



Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Schang, C;Lintern, A;Cook, PLM;Rooney, G;Coleman, R;Murphy, HM;Deletic, A;McCarthy, D

Title:

Escherichia coli survival and transfer in estuarine bed sediments

Date:

2018-07-01

Citation:

Schang, C., Lintern, A., Cook, P. L. M., Rooney, G., Coleman, R., Murphy, H. M., Deletic, A. & McCarthy, D. (2018). Escherichia coli survival and transfer in estuarine bed sediments. *River Research and Applications*, 34 (6), pp.606-614. <https://doi.org/10.1002/rra.3281>.

Persistent Link:

<https://hdl.handle.net/11343/283962>

Lintern Anna (Orcid ID: 0000-0002-2121-0301)

***E. coli* survival and transfer in estuarine bed sediments**

Christelle Schang¹, Anna Lintern^{1#}, Perran Cook², Graham Rooney³, Rhys Coleman³, Heather M. Murphy⁴, Ana Deletic¹ and David McCarthy¹

1. Environmental and Public Health Microbiology Laboratory (EPHM Lab), Department of Civil Engineering, Monash University, Wellington Road, Clayton 3800, Victoria, Australia.
2. Water Studies Centre, School of Chemistry, Monash University, Wellington Road, Clayton 3800, Australia.
3. Melbourne Water Corporation, La Trobe Street, Docklands 3008, Australia.
4. Water, Health and Applied Microbiology (WHAM) Lab, College of Public Health, Temple University, Philadelphia, 19122, USA

Corresponding Author Information:

Email: anna.lintern@monash.edu

Address: Department of Civil Engineering, Monash University, Wellington Road, Clayton 3800, Victoria, Australia

Running Head: *E. coli* survival and transfer in estuarine bed sediments

HIGHLIGHTS

- Bed sediment resuspension can lead to higher *E. coli* levels in the water column
- *E. coli* can survive for up to two weeks in estuarine bed sediments
- Management strategies should consider bed sediments as a source of microorganisms

KEYWORDS

E. coli, estuary, faecal contamination, resuspension, sediments, survival

ABSTRACT

Bed sediment resuspension is a potential source of faecal microorganisms in the water column of estuaries. As such, it is important to identify the survival of faecal microorganisms in these bed

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/rra.3281](https://doi.org/10.1002/rra.3281)

sediments and understand how bed sediment resuspension impacts the quality of estuarine waters. This study explores the effect of bed sediment resuspension on *Escherichia coli* concentrations in the water column and the persistence of *E. coli* in the water column and bed sediments of the Yarra River estuary in South-Eastern Australia. Using sediment cores, we identified that the resuspension of both surficial sediments (e.g. by tidal movements) and deeper bed sediments (e.g. by large storm events) can increase *E. coli* concentrations in the water column by up to 20 times in estuaries in oceanic climates. Bed sediment resuspension can result in increased *E. coli* concentrations in the water column even up to 24 days after *E. coli* first enters the estuarine water. This study demonstrates that faecal microorganisms, such as *E. coli*, can persist for extended periods in estuarine bed sediments, which may then be re-entrained into the water column via recreational activities, high flow events or tidal fluctuations. If the survival and resuspension processes observed here hold true for pathogenic microorganisms, the resuspension of bed sediments may indeed represent an increased public health risk.

1 INTRODUCTION

Faecal contamination is a significant cause of poor water quality in many urban estuaries across the world (Bernhard et al., 2003; Burton Jr and Pitt, 2001; Walsh, 2000). Microbial pollution of urban estuaries is problematic because it (1) threatens the health of people who come into contact with the estuaries during recreational or commercial activities and (2) undermines the aesthetic function of urban estuaries (Green et al., 2011). Successful mitigation of faecal pollution in urban estuaries requires sound knowledge of the primary sources of faecal microorganisms.

One potentially important source of faecal microorganisms in aquatic systems are bed sediments (Pachepsky and Shelton, 2011). Protected from inactivation and predation, microorganisms such as *Escherichia coli* can survive and potentially grow in bed sediments (Desmarais et al., 2002; Fries et al., 2008; Ishii et al., 2006; Solo-Gabriele et al., 2000) and be resuspended into the water column. Resuspension of sediments can occur due to currents, storms, high winds, boats and dredging (Gerba and McLeod, 1975). Specifically in estuarine and oceanic environments, sediment resuspension can result from movement of the salt wedge and tides. Indeed, bed sediment resuspension has previously been shown to contribute to higher concentrations of faecal

microorganisms within the water column of sub-tropical estuaries (e.g. Fries *et al.*, 2008). However, the survival of *E. coli* in bed sediments of estuaries of other climates (e.g. oceanic) is still not well understood. This distinction is important because the survival rates of *E. coli* depend on parameters such as temperature, nutrients, sunlight, and organic matter (Pachepsky and Shelton, 2011), which can vary significantly depending on the climate of the aquatic system.

Faecal microorganisms can survive, and potentially grow, in both the water column and the bed sediments. This process is particularly important when modelling and estimating human health risks. Interestingly, most water quality models and microbial risk assessments neglect the potential for faecal bacteria to regrow or survive for extended periods (de Brauwere *et al.*, 2014), potentially due to the lack of empirical evidence available for various climates, water (including estuarine) and sediment types.

This study aims to identify how sediment resuspension can affect *E. coli* concentrations in the overlying water column and assess the survival of *E. coli* in the bed sediment in an estuary in an oceanic climate zone. This investigation therefore fills an important gap in our understanding of the impact of sediment resuspension on the level of *E. coli* (and therefore, potentially pathogenic bacteria) on estuarine water quality, and for how long these effects will persist in oceanic climates. Whilst studies of the effects of sediment resuspension on microbial water quality have been conducted on freshwater streams (Grant *et al.*, 2011; Park *et al.*, 2017) and oceans (Phillips *et al.*, 2014; Vogel *et al.*, 2016), there is limited data for estuaries, particularly those located in an oceanic climate zone. More data about indicator bacteria persistence and survival are required under varying environmental conditions representative of conditions when humans are likely to be exposed to pathogens in waterways. As such, this study provides data that are essential for developing assessments of public health risk in estuaries. Given that recreational activities can take place in estuarine systems in oceanic climate zones, a sound understanding of the public health risk posed by persistence of pathogenic bacteria in bed sediments of marine environments, and the resuspension of these bed sediments is critical. The Yarra River estuary, located in the oceanic climate of Melbourne, Australia is used as a case study. This micro-tidal estuary is notorious for its high levels of *E. coli* (e.g. Bate *et al.*, 1997; Daly *et al.*, 2013; Nguyen, 2005). The impact of bed sediment resuspension on estuarine water quality is explored using sediment cores from the Yarra River and

measuring the impact of sediment resuspension on the concentration of *E. coli* in the water column above the core over time to infer survival of *E. coli* in the bed sediments.

