The Effects of Temperature and Sediment Characteristics on Survival of *Escherichia Coli* in Recreational Coastal Water and Sediment

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The survival of the faecal indicator organism Escherichia coli (E. coli) *in recreational coastal water and sediment was investigated using laboratory based microcosms. Intact sediment cores from three distinct coastal sites in metropolitan Adelaide were inoculated with known concentrations of* E. coli *and incubated at temperatures of 10°C, 20°C and 30°C. Enumeration of* E. coli *in sediment and overlying water was undertaken by the membrane filtration method on Days 0, 1, 2, 7, 14 and 28 following inoculation. Under all conditions studied, the decay rate of* E. coli *was greater in overlying water compared with the rate in sediment.* E. coli *survival was found to have an inverse relationship with temperature. Sediment characteristics (particle size and organic carbon content) were found to influence the decay of* E. coli. *The decay rate of* E. coli *was demonstrated to be lowest in sediment consisting of small particle size and high organic carbon content. Resuspension of* E. coli *into the water column was demonstrated when the top 10-20 millimetres of sediment was stirred immediately prior to enumeration, mimicking recreational activity. Greatest resuspension of* E. coli *was observed from sediment consisting mainly of sand when incubated at 10°C.*

Key Words: *Recreational Water, Faecal Coliforms,* Escherichia Coli*, Sediment, Survival, Resuspension*

The assessment of microbiological water quality for recreational coastal waters in Australia is primarily undertaken by enumeration of faecal coliforms and *Escherichia coli* from the water column. The presence of these indicator organisms is used to estimate the risk of other pathogenic organisms of faecal origin being present in the water body. Organisms released into the coastal environment are, however, subjected to numerous stressors such as temperature change, salinity, nutrient deficiencies, sunlight and predation (Davies et al. 1995; Mezrioui, Baleux & Troussellier 1995; Özkanca & Flint 1997; Thomas, Hill & Mabey 1999). Studies have demonstrated that organisms associated with suspended particles and sediment contribute greater numbers than in the water column under many in-situ conditions (Davies et al. 1995; Goulder 1977; Obiri-Danso & Jones 2000; Shiaris et al. 1987). It has been suggested that sediment characteristics such as particle size and organic carbon content influences the survival of microorganisms in sediment (Howell, Coyne & Cornelius 1996; Irvine & Pettibone 1993).

The possibility of resuspension of indicator microorganisms under environmental conditions has been discussed by a number of researchers (Irvine & Pettibone 1993; Obiri-Danso & Jones 2000). Studies have demonstrated exposure to recreational waters subjected to faecal contamination results in an increased risk of disease, in particular gastroenteritis (Fleisher et al. 1993; Fleisher et al. 1996; Kay et al. 1994). Resuspension of indicator organisms from the sediment into the water column will therefore indicate an increased

risk of exposure to pathogenic organisms of faecal origin during recreational activity. It is unclear, however, the extent to which bacteria are resuspended from sediment of various compositions into the water column at recreational coastal sites, and thus the increased health risk which may be posed is unknown.

Microcosms of varying complexity have been used to estimate the survival of microorganisms in water and sediment (Bordalo 1993; Brenner, Muller & McBride 1999; Davies et al. 1995; Gerba & McLeod 1976; Thomas, Hill & Mabey 1999; Wagner-Döbler et al. 1992). Unlike many other studies, this study utilised intact, non-sterile sediment cores to determine the persistence of *E. coli* in different sediment types incubated at various temperatures. The ability of *E. coli* to be resuspended into the water column following disruption of the sediment was also investigated.

