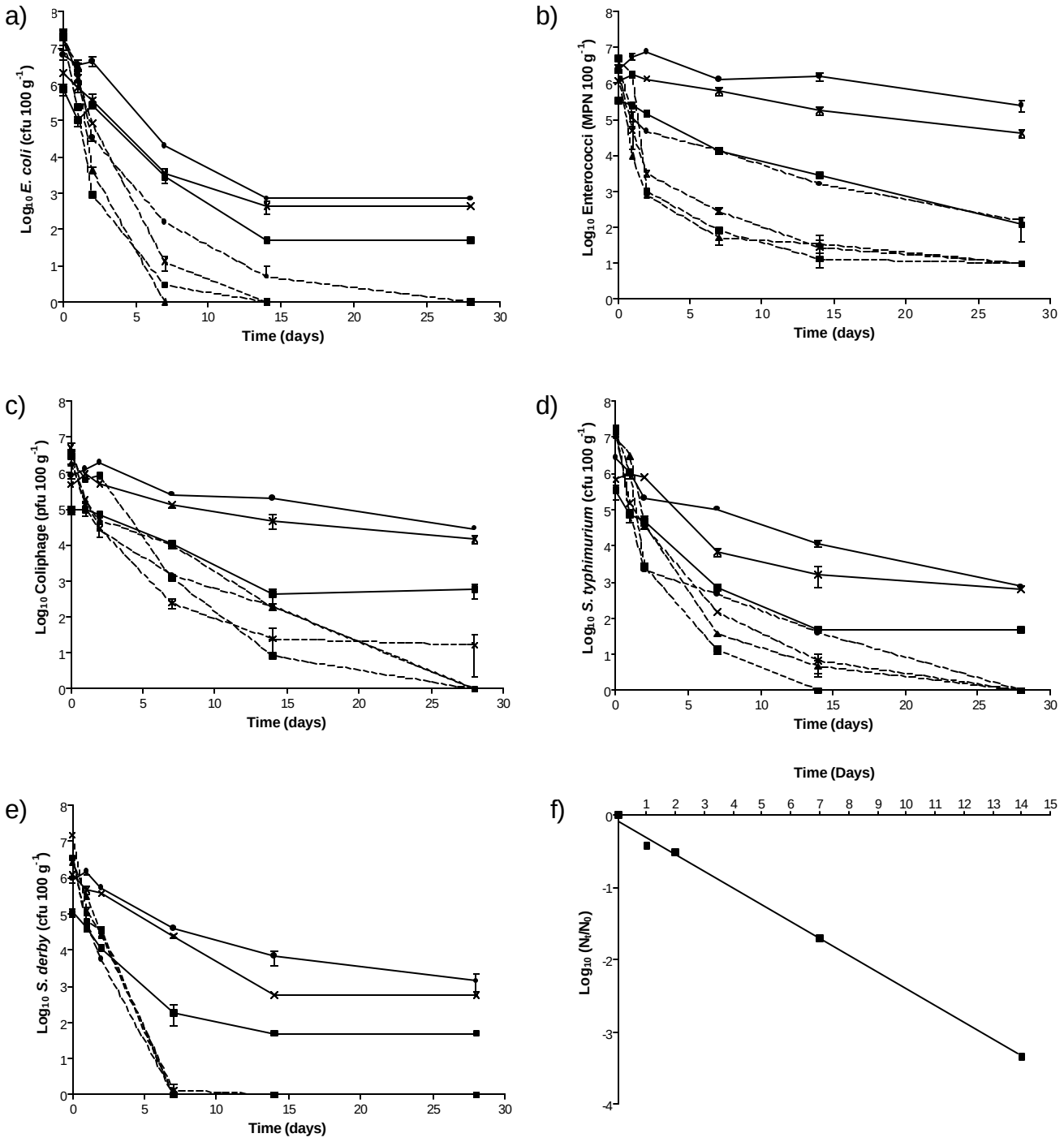

Fig. 2 Survival curves for *E. coli* (a), enterococci (b), coliphage (c), *S. typhimurium* (d) and *S. derby* (e) in sediment (—) and water (----) from Henley Beach ( ), Onkaparinga ( ), Pt. Adelaide ( ) and control column containing water only ( ) all incubated at 20°C (mean ± SD). Figure 2 (f) an example of an exponential decay plot used to calculate decay rate constants (*S. derby* decay in Onkaparinga sediment incubated at 20°C).

