USC RESEARCH BANK

University of the Sunshine Coast
Queensland, Australia

Relative Inactivation of Faecal Indicator Bacteria and Sewage Markers in Freshwater and Seawater Microcosms

Link to publication record in USC Research Bank:
http://research.usc.edu.au/vital/access/manager/Repository/usc:13101

Document Version:
Author accepted manuscript (postprint)

Citation for published version:
Ahmed, W, Gyawali, P, Sidhu, J P S, Toze, S (2014) Relative Inactivation of Faecal Indicator Bacteria and Sewage Markers in Freshwater and Seawater Microcosms. *Letters in Applied Microbiology,* Vol. 59, No. 3, pp.348-354.

Copyright Statement:
Copyright © 2014 Wiley-Blackwell Publishing Ltd. This is the accepted version of the following article: Ahmed, W., Gyawali, P., Sidhu, J.P.S. and Toze, S. (2014), Relative inactivation of faecal indicator bacteria and sewage markers in freshwater and seawater microcosms. Letters in Applied Microbiology, 59: 348–354. doi: 10.1111/lam.12285, which has been published in final form at http://dx.doi.org/10.1111/lam.12285

General Rights:
Copyright for the publications made accessible via the USC Research Bank is retained by the author(s) and / or the copyright owners and it is a condition of accessing these publications that users recognize and abide by the legal requirements associated with these rights.

Take down policy
The University of the Sunshine Coast has made every reasonable effort to ensure that USC Research Bank content complies with copyright legislation. If you believe that the public display of this file breaches copyright please contact research-repository@usc.edu.au providing details, and we will remove the work immediately and investigate your claim.

OPEN ACCESS

Accepted Article

Received Date : 04-Apr-2014
Revised Date : 13-May-2014
Accepted Date : 14-May-2014
Article type : Original Article

Relative Inactivation of Faecal Indicator Bacteria and Sewage Markers in Freshwater and Seawater Microcosms

W. Ahmed[1,2], P. Gyawali[1,3], J.P.S Sidhu[1,2] and S. Toze[1,3]

1 CSIRO Land and Water, Ecosciences Precinct, 41 Boggo Road, Qld 4102, Australia

2 Faculty of Science, Health and Education, University of the Sunshine Coast, Maroochydore, DC, Qld 4558, Australia

3 School of Population Health, University of Queensland, Herston Road, Qld 4006, Australia

Running title: Inactivation of sewage markers

Correspondence
Warish Ahmed.
CSIRO Land and Water, Ecosciences Precinct.
41 Boggo Road, Brisbane 4102, Australia.
E-mail: Warish.Ahmed@csiro.au (W. Ahmed).

Significance and Impact of the Study

In this study, we have shown that the persistence of the *Bacteroides* HF183 marker in freshwater and seawater microcosms was similar to faecal indicator bacteria (*Escherichia coli* and enterococci), whereas human adenoviruses (HAVs) persisted relatively longer. These findings suggest the suitability of both the markers to identify sewage pollution in environmental waters. However, HF183 marker appeared to be more useful than HAVs in identifying recent sewage pollution. Since, HAVs may remain infective for lengthy periods, it should

This article has been accepted for publication and 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 an 'Accepted Article', doi: 10.1111/lam.12285

This article is protected by copyright. All rights reserved.

be used in conjunction with the HF183 marker to obtain information on the potential human health risks associated with sewage polluted waters.

Abstract

In this study, the relative inactivation of faecal indicator bacteria (FIB) namely *Escherichia coli* and enterococci and sewage markers [*Bacteroides* HF183 and human adenoviruses (HAVs)] were assessed in sewage spiked freshwater and seawater microcosms under ambient sub-tropical climatic conditions. The numbers of declining FIB were measured with culture-based methods whereas the numbers of sewage markers were measured with qPCR assays. The T_{90} inactivation times of *E. coli*, enterococci and the HF183 markers in both freshwater and seawater microcosms were < 3.5 days suggesting the suitability of the HF183 marker to identify recent sewage pollution events. The T_{90} value of HAVs (9.4-13 days), however, was significantly higher than FIB and the HF183 marker in both freshwater ($P < 0.001$) and seawater ($P < 0.05$) microcosms. Therefore, we recommend that HAVs should be used as an additional marker in order to adequately assess the potential health risks associated with longer term sewage polluted environmental waters.