2 METHODS

2.1 Site description

This study was conducted in the Yarra River estuary (Figure 1), a 22 km long highly stratified salt-wedge estuary (Beckett et al., 1982) that flows through the city of Melbourne in South-East Australia. Three sampling sites along the estuary were used for this study: Morell Bridge (MB), Scotch College (SC) and Bridge Road (BR). The selection of these sampling sites was based on their differing oxygen and salinity levels (Table 1).

Figure 1 to be placed here

2.2 Sample collection and laboratory analysis

Bed sediment cores were collected from MB, SC and BR on five sampling occasions between November 2010 and March 2011 (Sample Run 1: November 2010, Sample Run 2: December 2010, Sample Run 3: January 2011, Sample Run 4: February 2011, Sample Run 5: March 2011). The temperature, antecedent rainfall and salinity levels at the three sites on the days that the five cores were collected are described in Table 1. The salinity condition at each site for each run was classified as fresh, brackish or saline based on the Venice System ("Symposium on the classification of brackish waters," 1958b). These cores were used to evaluate the effect of bed sediment resuspension on water column *E. coli* levels.

The cores were collected by inserting clean and sterile Perspex columns approximately 200 mm long into the bed sediment, also trapping approximately 50 to 100 mm of the lower water column. These columns were mounted on the end of an extension pole and gently lowered from a boat. The columns had sharp edges to minimize sediment disturbance. Clean and sterile stoppers were placed at both ends of the cores as soon as they were removed from the sediment, to minimise mixing of the sediments and water during transport. At each site, the cores were collected where the water depth was approximately 2 to 3.5 m. On the day of core collection, temperature, pH, dissolved

oxygen (DO), electrical conductivity (EC), salinity and turbidity were measured in situ using a Horiba multi-probe (Horiba, Japan) at the top and bottom of the water column just before the cores were collected. Samples were also taken from the top and bottom of the overlying water column and analysed for nutrients (NH_4^+ , NO_x , FRP) (APHA-AWWA-WEF, 2005) using a Lachat Quick-Chem 8500 (HACH, USA).

The three cores were transported to the laboratory and immediately placed in a water bath, the temperature of which was kept as close as possible to the temperature measured in situ at the bottom of the water column immediately prior to sediment core sampling. The core collection and transport process created some disturbance of the loose sediments present at the top of the sediment layer, which could not be controlled. Therefore, once transported to the lab, the cores were left to settle for 24 hours before the first sample was taken from the water column. The cores were also kept in the dark to mimic the low light exposure at the bottom of the estuary's turbid water column. A small water sample (10 mL) was taken from the overlying water in each core every 48 hours. After the water was sampled, the columns were tilted to roughly 30° to disturb the top layer of sediment (i.e., the loose particles at the surface of the bed sediment) (Figure 2). This tilting did not cause slumping of the sediment due to the cohesive nature of the sediments as a result of their high clay content. The intensity of the tilting reflected visual observations of tidal and boat movements in the Yarra River during our monitoring period and we aimed to produce the same water column colour (i.e., turbidity) created during these actual events. 10 mL of water was taken immediately following this inversion.

After approximately a week of testing the effect of gentle inversion on *E. coli* concentrations in the water column, the columns were vigorously shaken until the water and all the sediment was uniformly mixed (Figure 2). The intensity of the mixing aimed to represent a large resuspension event caused by either high flows, dredging or recreational activities (i.e., boat launching or primary contact recreation). Bathymetry data measured at multiple time points has demonstrated that it is possible for over 20 cm of sediment (i.e., the depth of our cores) to be transported in the Yarra River estuary (Red Mapping, personal communication, October 2012).

Figure 2 to be placed here

A 1 mL sample of this suspension was taken from the water column for analysis. The vigorous shaking was conducted every two to three days for approximately five to nine days (for the November 2010, December 2010, January 2011 and February 2011 runs) and for 22 days during the February 2011 run (Figure 3). All samples were analysed for *E. coli* using the Colilert-24 (IDEXX) method with a 1:10 dilution as per AS4726.21-2005-Method 21 (Standards Australia, 2005). This method has been previously found to reliably quantify *E. coli* levels (Eccles et al., 2004).

Figure 3 to be placed here

2.3 Data analysis

Statistical tests were used to: (1) identify the effect of sediment resuspension on *E. coli* concentrations in the estuarine water; and (2) determine how the *E. coli* concentrations in these sediment resuspensions changed over time.

Identifying the effect of sediment resuspension on E. coli concentrations in the estuarine water. The paired t-test was used to identify whether there were significant differences in *E. coli* concentrations in estuarine water prior to and after both gentle inversion and vigorous shaking (i.e., prior to and after resuspension of surface and deep bed sediments). As the *E. coli* concentrations in the water column before and after gentle inversion and vigorous shaking (grouped across all runs and sites) are not normally distributed ($p < 0.0009$ according to the Shapiro-Wilk test), *E. coli* concentrations were log (base 10) transformed prior to the t-test. The data were more normally distributed after transformation ($p > 0.04$ according to the Shapiro-Wilk test).

To better understand the environmental factors affecting the *E. coli* concentrations in the water column before and after sediment resuspension, Spearman Rank Correlation Coefficients (Spearman, 2010) were used to assess the correlation between the initial *E. coli* concentration in the water column prior to sediment resuspension, the *E. coli* concentration in the water column containing resuspended surface bed sediments, and the water quality of the water column on the days that the cores were collected (temperature, pH, DO, EC, salinity and turbidity, NH_4^+ , NO_x and FRP). This analysis was not conducted using *E. coli* concentrations of the water column containing

resuspended deeper bed sediments because these samples were taken from the cores approximately a week after the quality of the water column was measured.

Determining how the E. coli concentrations in these sediment resuspensions changed over time. Log transformed (base 10) concentrations of *E. coli* were used to estimate the decay rates of *E. coli* in the estuarine water column without resuspended sediments, and in the estuarine water column containing sediment suspensions. Decay rates were calculated using the model of Chick (1908). For the water column without sediment resuspension, an initial decay rate (using only samples taken before vigorous shaking) as well as an overall decay rate (including samples taken both before and after vigorous shaking), were calculated to consider the complete restructuring of the core after vigorous shaking. A 2-way ANOVA was used to test the effect of (1) site; and (2) treatment type (i.e., water column without sediment resuspension, water column with resuspended surface sediments, water column with resuspended deeper bed sediments) on *E. coli* decay rates. The overall decay rate in the water column was used in this 2-way ANOVA. The 2-way ANOVA was appropriate for this case because the samples were independent, and the assumption of heteroscedasticity was met ($p=0.27$ in Levene's Test). The data were not transformed due to its normality ($p>0.05$ in the Shapiro-Wilk test).