Figure 2 demonstrates a comparison of survival curves for the faecal indicators *E. coli*, enterococci, coliphage and the pathogens *S. typhimurium* and *S. derby* at 20ºC from all sediment types and shows that survival is enhanced in sediment compared to the water column and particularly in sediment consisting of small particle size and high organic carbon content. The effect of temperature was also important in that survival was enhanced at lower temperatures (10ºC compared with 30ºC; Craig *et al*. 2001). Coliphage and enterococci decay was generally less than *E. coli* and *Salmonella* spp. at all sites.

Decay rate constants were calculated for all organisms tested at each site and temperature (see Fig 1d). An example of decay rates for *E. coli* at a range of temperatures (10 - 30ºC) in Henley Beach (sandy) sediments and water are shown in Table 2. In general, results of decay rates plotted as $\log_{10}$ ($N_t.N_0^{-1}$) against time were significantly linear ($P < 0.05$) indicating under most conditions, organisms followed first-order kinetics. Although no direct correlations were observed between decay rates in overlying water of *Salmonella* spp. and the faecal indicator organisms investigated ($P > 0.05$; data not shown), decay of the pathogenic microorganisms more closely resembled that of *E. coli* than the other indicators.

A period of high rainfall in the summer of 2000 provided an opportunity to compare the survival of faecal contaminants from stormwater output via the Torrens River onto Henley beach, with microcosms from the same site over a similar period. Table 4 shows a comparison between the decay rate constants of faecal coliforms which were calculated over a range of temperatures (10 - 30ºC) in the microcosms and these were compared with the decay rates from the environmental samples (average temp 26.1 ± 1.6°C).

Table 4 Decay rate constants (*k*; d^{-1}) and the regression coefficient (r^2) of the linear fit for *E. coli* determined using laboratory based microcosms containing sediment from Henley Beach and in-situ decay rates observed at Henley Beach; **P* <0.05.

	10°C		20°C		26°C		30°C	
	k	r^2	*k*	r^2	*k*	r^2	*k*	r^2
Microcosm water	-0.47	0.80*	-0.89	0.86			-1.74	0.95*
Microcosm sediment	-0.32	0.93*	-0.32	0.98*			-1.11	0.95*
In situ water					-0.78	0.80*		
In situ sediment					-0.60	0.96*		

Modelling the microcosm decay rate against temperature using an exponential relationship,

$$k = \alpha.e^{\beta T} \qquad \text{(Eq 3)}$$

where,

k = decay rate constant
T = temperature (°C)

resulted in values for α and β in overlying water of –0.24 and 0.07 respectively ($r^2 = 1.00$; Figure 3). For microcosm sediment, α was –0.05 and β was 0.11 ($r^2 = 0.94$). Using this exponential model, the estimated decay rate for *in situ* samples of overlying water and sediment at Henley Beach following the high rainfall event, was –1.34 and –0.86 respectively. The modelled estimate for *E. coli* decay rate in overlying water was somewhat lower than the measured value, whereas, the modelled estimate for sediment was much closer to the actual measured decay rate. It should be identified, however, that unlike the microcosm experiment undertaken in the current study, the natural environment is not static. Factors such as turbulence and tidal movement, exposure to sunlight as well as additional faecal coliforms inputs during the sampling period will influence decay rates.

These results are extremely useful and could be included in a QMRA model similar to the one described in this study, to estimate risk of infection following exposure to faecally contaminated coastal water at any given temperature and period of time following a contamination incident.