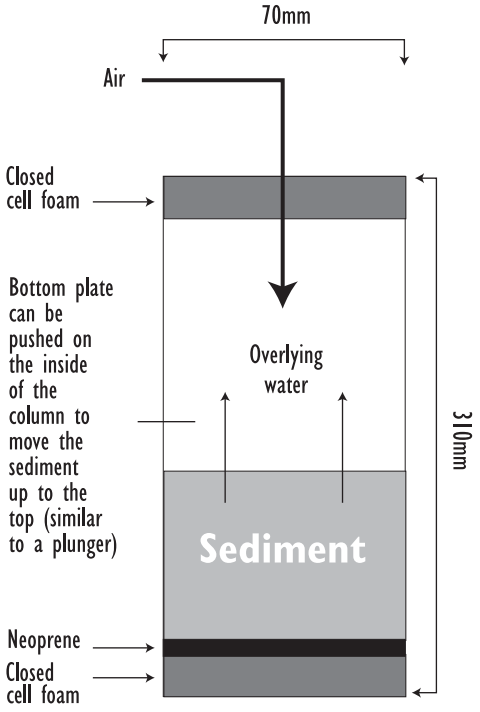
Methodology

Microcosm design

For each microcosm experiment, six intact sediment cores were collected from three sites in metropolitan Adelaide (Henley Beach, Onkaparinga River and Port Adelaide River) which represented distinct coastal sites with different physical characteristics. Sediment was characterised (percentage sand, silt and clay) using the pipette method (Sheldrick & Wang 1993). The percent of organic carbon present in the sediment was determined by the dichromate method (Tiessen & Moir 1993). In addition to these, six cores were filled only with overlying water to act as controls.

Perspex columns (70mm diameter, 310mm length) were inserted approximately 100mm into sediment and overlying water at respective sites. The top of the column was capped with a rubber bung to aid the removal of the core from the sediment. The sediment core was kept in place by inserting neoprene (5mm thick) and closed-cell foam (20mm thick) bungs into the bottom of the core (Figure 1). This prevented the movement of both sediment and water within and from the column.

Figure 1: Microcosm design.



To enable investigation of the effect of sediment composition on *E. coli* survival, the incubations were conducted with identical overlying water to remove the influence of conductivity changes. Columns were then placed in a water bath held at a constant temperature for a maximum of 28 days.

Inoculation of microcosm

A stock bacterial suspension was prepared by inoculating *E. coli* (ATCC 25922) into 10mL of nutrient broth and incubating overnight at 37°C. Cells were harvested by centrifugation at 2,500g for 10 minutes. The pelleted sample was resuspended in 1mL of 0.1M phosphate buffered saline (pH 7.2), washed by centrifugation at 8,000g in a microcentrifuge, followed by resuspension

in 1.5mL PBS. Stock *E. coli* suspension was stored at 4°C until use. Each column was inoculated by adding 50mL of stock *E. coli* suspension to overlying water giving a final concentration of approximately 1 x 10^7 CFU/100mL. Aeration of the overlying water in the column provided adequate oxygenation of the overlying water.

Determination of E. coli survival

Both sediment and water from the columns were analysed on Day 0 (1 hour after inoculation), and after 1, 2, 7, 14 and 28 days incubation. Due to there being a rapid decay of *E. coli* when incubated at 30°C, columns incubated at this temperature were analysed on Day 0, 1, 2, 4 and 7. In an effort to simulate disruption of sediment due to recreational activities, the microcosm experiment was repeated at all three temperatures with the top 10-20mm of sediment being gently mixed immediately prior to sampling. To identify the possible resuspension of *E. coli*, results were expressed as percent partitioning between the sediment and water column under both static and stirred conditions.

The number of *E. coli* present in the water was determined using the membrane filtration method (Australian Standard AS 4276.7, 1995). This involved filtering the overlying water sample through a membrane filter (GN-6, Gellman), placing the filter on membrane lauryl sulphate agar (Oxoid) and incubating at 30°C for 4 hours followed by 44°C for 18 hours. Results were expressed as number of colony forming units (CFU)/100mL.