3 RESULTS AND DISCUSSION

3.1 The effect of sediment resuspension on *E. coli* concentrations in the estuarine water

There appears to be no consistent spatial (between sampling sites) or temporal (between sample runs) pattern in the initial *E. coli* concentrations measured in the water column, which ranges from 135.8 organisms /100 mL to 13369 organisms/100 mL (Supplementary materials 1). However, when the initial *E. coli* concentrations in the water column are compared to the water quality parameters measured when sampling the cores (Table 2), there is a statistically significant positive correlation ($R_s=0.53$ and $p\leq 0.05$) between the initial FRP concentration measured in situ at the bottom of the water column on the day of sampling and *E. coli* concentrations measured in the water column of the cores (Supplementary material 2). This supports previous observations by Juhna et al. (2007), who found that higher phosphorus concentrations extend the survival of culturable *E. coli* in water.

A previous study of *E. coli* concentrations in the Yarra River estuary by Daly *et al.* (2013) also identified a positive correlation between phosphorus and *E. coli* concentrations in the water column.

The sediment resuspensions led to an increase in the *E. coli* concentrations present in the water column of the cores. The paired t-test comparing the *E. coli* concentrations in the water column before and after the first instance of gentle inversion of the cores indicated a statistically significant difference between the two ($p=5.0 \times 10^{-5}$). Similarly, the paired t-test comparing the *E. coli* concentrations in the water column before and after the first instance of vigorous shaking showed a statistically significant difference between the two ($p=0.007$; paired t-test). This may suggest that the resuspension of both surface and deeper bed sediments by tidal movements, storms or by anthropogenic activities (e.g. boating or dredging) has the potential to contribute to elevated *E. coli* concentrations of in the water column.

In fact, an increase in *E. coli* concentrations measured in the water column (immediately prior to sediment resuspension) was observed on several occasions (Figure 4) (e.g., *E. coli* at BR (Day 18) from run 4 was greater than the initial concentration). This is likely linked to the resuspension of bed sediments and associated *E. coli* that were previously buried, which occurred when vigorous shaking started. In fact, at all sites and during most sampling runs (all but runs 1 and 2), *E. coli* concentrations in the water column increased after the first time the cores were vigorously shaken (denoted by the dashed lined in Figure 4) and this increase was over one log in some cases.

The highly disturbed estuarine water had the greatest *E. coli* concentrations of the three treatment types (Figure 4). There was also minimal difference in *E. coli* concentrations between sites and sample runs for this treatment (Figure 4). This may be suggesting, that the *E. coli* concentrations buried deep in the bed sediments, which are being released into the water column whenever the vigorous shaking/agitation of the sediment columns occur are more consistent spatially and temporally, compared to *E. coli* concentrations in the water column and in the surface sediments of the Yarra River estuary, which can vary considerably.

Figure 4 to be placed here

3.2 Changing concentrations of *E. coli* in sediment resuspensions over time

Figure 4 suggests that the change in *E. coli* concentrations in the estuarine water column over time differs according to the treatment type (i.e., water column without resuspended sediment, water column with resuspended surface sediment, and water column with resuspended deeper bed sediment). This visual assessment can be substantiated by the 2-way ANOVA testing the effect of site (i.e., MB, BR or SC) and suspension type on *E. coli* decay rates (Table 3). This test suggests that there was no significant difference in rate of *E. coli* decrease across sites ($p=0.22$) but that there was a significant difference between the different types of sediment resuspensions ($p=0.019$). The p -value for the interaction term was 0.6.

E. coli in the water column without sediment resuspension generally decreased in concentration over time but in some cases, could still be detected after 15 days at concentrations up to 3700 organisms/100 mL (Site MB, Run 4; Supplementary material 1). The initial decay rates in the water column (i.e., before vigorous shaking started; Table 3) ranged between net increase (-0.1, Run 2, MB) and net decrease (maximum of 0.57, Run 4, MB). The decreasing *E. coli* concentrations in the water column (without sediment resuspensions – representing non-agitated water) (Table 3), are indicative of *E. coli* die-off rates found in the literature (0.06-0.10 log/day in Meng et al. (2016) as cited in Murphy (2017); 0.1-0.17 log/day in Schang et al. (2016)) and brackish water systems (1.47 log/ day initial decay rate and subsequent decay of 0.06 log/ day after 4.2 days in Zhang et al. (2015)).

There appears to be a consistent spatial trend in initial *E. coli* rate of decrease in the water column. In general, MB had the highest average rate of decrease combined with the most varied rates between sampling runs. This might be due to the variability in salinity levels that are observed over time at this site. This is consistent with a literature review by Murphy (2017) that found that bacteria are less persistent in brackish/ saltwater than freshwaters. The upstream sites (SC and BR) had lower average rates of decrease and much lower variability between runs (Table 3). These sites rarely had any detectable salinity (Table 2) and this lower salinity may be enhancing the survival of *E. coli* (Anderson et al., 2005; Atwill et al., 2007; Murphy, 2017). These findings are also consistent with observations by Boehm et al. (2012) and Chandran et al. (2013) who found that *Salmonella* persisted for shorter periods of time under saline conditions compared to fresh water conditions.

The lowest rate of decrease of *E. coli* concentrations initially in the water column without resuspended bed sediments, were detected in Run 2. This is most likely because the decay rates for Run 2 were calculated using *E. coli* concentrations measured over a shorter time period (six days) and only three time points. However, there was no other consistent seasonal trend in die-off rates observed across all three sites (Table 3).

Although *E. coli* concentrations in the resuspended surface bed sediments also decreased over time, levels of up to 4980 organisms/100 mL (Site MB, Run 5; Supplementary material 1) were still measured after 14 days. Some sample runs and sample sites (e.g. BR during sample run 4 and MB during sample run 5) had negative decay rates (Table 3), which suggests instances of *E. coli* growth. *E. coli* concentrations decreased less rapidly in the water column when surface sediments were resuspended, compared to when the water column did not contain resuspended bed sediments. The average ratio between the overall decay rate observed in the water column without re-suspended sediment and the decay rate observed in the water column with resuspended surface bed sediment was 1.3. When compared to the initial decay in the water column, this average ratio was 2. Another key difference between the inactivation trends in the water column with and without resuspended surface sediments is that *E. coli* was always detected in the resuspended samples regardless of the time after sampling (Figure 4). These results are similar to those of Garcia-Armisen *et al.* (2006) and Uttara *et al.* (2013) who found that the decay rate of free floating *E. coli* is twice the one of *E. coli* attached to suspended particles.