Keyword

Microbial source tracking, Sewage pollution, Inactivation, Human adenoviruses, Faecal indicator bacteria, Microcosms

Introduction

Faecal pollution is one of the major concerns in relation to water resources used for drinking, recreational activities, and seafood harvesting. This is due to potential exposure to a wide array of pathogenic bacteria, protozoa, and viruses (Hörman *et al.* 2004; Fong *et al.* 2005). Sources such as agricultural inputs, wild animals, combined sewer overflows, sewage treatment plants, malfunctioning septic systems, and industrial wastewater outlets are known to be potential sources of faecal pollution in environmental waters (Aslan-Yilmaz *et al.* 2004; Ahmed *et al.* 2010; Sidhu *et al.* 2012). The identification of the source(s) of faecal pollution in environmental waters is a critically important step in efforts to minimize public health risks. Human faecal pollution via sewage or septage poses the greater health risk due to exposure to a wide array of relevant pathogens from human sources.

Accepted Article

This article is protected by copyright. All rights reserved.

Accepted Article

Faecal indicator bacteria (FIB) such as *Escherichia coli* and enterococci have been commonly used to monitor the microbiological quality of water resources. These groups of bacteria are found in the gastrointestinal tracts of warm-blooded animals including humans. One major limitation of using FIB is that they provide limited information on the sources of faecal pollution. In addition, FIB can be present in the environment in the absence of faecal pollution, and sometimes regrowth is possible under suitable conditions (Desmarais *et al.* 2002; Power *et al.* 2005). Library independent microbial source tracking (MST) methods have been successfully used to detect human and animal faecal pollution in environmental waters using polymerase chain reaction (PCR) assays targeting sewage or animal wastewater specific molecular markers (Ufnar *et al.* 2006; McQuaig *et al.* 2009; Ahmed *et al.* 2012).

Ideally, a MST marker should have certain characteristics such as: (i) it should be specific to only a target host group, (ii) it should be present in all members within a host group, (iii) it should exhibit temporal and geographical stability, and (iv) the inactivation rate should be similar to those of FIB or pathogens (Stoeckel *et al.* 2007). None of the MST markers, however, poses all these desirable characteristics and, as such it has been recommended that a "Toolbox" approach (providing multiple lines of evidence) should be used for greater accuracy in the identification of pollution sources in environmental waters (Stricker *et al.* 2008; Tambalo *et al.* 2012).

We have used a "Toolbox" approach to identify sewage pollution in stormwater and reservoir waters in Southeast Queensland, Australia (Ahmed *et al.* 2012; Sidhu *et al.* 2012). Although the sewage markers were frequently detected in environmental water samples, little is known regarding their inactivation times in relation to each other and with FIB. Such information is important in order to further assess the suitability of the markers as sewage trackers and associated human health risks. The objective of this study was to investigate the relative inactivation of sewage associated markers, namely *Bacteroides* HF183 and human adenoviruses (HAVs) with culturable *E. coli* and enterococci in freshwater and seawater microcosms spiked with raw sewage.

Results and Discussion

Climatic conditions

The inactivation experiments of FIB and sewage markers were undertaken in July-August 2013 (winter season). The average atmospheric temperature, evaporation, relative humidity and wind speed ranged from 6.40 to 26.5°C (average 16.7 ± 7.06°C), 2.20 to 9.20 mm (average 3.92 ± 1.85 mm), 46.0 to 86.0% (average 61.7 ± 11.7%), 17.0 to 46.0 km h^{-1} (average 23.9 ± 7.98 km h^{-1}), respectively. No precipitation occurred during the inactivation experiment. The daily natural solar exposure ranged from 9.7 to 20.8 MJ m^{-2} (average 17.9 ± 3.31

This article is protected by copyright. All rights reserved.

Accepted Article

MJ m^{-2}). The water temperature in the microcosms (measured by data logger) ranged from 14.2 ± 0.58°C to 17.7 ± 4.28°C (average 15.8°C ± 1.2°C). The temperature ranged observed in the current study corresponded with the temperature of environmental waters (17.5°C ± 0.69°C) during the winter season in the region (Toze *et al.* 2012).