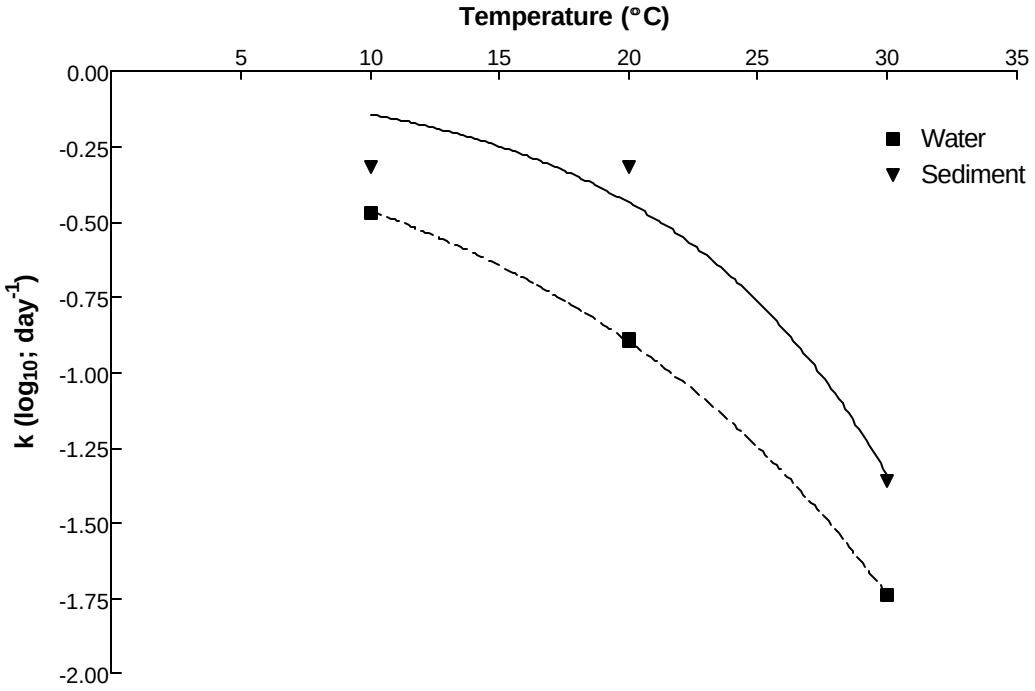

Figure 3 Relationship between decay rate (*k*; d^{-1}) of *E. coli* in Henley Beach sediment and water with temperature (r^2 = 0.94 and 1.00 respectively).

A preliminary quantitative microbial risk assessment for recreational coastal water was performed using currently available data on *Salmonella* spp. occurrence, water consumption during recreation and dose response data for non-typhi *Salmonella* spp. along with decay rates observed from the microcosm experiments. The risk of infection in the days following exposure to different faecal contamination scenarios was estimated and is summarized in Table 5.

It was demonstrated by the preliminary QMRA model described here, that the risk of infection from exposure to poor quality recreational water on the day of the faecal contamination event (day 0) was above 2.0×10^{-1} (> 20% risk of infection) and remained above 1×10^{-3} for three days following the initial high concentration. For the other two water quality scenarios, the maximum probability of infection was below 1×10^{-3} on day 0 (at the 95% level). The mean risk of infection by *S. derby* following exposure to coastal water that meets the guideline faecal coliform concentration was determined to be 6.30×10^{-6} (95^{th} percentile 1.99×10^{-5}). As demonstrated in the microcosm experiments, survival of *Salmonella* spp. was always greater in coastal sediment compared with overlying water. There may therefore be a greater risk of infection due to possible resuspension of this more highly contaminated sediment into the water column. To estimate this likely increase in exposure risk it would be necessary to determine the rate of resuspension of pathogenic microorganisms. The authors acknowledged, however, the estimations determined by this model are exclusive to the risk of infection of *Salmonella* spp. and do not take into account exposure to other bacterial, protozoan or viral pathogens which have been demonstrated to represent greater risk of disease than *Salmonella* spp. (Crabtree *et al*. 1997; Gerba *et al*. 1997; López-Pila and Szewzyk 2000). The model does, however, describe the change in infection risk over time following an incident of faecal contamination.

Table 5 Results of Monte Carlo simulations for estimating risk of infection with *S. derby*.