The decay rate constants of *E. coli* in the resuspended surface sediments correlate positively to NO_x concentrations ($R_s = 0.63$, $p = 0.011$) and negatively to FRP concentrations ($R_s = -0.46$, $p = 0.087$) in the overlying water column on the day of core sampling. A previous study (Chudoba *et al.*, 2013) found that the survival rates of faecal bacteria in aquatic systems increased with greater levels of phosphorus in the system, but found no correlation between survival and nitrogen levels. In a separate study on groundwater, *E. coli* survived the longest in high dissolved nitrogen conditions (Cook and Bolster, 2007). It is not clear why in the Yarra estuary sediments, survival of *E. coli* decreases with increasing NO_x concentrations in the bottom of the water column. There is a gap in the literature when it comes to relationships between *E. coli* survival in sediments and NO_x concentrations in the water column. Therefore, we recommend further investigation to explain the

positive correlation between *E. coli* decay rates in resuspended surface sediments and water column NO_x concentrations.

There was no significant difference between the initial and final *E. coli* concentrations in the water column with resuspended deeper bed sediments for each site (unequal variance *t*-test MB - $p=0.42$; SC - $p=0.07$; BR - $p=0.43$). *E. coli* continued to be detected in the water column when deeper bed sediments were resuspended even 14 days after commencement of the experiment, and up to 24 days in the case of run 4 (Figure 4). This suggests that the *E. coli* buried deep in the bed sediments, which are being released into the water column whenever the vigorous shaking/agitation of the sediment columns occur, are not dying off over the period of the experimental test (up to 24 days). The *E. coli* buried in deeper sediments are protected from fluctuations in climate and environmental conditions (Hartz et al., 2008) and hence can survive for extended periods of time. Gerba and McLeod (1976) previously explained that the sediments contain a large amount of leachable nutrients that promote the survival and growth of *E. coli*. Similarly, Craig et al. (2004) found that *E. coli* persisted for longer periods when it was placed in sediments with relatively small particle sizes and high levels of organic carbon (which holds true also for the Yarra River, which is well known for its clay sized particulate matter). Davies et al. (1995) suggested that marine sediments provide a favorable non-starvation environment for *E. coli* after they observed no reduction in culturable *E. coli* concentrations in sediments over a 68 day period.

Decay rates in the resuspensions of deeper bed sediments ranged from 0.02 to 0.12 log/day, which is comparable to the decay rates found by Schang et al. (2016) in the top 20 mm of bed sediment measured in the same Yarra River system in June 2011 (average of 0.1 log/day).

4 CONCLUSIONS

The objective of this study was to identify the effect of surface and deep bed sediment resuspension on the concentration of *E. coli* in an estuarine water column, and investigate the change in *E. coli* concentrations of these resuspensions over time. We identified that the resuspension of sediments can lead to impairment of quality of the overlying water column. *E. coli* in the water column could increase from approximately 20 organisms/100 mL to approximately 1500 organisms/100 mL after

resuspension of deep bed sediments, even without the addition of nutrients for 17 days. It appears that *E. coli* in the bed sediments can survive, and even multiply without the addition of nutrients. Additionally, the observation that *E. coli* was able to survive in the bed sediments at Morrell Bridge, where the water column is often saline, demonstrates the resilience to salinity. The ability of faecal microbes to be resuspended by tidal movements (which the gentle inversion of the column represented), even up to 18 days after *E. coli* entered the estuarine system confirms that a potential public health risk can exist for long after the sediments are first contaminated.

The decrease in *E. coli* concentrations in the water column containing resuspended bed sediments was less than that of the water column without resuspended bed sediments. This can be used to infer that the survival rates of *E. coli* in bed sediments (especially deep bed sediments) is greater than that of free-floating *E. coli* in the water column. However, further experimental work with separate mesocosms is required to confirm this inference. In addition, we recommend that more investigation be done to characterise the factors affecting the survival of *E. coli* in the bed sediments. Reactive phosphorus levels (FRP) correlate positively to the survival of *E. coli* in resuspended surficial bed sediments, which suggests that FRP is conducive to the survival of *E. coli* in surficial bed sediments. However, other characteristics that may affect the survival of *E. coli* in bed sediments and in the water column in oceanic climates (e.g., other physical and chemical conditions, seasonal and spatial variability) are still poorly understood. Better understanding of these factors would enable us to more accurately identify high risk areas and develop more effective mitigation strategies. Also, further work should focus on using more intensive protocols which can better mimic the effect of tidal movements, high flow events and recreational activities on bed and bank sediment resuspension. Finally, further studies that compare the survival of *E. coli* in bed sediments with other reference bacterial pathogens such as *Campylobacter* and *Salmonella*, would help improve estimates of public health risks.

ACKNOWLEDGEMENTS

The authors acknowledge the contributions made by the Australian Research Council and Melbourne Water through the Linkage Program (LP120100718). We also acknowledge the work of the Monash University Water Studies Centre, Keralee Brown, Vera Eate, Peter Kolotelo and Richard Williamson for the collection and laboratory analysis of samples. Dr Peter Bach assisted in producing Figure 1.