Sediment samples for microbiological analysis were obtained by first removing the remaining overlying water. The column was then placed on a coring device that extruded the sediment at controlled intervals. The top 10mm of sediment was removed and placed into a sterile beaker. Of this sediment, 25g (wet weight) was placed into 75mL of sterile 0.1% peptone water (Oxoid). This sample was then placed into a sonication bath (Cooper Vision Model 895, 700W, 35kHz) for 10 minutes, stirred and sonicated for a further 10 minutes (Craig, Fallowfield & Cromar 1999). *E. coli* were enumerated from the supernatant removed from the sonicated sample (by the membrane filtration method).

For all microcosm experiments, a known weight of sediment was placed in a pre-weighed dish and dried at 105°C for 24 hours to determine the sediment dry weight. The results for *E. coli* concentration in sediment were expressed as number of CFU/100g (dry weight) sediment. Overlying water from the microcosms was monitored for conductivity and pH over the course of the experiment to indicate the relative stability of the cores.

The decay rate constant (k) 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 & Evison 1991). All analyses were undertaken in triplicate and expressed as the mean ± standard deviation.

Results

Sediment from Henley Beach consisted mainly of sand, with very little silt and clay, and low percentage of organic carbon (Table 1). In comparison Port Adelaide sediment contained much higher proportions of silt, clay and organic carbon. Sediment from the Onkaparinga River could be described as intermediate. At the Port Adelaide River the sediment was distinctly stratified, with the top two centimetre layer comprising anaerobic horizon and below that a layer consisting of a mixture of sand and silt/clay. Only the surface layer of each column was examined, as this would provide the main source of exposure to microorganisms in any recreational activity.

Table 1: Sediment characterisation

Site	%Sand	%Silt	%Clay	%Organic C
Henley Beach	98.54	0.08	1.41	0.046
Onkaparinga	93.20	1.23	2.84	0.346
Pt Adelaide (upper horizon)	81.80	4.18	10.17	2.348

Monitoring of overlying water

conductivity and pH suggested the microcosms remained relatively stable over the sampling period of 28 days. Macro-invertebrates were present in the overlying water throughout the study; thus conditions were sustained similar to that of the natural environment to support in-situ organisms.

Results of the microcosm experiment illustrated at all temperatures there was a greater decay rate of *E. coli* in water compared with sediment for all sediment types (Table 2). It was also demonstrated that the greatest decay of *E. coli* (-2.13 d^{-1}) occurred in the column containing water only at 30°C. Increased temperature resulted in increased decay rates in both sediment and water from all sites, with a significant increase in decay rates in water incubated at 30°C. Of the microcosms containing sediment, greatest decay was observed in sediment from Henley Beach (high sand content). In contrast, persistence of *E. coli* was prolonged in sediment from Port Adelaide (high silt, clay and organic carbon content).

Table 2: Decay rate constants (k; days^{-1}) for E. coli in water and sediment under static conditions.

	Water			Sediment		
	10ºC	20ºC	30ºC	10ºC	20ºC	30ºC
Henley Beach	-0.45	-0.89	-2.40	-0.32	-0.32	-1.36
Onkaparinga	-0.24	-0.52	-2.09	-0.13	-0.22	-0.91
Port Adelaide	-0.21	-0.45	-2.10	-0.14	-0.49	-0.58
Water only	-1.04	-1.03	-2.13			

Note - more negative decay rate constant equals more rapid death of microorganisms.

Results expressed as decay rate constants for the stirred microcosms indicated a lower decay rate of *E. coli* (larger numbers of organisms) in the water column when incubated at 20°C and 30°C compared with static conditions (Table 3). Under static conditions at these higher temperatures, the concentration of *E. coli* in the water column declined very rapidly. In contrast, the concentration of *E. coli* in the sediment remained relatively high (approximately 2-log higher than the water column).