Recreational Water Quality	Day	Probability of infection for individual exposure		
		5% Level	Mean	95% Level
Low	0	5.74×10^{-4}	4.42×10^{-2}	2.17×10^{-1}
	1	7.18×10^{-5}	9.91×10^{-3}	4.20×10^{-2}
	2	8.87×10^{-6}	1.75×10^{-3}	5.63×10^{-3}
	3	1.10×10^{-6}	2.56×10^{-4}	7.11×10^{-4}
	4	1.36×10^{-7}	3.31×10^{-5}	8.76×10^{-5}
	5	1.66×10^{-8}	4.16×10^{-6}	1.08×10^{-5}
	6	2.04×10^{-9}	5.20×10^{-7}	1.34×10^{-6}
	7	2.49×10^{-10}	6.51×10^{-8}	1.70×10^{-7}
Medium	0	6.34×10^{-7}	3.15×10^{-4}	4.57×10^{-4}
	1	7.91×10^{-8}	4.39×10^{-5}	5.70×10^{-5}
	2	9.80×10^{-9}	5.53×10^{-6}	7.04×10^{-6}
	3	1.21×10^{-9}	6.85×10^{-7}	8.84×10^{-7}
	4	1.49×10^{-10}	8.47×10^{-8}	1.10×10^{-7}
	5	1.86×10^{-11}	1.05×10^{-8}	1.37×10^{-8}
	6	2.28×10^{-12}	1.30×10^{-9}	1.72×10^{-9}
	7	2.82×10^{-13}	1.61×10^{-10}	2.13×10^{-10}
High	0	2.07×10^{-7}	6.30×10^{-6}	1.99×10^{-5}
	1	2.58×10^{-8}	7.83×10^{-7}	2.44×10^{-6}
	2	3.19×10^{-9}	9.72×10^{-8}	3.03×10^{-7}
	3	3.95×10^{-10}	1.21×10^{-8}	3.78×10^{-8}
	4	4.85×10^{-11}	1.50×10^{-9}	4.71×10^{-9}
	5	5.96×10^{-12}	1.87×10^{-10}	5.92×10^{-10}
	6	7.32×10^{-13}	2.34×10^{-11}	7.42×10^{-11}
	7	9.02×10^{-14}	2.92×10^{-12}	9.38×10^{-12}

More data is required for a number of model inputs to more accurately estimate infection risk at recreational coastal sites. Limited data is available for the occurrence of *Salmonella* spp. in coastal waters. This is partly due to their usually low concentrations in such environments, as well as difficulties in enumeration. Under most circumstances, an enrichment step is required and therefore results are often expressed as the presence or absence of *Salmonella* spp. Dose-response data is generally gained using healthy adults and does not take into account previous exposure, immunity or exposure do different strains of *Salmonella* spp. The influence of exposure to low nutrient conditions (and subsequent stress) on the infectivity of *Salmonella* spp. also needs to be investigated. Despite these limitations, QMRA is nonetheless a useful tool in the estimation of infection risk for waters of varying quality and is extremely useful in the management of health risk for recreational coastal sites.

CONCLUSION

This research demonstrated under both laboratory and in situ conditions, coastal sediments may act as a reservoir for both faecal indicator and pathogenic microorganisms released into the coastal environment. If other pathogenic bacterial, viral or protozoan organisms behave more similarly to enterococci or coliphage in particular, the use of prescribed faecal coliform concentrations in the water column alone may underestimate exposure risk. This research demonstrated the usefulness of QMRA in the estimation of infection risk at recreational coastal sites under different faecal contamination scenarios.

ACKNOWLEDGEMENTS

The Australian Commonwealth Government under the Public Health Education Research Program (Environmental Health Specialty Program) funded this project. The authors would like to thank the Institute of Medical and Veterinary Science, Adelaide for supplying the *S. derby* culture, the Dept of Microbiology and Infectious Diseases, Flinders Medical Centre for supplying the *E. coli* and *Enterococcus faecium* cultures and the Biomedical Engineering Department, Flinders Medical Centre, for their support in construction of the microcosms. The authors would also like to acknowledge the Bureau of Meteorology, Adelaide for access to the weather data.

REFERENCES

Ashbolt, N.J., Riedy, C. and Haas, C. N. (1997) Microbial health risk at Sydney's coastal bathing beaches. In: *Proceedings of the 17th Federal Convention of the AWWA, March 1997, Melbourne* **2**, 104-111.

Australian Standard AS4276.7 (1995). Thermotolerant coliforms and *Escherichia coli* – membrane filtration method. Standards Australia, Australia.

Baudart, J., Grabulos, J., Barusscau, J.-P. and Lebron, P. (2000) *Salmonella* spp. and fecal coliform loads in coastal waters from a point vs. nonpoint source of pollution. *Journal of Environmental Quality* **29** 241-250.

Crabtree, K. D., Gerba, C. P., Rose, J. B. and Haas, C. N. (1997) Waterborne Adenovirus - a Risk Assessment. *Water Science & Technology* **35** (11-12), 1-6.

Craig, D. L., Cromar, N. J. and Fallowfield, H. J. (1999). Comparison of techniques for the separation of bacteria from coastal sediments. *Proceedings of Australian Water and Wastewater Association, 18th Federal Convention*. AWWA. 6pp.