REFERENCES

- Anderson, K.L., Whitlock, J.E., Harwood, V.J., 2005. Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. *Appl. Environ. Microbiol.* 71, 3041–3048.
- Atwill, R., Lewis, D., Pereira, M., Huerta, M., Bond, R., Ogata, S., Bass, P., 2007. Characterizing Freshwater Inflows and Sediment Reservoirs of Fecal Coliforms and *E. coli* at Five Estuaries in Northern California. *Univ. Calif. Sch. Vet. Med. Coop. Ext. Sonoma Mar. Counties, Davis, Calif.*
- Bate, N., Hewlett, R., Leeming, R., Reed, J., 1997. Origins of faecal contamination of the Lower Yarra river - an investigation using faecal sterols and bacteria. Environmental Protection Agency, Melbourne.
- Beckett, R., Easton, A.K., Hart, B.T., McKelvie, I.D., 1982. Water movement and salinity in the Yarra and Maribyrnong estuaries. *Mar. Freshw. Res.* 33, 401–415.
- Bernhard, A.E., Goyard, T., Simonich, M.T., Field, K.G., 2003. Application of a rapid method for identifying fecal pollution sources in a multi-use estuary. *Water Res.* 37, 909–913. [https://doi.org/http://dx.doi.org/10.1016/S0043-1354\(02\)00384-6](https://doi.org/http://dx.doi.org/10.1016/S0043-1354(02)00384-6)
- Boehm, A.B., Soetjijto, C., Wang, D., 2012. Solar inactivation of four *Salmonella* serovars in fresh and marine waters. *J. Water Health* 10, 504–510.
- Burton Jr, G.A., Pitt, R., 2001. Stormwater effects handbook: A toolbox for watershed managers, scientists, and engineers. CRC Press, Boca Raton.
- Chandran, A., Suson, P.S., Thomas, A.P., Hatha, M., Mazumder, A., 2013. Survival of multi-drug resistant enteropathogenic *Escherichia coli* and *Salmonella paratyphi* in Vembanadu lake as a function of saltwater barrier along southwest coast of India. *J. Water Health* 11, 324–332. <https://doi.org/10.2166/wh.2013.221>
- Chick, H., 1908. An investigation of the laws of disinfection. *J. Hyg. (Lond).* 8, 92–158.
- Chudoba, E.A., Mallin, M.A., Cahoon, L.B., Skrabal, S.A., 2013. Stimulation of fecal bacteria in ambient waters by experimental inputs of organic and inorganic phosphorus. *Water Res.* 47, 3455–3466. <https://doi.org/http://dx.doi.org/10.1016/j.watres.2013.03.047>
- Cook, K.L., Bolster, C.H., 2007. Survival of *Campylobacter jejuni* and *Escherichia coli* in groundwater during prolonged starvation at low temperatures. *J. Appl. Microbiol.* 103, 573–583. <https://doi.org/10.1111/j.1365-2672.2006.03285.x>
- Craig, D.L., Fallowfield, H.J., Cromar, N.J., 2004. Use of microcosms to determine persistence of *Escherichia coli* in recreational coastal water and sediment and validation with in situ measurements. *J. Appl. Microbiol.* 96, 922–930.
- Daly, E., Kolotelo, P., Schang, C., Osborne, C.A., Coleman, R., Deletic, A., McCarthy, D.T., 2013. *Escherichia coli* concentrations and loads in an urbanised catchment: The Yarra River, Australia. *J. Hydrol.* 497, 51–61. <https://doi.org/http://dx.doi.org/10.1016/j.jhydrol.2013.05.024>

- Davies, C.M., Long, J.A.H., Donald, M., Ashbolt, N.J., 1995. Survival of fecal microorganisms in marine and freshwater sediments. *Appl. Environ. Microbiol.* 61, 1888–1896.
- de Brauwere, A., Ouattara, N.K., Servais, P., 2014. Modeling Fecal Indicator Bacteria Concentrations in Natural Surface Waters: A Review. *Crit. Rev. Environ. Sci. Technol.* 44, 2380–2453. <https://doi.org/10.1080/10643389.2013.829978>
- Desmarais, T.R., Solo-Gabriele, H.M., Palmer, C.J., 2002. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Appl. Environ. Microbiol.* 68, 1165–1172.
- Eccles, J.P., Searle, R., Holt, D., Dennis, P.J., 2004. A comparison of methods used to enumerate *Escherichia coli* in conventionally treated sewage sludge. *J. Appl. Microbiol.* 96, 375–383. <https://doi.org/10.1046/j.1365-2672.2004.02165.x>
- Fries, J.S., Characklis, G.W., Noble, R.T., 2008. Sediment–water exchange of *Vibrio* sp. and fecal indicator bacteria: Implications for persistence and transport in the Neuse River Estuary, North Carolina, USA. *Water Res.* 42, 941–950.
- Garcia-Armisen, T., Thouvenin, B., Servais, P., 2006. Modelling faecal coliforms dynamics in the Seine estuary, France. *Water Sci. Technol.* 54, 177–184.
- Gerba, C.P., McLeod, J.S., 1976. Effect of sediments on the survival of *Escherichia coli* in marine waters. *Appl. Environ. Microbiol.* 32, 114–120.
- Grant, S.B., Litton-Mueller, R.M., Ahn, J.H., 2011. Measuring and modeling the flux of fecal bacteria across the sediment-water interface in a turbulent stream. *Water Resour. Res.* 47, n/a-n/a. <https://doi.org/10.1029/2010WR009460>
- Green, H.C., Shanks, O.C., Sivaganesan, M., Haugland, R.A., Field, K.G., 2011. Differential decay of human faecal *Bacteroides* in marine and freshwater. *Environ. Microbiol.* 13, 3235–3249. <https://doi.org/10.1111/j.1462-2920.2011.02549.x>
- Hartz, A., Cuvelier, M., Nowosielski, K., Bonilla, T.D., Green, M., Esiobu, N., McCorquodale, D.S., Rogerson, A., 2008. Survival Potential of *Escherichia coli* and Enterococci in Subtropical Beach Sand: Implications for Water Quality Managers All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical. *J. Environ. Qual.* 37, 898–905. <https://doi.org/10.2134/jeq2007.0312>
- Ishii, S., Ksoll, W.B., Hicks, R.E., Sadowsky, M.J., 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Appl. Environ. Microbiol.* 72, 612–621.
- Juhna, T., Birzniece, D., Rubulis, J., 2007. Effect of phosphorus on survival of *Escherichia coli* in drinking water biofilms. *Appl. Environ. Microbiol.* 73, 3755–3758.
- Meng, Z., Chandrasena, G.I., Rebekah Henry, Deletic, A., McCarthy, D.T., 2016. Survival of *E. coli* and *Campylobacter* in surface flow constructed stormwater wetlands. Clayton, Australia.
- Murphy, H.M., 2017. Persistence of Pathogens in Sewage and Other Water Types, in: Rose, J.B.,

Jiménez-Cisneros, B. (Eds.), Global Water Pathogens Project, [Http://www.waterpathogens.org](http://www.waterpathogens.org) (M. Yates (Eds) Part 4 Management of Risk from Excreta and Wastewater) [Http://www.waterpathogens.org/book/persistence-in-Sewage](http://www.waterpathogens.org/book/persistence-in-Sewage). Michigan State University, East Lansing, MI, UNESCO.