Resuspension of *E. coli* into the water column following disruption of the sediment was most noticeable for sediment from Henley Beach (high sand content) incubated at 10°C (Figure 2). Under static conditions the concentration of *E. coli* in the water column contributed to only 3.0% of the total concentration (sediment and water) by Day 2. After the same period of time under stirred conditions, when the top two centimetres of sediment was mixed immediately prior to enumeration, the concentration of *E. coli* in the water column contributed 85.3% of the total load. The effect of stirring on resuspension was time dependent, as by Day 7 under stirred conditions the number of *E. coli* in the water column had declined to 25.9% of the total number (compared with 3.9% for static). When incubated at 20°C, stirring the Henley Beach sediment resulted in a much more rapid resuspension of *E. coli* into the water column when compared with 10°C in the initial two day period (Figure 3), with most of the inoculum being resuspended on Day 1.

Resuspension of *E. coli* from both Onkaparinga (Figure 4) and Port Adelaide sediment (not shown) into the water column was observed at 10°C. Under static

Table 3: Decay rate constants (k; days^{-1}) for** E. coli **in water and sediment following resuspension by agitation.

	Water			Sediment		
	10ºC	20ºC	30ºC	10ºC	20ºC	30ºC
Henley Beach	-0.48	-0.39	-1.46	-0.21	-0.28	-2.09
Onkaparinga	-0.31	-0.45	-1.29	-0.09	-0.24	-0.84
Port Adelaide	-0.30	-0.36	-1.32	-0.22	-0.24	-0.68
Water only	-0.51	-1.02	-2.59			

Figure 2: Partitioning between sediment and water for Henley Beach sediment incubated at 10ºC.

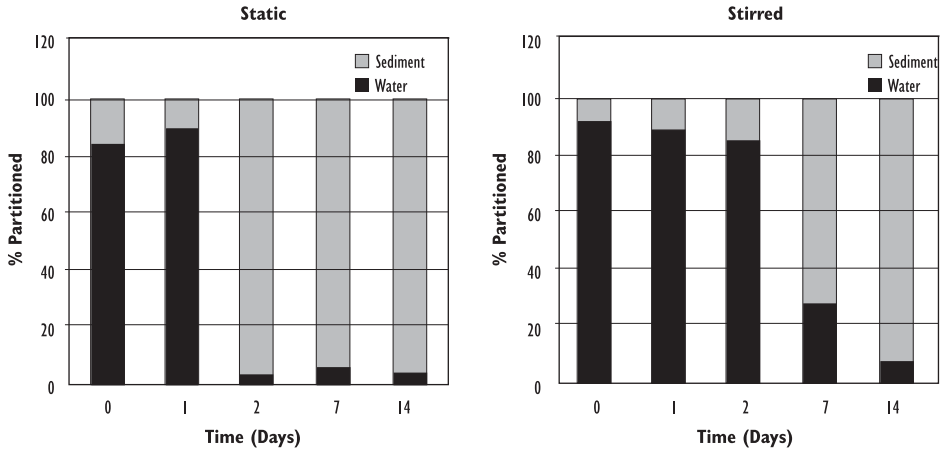


Figure 3: Henley Beach sediment incubated at 20ºC.