Craig, D. L., Fallowfield, H. J. and Cromar, N. J. (2001) Comparison of decay rates of faecal indicator organisms in recreational coastal water and sediment. In*: Proceedings of the International Water Association,* 2nd World Water Congress, Berlin, Germany. IWA. 8 pp.

Davies, C. M. and Evison, L. M. (1991). Sunlight and the survival of enteric bacteria in natural waters. *Journal of Applied Bacteriology* **70,** 265-274.

Davies, C. M., Long, J. A. H., Donald, M. and Ashbolt, N. J. (1995) Survival of fecal microorganisms in marine and freshwater sediments. *Applied and Environmental Microbiology* **61,** 1888-1896.

Dionisio, L. P. C., Rheinheimer, G. and Borrego, J. J. (2000) Microbiological pollution of Ria Formosa (South of Portugal). *Marine Pollution Bulletin* **40** (2), 186-193.

Fazil, A. M. and Haas, C. N. (1996) A quantitative risk assessment model for *Salmonella*. In: *Proceedings of the 1996 Annual Conference, American Water Works Association*. Volume C: 367-390.

Ferguson, C. M., Coote, B. G., Ashbolt, N. J. and Stevenson, I. M. (1996) Relationships between indicators, pathogens and water quality in an estuarine system. *Water Research* **30,** 2045-2054.

Fleisher, J. M., Kay, D., Salmon, R. L., Jones, F., Wyer, M. and Godfree, A. F. (1996) Marine waters contaminated with domestic sewage: nonenteric illnesses associated with bather exposure in the United Kingdom. *American Journal of Public Health* **86** (9), 1228-1234.

Gerba, C. P., Rose, J. B., Haas, N. and Crabtree, K. D. (1996) Waterborne Rotavirus - a Risk Assessment. *Water Research* **30** (12), 2929-2940.

Goulder, R. (1977) Attached and free bacteria in an estuary with abundant suspended solids. *Journal of Applied Bacteriology* **43,** 399-405.

Hood, M. A. and Ness, G. E. (1982). Survival of *Vibrio cholerae* and *Escherichia coli* in estuarine waters and sediments. *Applied and Environmental Microbiology*, **43**(1), 578-284.

Irvine, K. N. and Pettibone, G. W. (1993). Dynamics of indicator bacteria populations in sediment and river water near a combined sewer outfall. *Environmental Technology*, **14**(6), 531-342

Kay, D., Fleisher, J. M., Salmon, R. L., Jones, F., Wyer, M. D., Godfree, A. F., Zelenauch-Jacquotte, Z. and Shore, R. (1994) Predicting likelihood of gastroenteritis from sea bathing: results from randomised exposure. *The Lancet.* **344(8927),** 905-909.

Koh, E. G. L., Huyn, J.-H. and LaRock, P. A. (1994) Pertinence of indicator organisms and sampling variables to Vibrio concentrations. *Applied and Environmental Microbiology* **60,** 3897-3900.

López-Pila, J. M. and Szewzyk, R. (2000) Estimating the infection risk in recreational waters from the faecal indicator concentration and from the ratio between pathogens and indicators. *Water Research* **34** (17), 4195-4200.

NHMRC. (1990) Australian guidelines for recreational use of water. National Health and Medical Research Council, Canberra.

Obiri-Danso, K. and Jones, K. (2000). Intertidal sediments as reservoirs for hippurate negative campylobacters, Salmonellae and faecal indicators in three EU recognised bathing waters in North West England. *Water Research*, **34**(2), 519-527.

Sheldrick, B. H. and Wang, C. (1993). Particle size distribution. In*: Soil sampling and methods of analysis*. Carter, MR (ed). Lewis Publishers, USA

Shiaris, M. P., Rex, A. C., Pettibone, R. W., Keay, K., McManus, P., Rex, M. A. and Ebersole, J. (1987) Distribution of indicator bacteria and *Vibrio parahaemolyticus* in sewage-polluted intertidal sediments. *Applied and Environmental Microbiology* **53,** 1756-1761.

Tiessen, H. and Moir, J. O. (1993). Total and organic carbon. In: *Soil sampling and methods of analysis*, M. R. Carter (ed.), Lewis Publishers, USA, pp. 187-191.

Van Donsel, D. J. and Geldreich, E. E. (1971) Relationships of Salmonellae to fecal coliforms in bottom sediments. *Water Research* **5** 1079-1087.