- Nguyen, K., 2005. Yarra River probe as man falls seriously ill. *Age* (Omaha).
- Ouattara, N.K., de Brauwere, A., Billen, G., Servais, P., 2013. Modelling faecal contamination in the Scheldt drainage network. *J. Mar. Syst.* 128, 77–88. <https://doi.org/http://dx.doi.org/10.1016/j.jmarsys.2012.05.004>
- Pachepsky, Y.A., Shelton, D.R., 2011. *Escherichia Coli* and Fecal Coliforms in Freshwater and Estuarine Sediments. *Crit. Rev. Environ. Sci. Technol.* 41, 1067–1110.
- Park, Y., Pachepsky, Y., Hong, E.-M., Shelton, D., Coppock, C., 2017. *Escherichia coli* Release from Streambed to Water Column during Baseflow Periods: A Modeling Study. *J. Environ. Qual.* 46, 219–226. <https://doi.org/10.2134/jeq2016.03.0114>
- Phillips, M.C., Feng, Z., Vogel, L.J., Reniers, A.J.H.M., Haus, B.K., Enns, A.A., Zhang, Y., Hernandez, D.B., Solo-Gabriele, H.M., 2014. Microbial release from seeded beach sediments during wave conditions. *Mar. Pollut. Bull.* 79, 114–122. <https://doi.org/https://doi.org/10.1016/j.marpolbul.2013.12.029>
- Schang, C., Lintern, A., Cook, P.L.M., Osborne, C., McKinley, A., Schmidt, J., Coleman, R., Rooney, G., Henry, R., Deletic, A., McCarthy, D., 2016. Presence and survival of culturable *Campylobacter* spp. and *Escherichia coli* in a temperate urban estuary. *Sci. Total Environ.* 569–570, 1201–1211.
- Solo-Gabriele, H.M., Wolfert, M.A., Desmarais, T.R., Palmer, C.J., 2000. Sources of *Escherichia coli* in a coastal subtropical environment. *Appl. Environ. Microbiol.* 66, 230–237.
- Spearman, C., 2010. The proof and measurement of association between two things. *Int. J. Epidemiol.* 39, 1137–1150.
- Standards Australia, 2005. AS 4276.21-2005: Water microbiology. Standards Australia Limited, NSW, Australia.
- Symposium on the classification of brackish waters, 1958a. , in: *Oikos*. Wiley-Blackwell, pp. 311–312.
- Symposium on the classification of brackish waters, 1958b. , in: *Oikos*. Wiley-Blackwell, pp. 311–312.
- Vogel, L.J., O'Carroll, D.M., Edge, T.A., Robinson, C.E., 2016. Release of *Escherichia coli* from Foreshore Sand and Pore Water during Intensified Wave Conditions at a Recreational Beach. *Environ. Sci. Technol.* 50, 5676–5684. <https://doi.org/10.1021/acs.est.6b00707>
- Walsh, C.J., 2000. Urban impacts on the ecology of receiving waters: a framework for assessment, conservation and restoration. *Hydrobiologia* 431, 107–114. <https://doi.org/http://dx.doi.org/10.1023/A:1004029715627>
- Zhang, Q., He, X., Yan, T., 2015. Differential Decay of Wastewater Bacteria and Change of Microbial

Table 1. Air temperature, antecedent rainfall, and salinity at the bottom of the water column during each sampling run. Air temperature and rainfall data were obtained from the Bureau of Meteorology for the Melbourne Olympic park station and salinity was measured at each site on the day of collection using a Horiba multi-probe.

Run	Start date	Outside temperature (°C)	Rainfall (mm, 8 days prior to sampling)	Salinity classification at bottom of water column*		
				MB	SC	BR
1	17/11/2010	24	39.9	brackish	fresh	fresh
2	15/12/2010	23	38.6	brackish	fresh	fresh
3	18/01/2011	22	54.0	Fresh	fresh	fresh
4	15/02/2011	30	115.4	brackish	fresh	fresh
5	15/03/2011	20	17.3	brackish	fresh	fresh

* Salinity classification was done using the Venice System (1958): <0.5ppt = fresh water, 0.5-30ppt = brackish water, 30-50ppt = saline water.

Table 2: Water quality observed in situ at the bottom of the water column prior to core collection on the day of sampling. Depth, salinity, pH, DO, turbidity, temperature and EC were measured in situ. Nutrients (NH₄⁺, NO_x and FRP) were tested in the laboratory.

		Depth [m]	NH ₄ ⁺ [mg/L]	FRP [mg/L]	NO _x [mg/L]	Salinity [ppt]	pH	Turbidity [NTU]	DO [mg/L]	Temp [°C]	EC [mS/cm]
MORELL BRIDGE (MB)	Run 1	2.0	0.112	0.024	0.683	14.7	7.52	16	6.02	17.6	NA
	Run 2	3.0	0.160	0.031	0.184	27.3	7.41	14	3.68	18.9	42.5
	Run 3	2.5	0.060	0.020	0.373	0.0	6.83	63	7.18	21.8	0.202
	Run 4	2.5	0.331	0.044	0.191	28.9	7.46	5	2.04	21.3	44.6
	Run 5	2.5	0.132	0.048	0.287	27.6	7.58	15	3.8	20.0	44.3
SCOTCH COLLEGE (SC)	Run 1	3.5	0.062	0.017	0.772	0.1	7.27	40	7.77	17.6	NA
	Run 2	3.0	0.074	0.027	0.401	0.0	6.99	33	7.38	19.7	0.205
	Run 3	2.0	0.057	0.020	0.371	0.0	6.90	67	7.32	21.5	0.180
	Run 4	3.0	0.071	0.021	0.449	0.0	7.15	24	7.17	19.9	0.234
	Run 5	3.5	0.050	0.018	0.627	0.0	6.96	78	6.27	20.4	0.211
BRIDGE ROAD (BR)	Run 1	3.5	0.064	0.018	0.762	0.0	7.27	46	8.71	17.9	NA
	Run 2	5.0	0.067	0.027	0.410	0.0	6.91	35	7.47	19.6	0.202
	Run 3	2.0	0.052	0.018	0.364	0.0	6.90	69	7.57	21.5	0.171
	Run 4	3.0	0.069	0.020	0.444	0.0	7.09	27	7.57	20.0	0.227
	Run 5	2.0	0.066	0.019	0.550	0.0	6.91	192	6.28	20.5	0.196

NA – data Not Available

Table 3. Decay rate constant (k ; days⁻¹) for *E. coli* in the water column before vigorous shaking occurred (initial) and including all data (overall), the water column containing resuspended surface sediments and the water column containing resuspended deeper bed sediments. 'NA' indicates when there was insufficient data to calculate the decay rate or when the test was not performed.