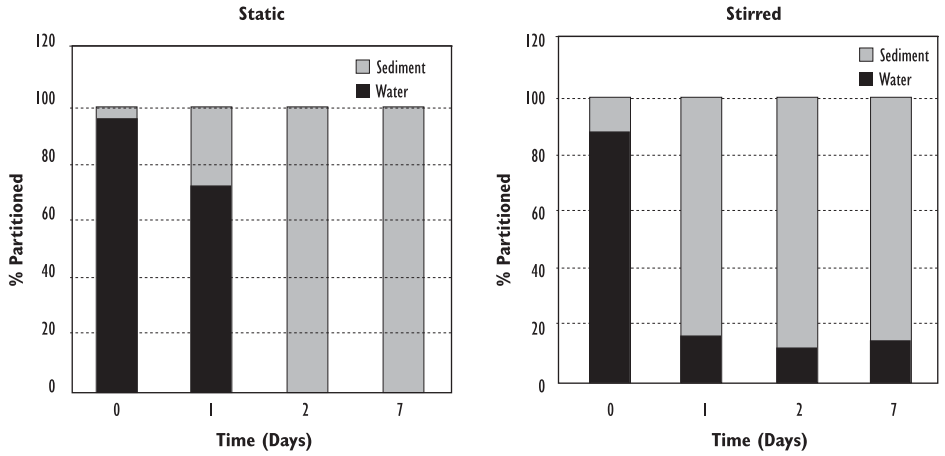
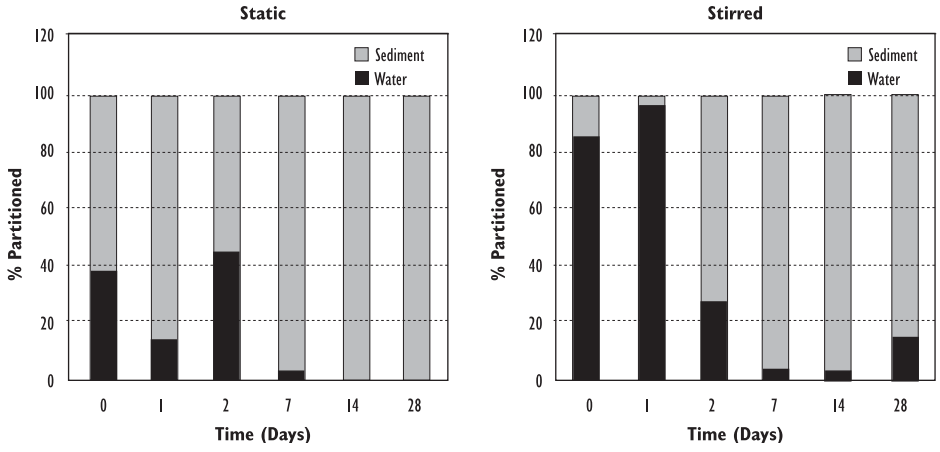


Figure 4: Onkaparinga sediment incubated at 10ºC.



conditions at 10°C the proportion of *E. coli* in these sediments were much greater than in the water column during the initial three days following inoculation. Results demonstrated under stirred conditions an elevated proportion of *E. coli* in the water column during the initial two days following inoculation compared with static conditions (Figure 4). Resuspension was, however, not evident for Onkaparinga or Port Adelaide sediment incubated at 20°C and 30°C (results not shown) when expressed as percent partitioning.

Discussion

Our results demonstrated that the microcosms remained relatively stable over the sampling period of 28 days. A study by Wagner-Döbler et al. (1992) demonstrated conditions of microcosms of a similar design closely resembled in-situ measurements over the same time period.

The highest decay rate of *E. coli* (-2.13 d^{-1}) was observed in the column containing water only incubated at 30°C. A study by Flint (1987) demonstrated a significantly increased decay rate of *E. coli* in unfiltered river water compared with 0.45μm filtered water and autoclaved water, which suggests that competition with natural microorganisms influences survival of *E. coli* in aquatic environments. The water used in these experiments was unfiltered and thus would also experience competition with natural microorganisms, however, numbers of the test organism inoculated into the water column were so large that competition would exert only a very minor influence.

Increases in temperature resulted in increased decay rates in both sediment and water from all sites. The influence of increased temperature was, however, less pronounced in sediment (particularly in the highly organic loaded sediment from Port Adelaide). Özkanca and Flint (1997) demonstrated that viability of *E. coli*, as determined by plate counts, in filtered-autoclaved river water declined faster at 37°C than other temperatures investigated and that numbers of organisms were highest at 4°C. In addition, under starvation, stress respiratory enzyme activity of *E. coli* declined more rapidly at 37°C than at 4°C. This might explain in part the increased effect of higher temperature on decay rates in water compared with sediment for this study. *E. coli* present in the water column would be under more starvation stress due to lower nutrient availability than for those in the sediment.