Run	Water (initial)			Water (overall)			Resuspended surface sediments			Resuspended deeper bed sediments		
	MB	SC	BR	MB	SC	BR	MB	SC	BR	MB	SC	BR
1	0.18	0.25	0.10	0.17	0.11	0.13	0.19	0.16	0.14	0.13	0.04	0.08
2	-0.03	0.18	0.06	-0.03	0.18	0.06	0.05	0.14	0.07	NA	NA	NA
3	0.20	0.18	0.12	0.13	0.07	0.09	0.14	0.09	0.08	0.07	0.02	0.05
4	0.57	0.28	0.16	0.10	0.11	-0.05	0.08	0.13	-0.01	0.04	0.02	0.03
5	0.21	0.20	0.15	-0.07	0.04	0.09	-0.03	0.10	0.08	0.10	NA	0.05
Mean	0.23	0.22	0.12	0.06	0.10	0.06	0.09	0.12	0.07	0.08	0.03	0.05
Standard Deviation	0.22	0.04	0.04	0.10	0.05	0.07	0.08	0.03	0.06	0.04	0.01	0.02

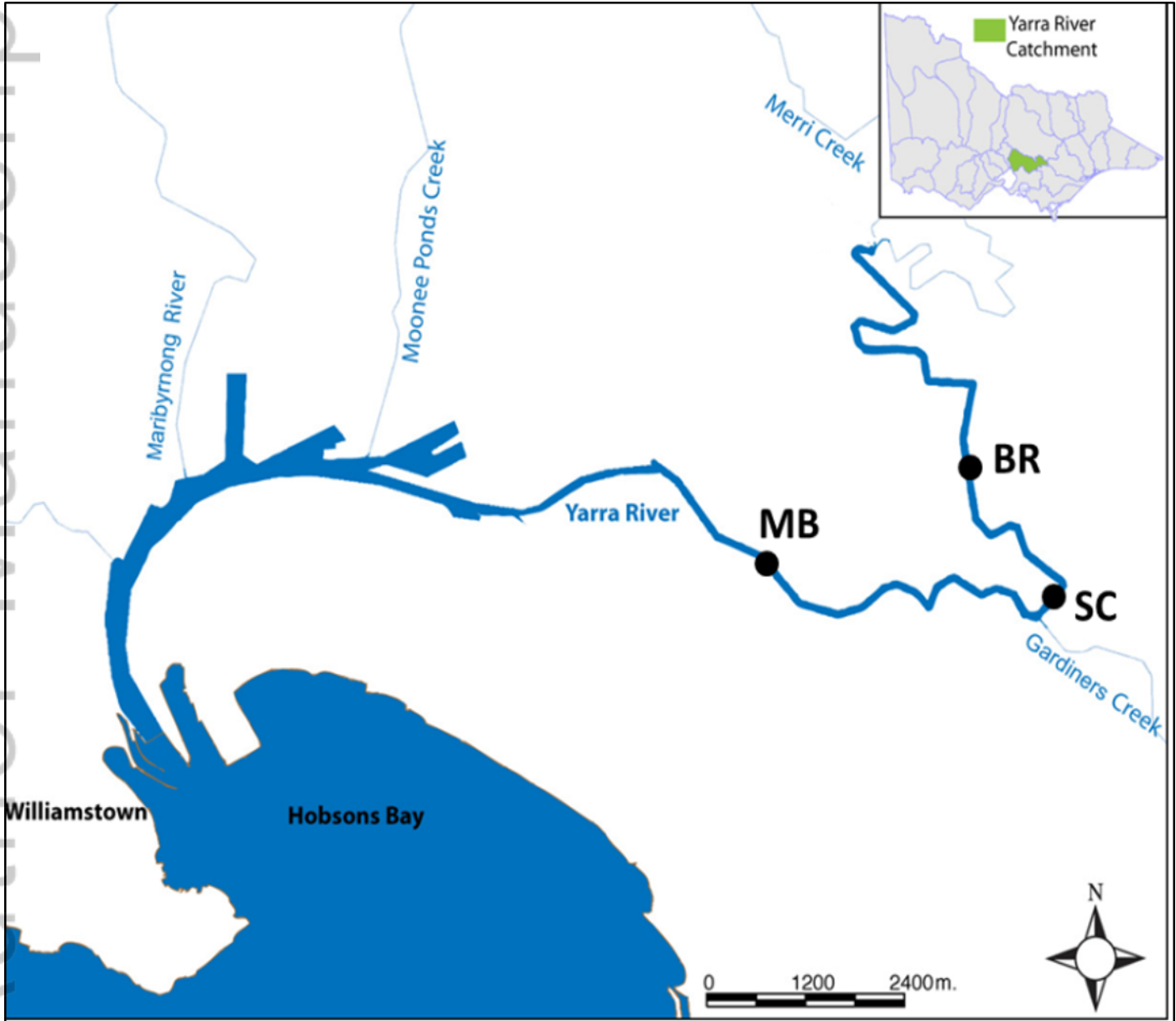
Figure Legend:

Figure 1. Yarra River estuary. Insert shows the location of the Yarra River catchment in Victoria, South-East Australia. The three study sites were Morrell Bridge (MB), Scotch College (SC) and Bridge Road (BR).

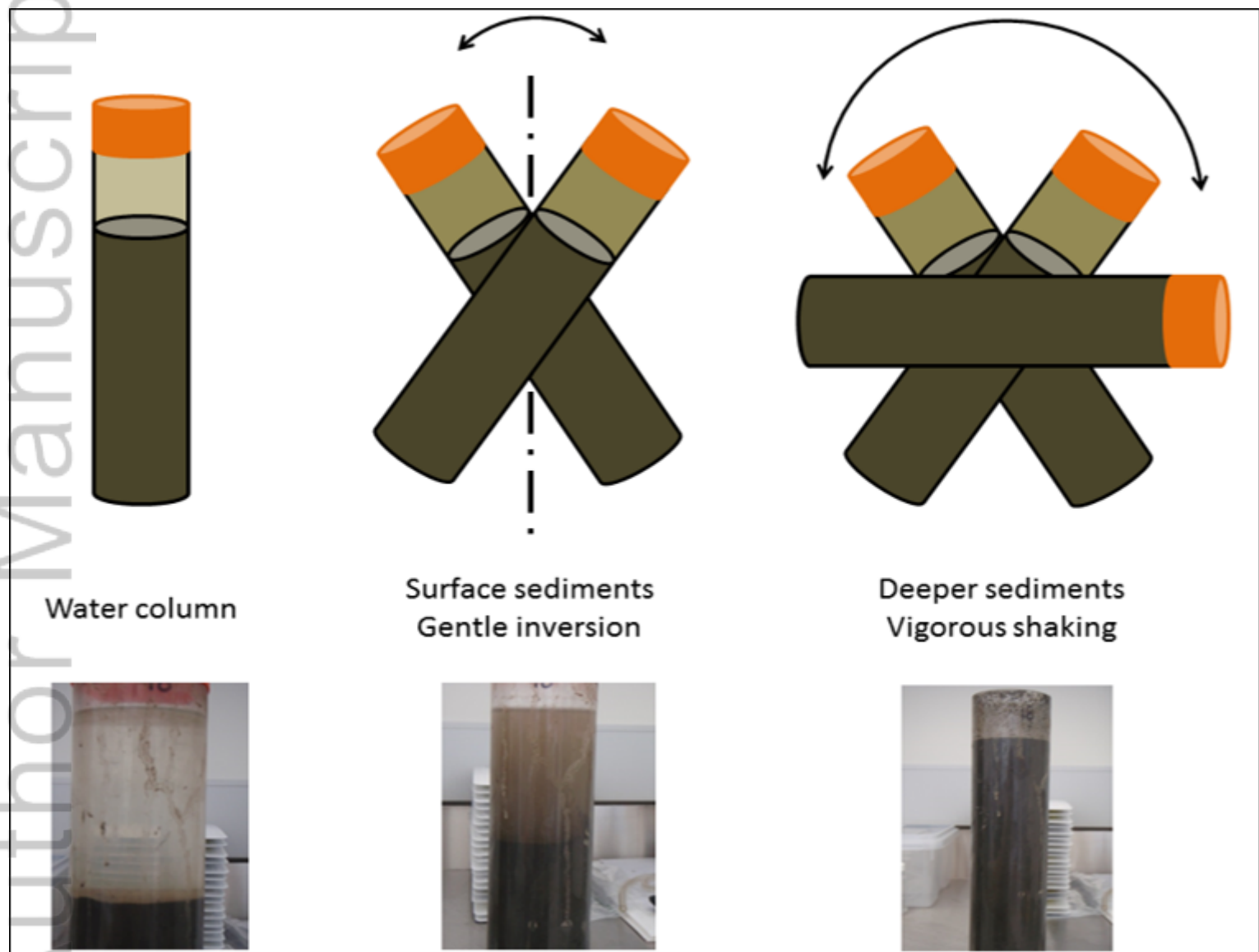
Figure 2. Experimental protocol showing treatment type, and level of turbidity after each treatment.

Figure 3. Sampling schedule for each sample run, showing days on which each treatment was conducted (Run 1: November 2010, Run 2: December 2010, Run 3: January 2011, Run 4: February 2011, Run 5: March 2011).

Figure 4. E. coli concentration in the water column without resuspended bed sediments (top) with resuspended surface sediments (middle) and with resuspended deeper bed sediments (bottom) at MB - Morell Bridge (left), SC - Scotch College (middle) and BR - Bridge Road (right) over time. The * indicates when the water column was saline at MB. The vertical dashed lines indicate when the vigorous shaking began.



RRA_3281_F1.tif



RRA_3281_F2.tif

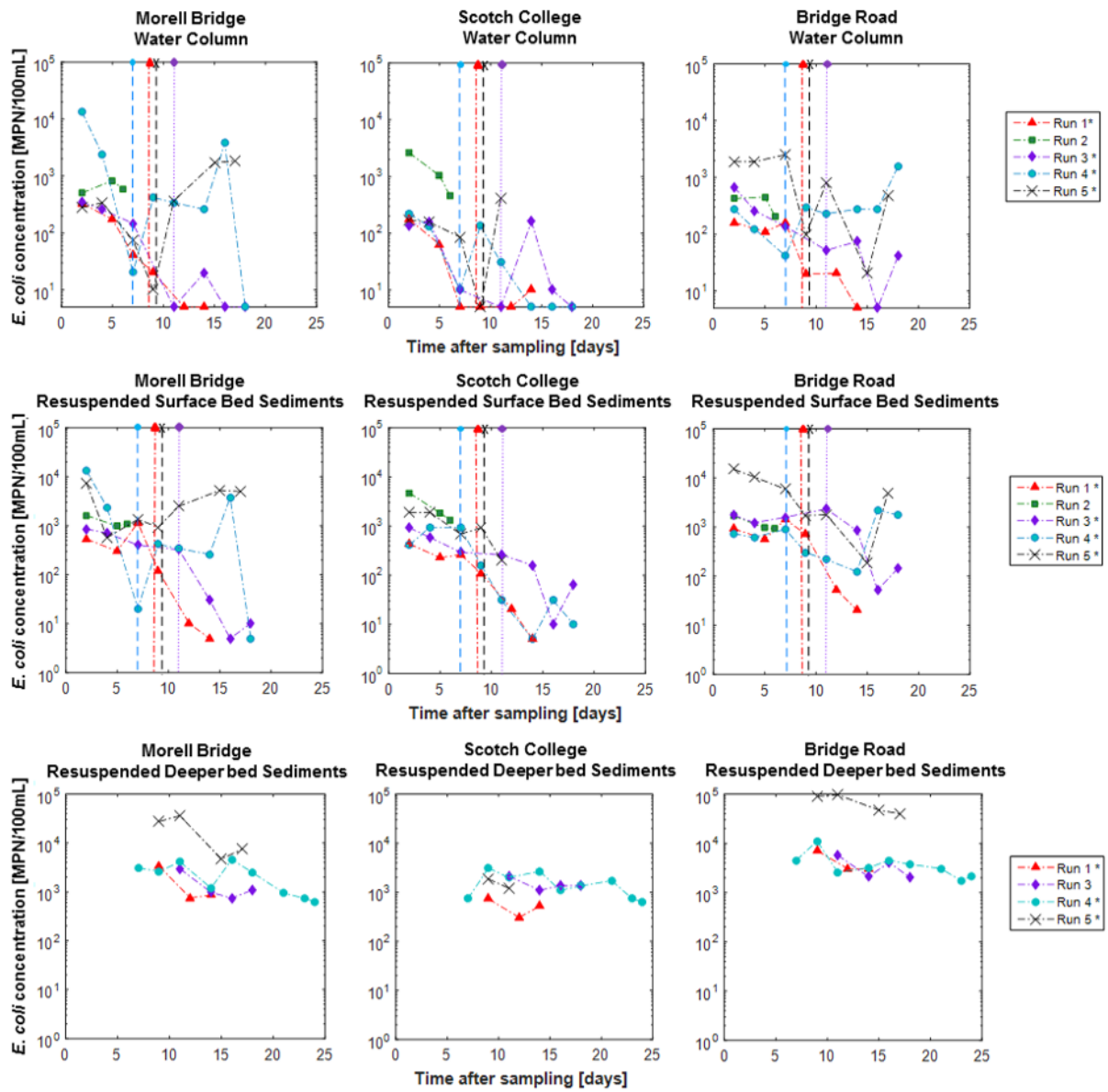
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
Run 1		*			*		*		*			*		*												
Run 2		*			*	*																				
Run 3		*		*			*				*			*		*		*								
Run 4		*		*			*		*		*			*		* ₁		* ₁					•		•	•
Run 5		*		*			*		*		*				*		*									

*Water sample taken, followed by gentle inversion, followed by sampling of suspension

• Vigorous shaking, followed by sampling of suspension (water sample taken prior to vigorous shaking on days where gentle inversion does not occur)

¹ Not enough water left to take a water sample and a resuspended sample (top sediments) for SC samples

RRA_3281_F3.tif



RRA_3281_F4.tif