Greatest survival of *E. coli* was observed in Port Adelaide sediment (high silt, clay and organic carbon content). The influence of sediment type on *E. coli* survival reflects intrinsic differences such as particle size, organic carbon content and nutrients. A study by Irvine and Pettibone (1993) investigating in-situ populations of indicator bacteria in river water and sediment found a weak correlation between bacteria density with particle size and organic carbon content. Howell et al. (1996) also demonstrated a significant relationship between sediment particle size and faecal coliform mortality using a microcosm experiment consisting of 50g non-sterile sediment and 200mL sterile physiological saline. Greatest survival was observed in sediment consisting of small particle size incubated at lower temperatures (4°C > 25°C > 35°C).

It is difficult to compare directly results of this study with other microcosm studies investigating the effect of sediment type on the persistence of *E. coli*. Many previous studies used sterile sediment and water (Brenner, Muller & McBride 1999; Thomas, et al. 1999). The microcosms used in this study consisted of non-sterile, intact sediment cores and therefore included the added pressures to *E. coli* survival of competition with natural organisms and predators. It has also been suggested that the act of sterilising sediment by autoclaving might result in the increased transfer of

nutrients from sediment into the water and this might therefore influence survival in the water column (Gerba & McLeod 1976). Davies and Evison (1991) demonstrated exposure of *E. coli* to natural sunlight had a greater effect of reducing survival in marine water compared with fresh water. As the current study was undertaken without exposure to sunlight, the decay rates observed can be considered a conservative estimate of in-situ rates.

The effect of stirring the sediment immediately prior to sampling resulted in greater numbers of *E. coli* being enumerated from the water column, therefore producing lower overall decay rates compared with static conditions, when incubated at 20°C and 30°C. Results expressed as percent partitioning demonstrated greatest resuspension of *E. coli* into the water column occurred from Henley Beach sediment when incubated at 10°C. Incubation at higher temperatures resulted in a much more rapid resuspension of *E. coli* into the water column, with greatest resuspension occurring on Day 1. This could be explained by the higher decay rate of *E. coli* in the water column at 20°C. Under static conditions, the number of *E. coli* present in the water column was at the limit of detection by Day 7, however, a concentration of 2.9 x 10^3 CFU/100mL remained in the sediment. Therefore, immediately prior to stirring, only a small number of *E. coli* would have been present in the water column of the stirred experiment. Results suggest the organisms detected in the water column following stirring of the sediment were present as a result of resuspension.

The current National Health and Medical Research Council guideline for the microbiological quality of water used for primary contact recreation is a median value (from five samples) of less than 150 faecal coliforms (cfu)/100mL (NHRMC 1990). As this study has demonstrated, the concentration of organisms in the sediment may be one to two orders of magnitude higher than in the overlying water. If this is found to be the case, then this may indicate a significantly increased risk of exposure, and therefore disease. Research is ongoing to determine whether pathogenic and other indicator organisms behave similarly to *E. coli* and we may have to question the effectiveness of setting guideline faecal indicator organism values for the water column alone in estimating health risk at recreational coastal sites.

Conclusion

Results demonstrated a lower decay rate (higher survival) of *E. coli* in coastal sediment compared with overlying water. Sediment characteristics influenced decay rates, with high organic carbon content and small particle size found to be more conducive to *E. coli* persistence. Resuspension of *E. coli* into the water column during stirring of the surface layer was illustrated for sediment consisting of mainly sand, particularly at low temperatures (10°C). The greater resuspension of *E. coli* observed from the sandy Henley Beach sediment may be due to differences in the physical attachment of the organisms to the larger particle size. These findings have significant implications in regard to the estimation of risk of infection during recreational activities. If a recreational coastal site has been subjected to faecal contamination, the concentration of indicator organisms in the water column may rapidly decrease to below guideline values. Results of this study, however, demonstrated that *E. coli* can persist in coastal sediments for prolonged periods of time and be resuspended into the water column under certain conditions.

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