T_{90} inactivation of FIB and MST markers in freshwater microcosms

The T_{90} inactivation times of FIB and sewage markers (HF183 and HAVs) were evaluated in freshwater microcosms spiked with sewage (Fig. 1). The numbers of FIB and sewage markers were measured with culture-based methods and qPCR assays, respectively. The average T_{90} inactivation time of *E. coli* and enterococci were 2.2 and 1.9 days, respectively (Table 1). Solecki *et al.* (2011) investigated the T_{90} inactivation times of FIB in freshwater microcosms spiked with pig manure. The T_{90} inactivation time of enterococci they determined was similar to this study. However, the T_{90} inactivation time of *E. coli* (T_{90} = 5.4 days) was higher compared to this study (T_{90} = 2.2 days). Such discrepancy could be attributed to the fact that the microcosms in our study were exposed to diurnal solar insolation compared to Solecki and colleagues who incubated their experimental microcosms in the dark. In addition, climatic conditions such as temperature and solar exposure may have accelerated a faster inactivation time of *E. coli* in the current study.

The average T_{90} inactivation time of the HF183 was 3.5 days in freshwater microcosms under our experimental conditions which was similar to the T_{90} value (< 4 days) reported in previous studies in Southern Saskatchewan, Canada and Ohio, USA (Dick *et al.* 2010; Tambalo *et al.* 2012). The average T_{90} inactivation time of the HAVs was 13 days which was relatively slower than a previous study where HAVs persisted up to 5 days (also determined using qPCR) in freshwater microcosms under sunlight and dark conditions at 22°C (Bae *et al.* 2011). The difference in results could be attributed to the fact that in our study, the temperature of the microcosms was around 5°C lower than the study undertaken by Bae and colleagues (Bae *et al.* 2011).

The T_{90} inactivation time of the HAVs observed in the current study was similar to the T_{90} inactivation time (< 18 days) of the HAVs assessed in diffusion chambers in a reservoir in the region (Toze *et al.* 2012). Caution should be exercised when comparing the results of T_{90} inactivation times of the FIB and sewage markers among studies undertaken in various geographical regions because inactivation times can be influenced by factors such as predation, higher temperature and sunlight, types of seeding materials, variation in assays, and statistical analysis (Sinton *et al.* 2002; Noble *et al.* 2004; Walters *et al.* 2009; Dick *et al.* 2010; Green *et al.* 2011; Schulz *et al.* 2011; Sokolova *et al.* 2012). All these factors could attribute the differences in inactivation times and thus making direct comparison difficult.

This article is protected by copyright. All rights reserved.

The T_{90} inactivation time of the HAVs was significantly higher than both FIB and the HF183 marker under our experimental conditions (Table 2). Although, the HF183 had slightly higher T_{90} inactivation time compared to both FIB. These results were not statistically significant. Caution should be exercised when comparing the results of T_{90} inactivation times of the FIB (determined using culture-based methods) with sewage markers (determined using qPCR based assays) because two different approaches were used to measure the numbers of target organisms in the microcosm. Further research is needed to compare the inactivation times of FIB and sewage markers with qPCR based assays.

T_{90} inactivation of FIB and MST markers in seawater microcosms

The T_{90} inactivation times of FIB and sewage markers were also evaluated in seawater microcosms spiked with sewage (Fig. 2). The average T_{90} inactivation time of *E. coli* and enterococci were 1.7 and 1.9 days, respectively. A previous study also reported the rapid inactivation of FIB (*E. coli* T_{90} = 1.7 days and enterococci T_{90} = 3.6 days) in seawater microcosms, which is comparable to the results obtained in this study (Jeanneau *et al.* 2012). The T_{90} inactivation time of HF183 in the seawater microcosm was 2.7 days. Similar results were reported in sewage spiked seawater microcosms exposed to sunlight (Walters *et al.* 2009; Jeanneau *et al.* 2012). This could be due to the fact that the HF183 is more sensitive to salinity. The negative effect of salinity on the persistence of *Bacteroides* 16S rRNA genetic marker in seawater has been reported (Okabe and Shimazu 2007; Schulz *et al.* 2011). The average T_{90} inactivation time of HAVs was 9.4 days. Similar to freshwater microcosms, the T_{90} inactivation time of HAVs in seawater microcosms was significantly higher than both FIB and the HF183 marker under our experimental conditions (Table 2). Although, the HF183 had slightly higher T_{90} inactivation time compared to both FIB. The results were similar to the results obtained in the freshwater microcosms and were not statistically significantly (Table 2).

Comparison of the T_{90} inactivation times of FIB and sewage markers (HF183 and HAVs) between the freshwater and seawater microcosms found that the T_{90} inactivation times of FIB and sewage markers were slightly higher in the freshwater microcosms compared to those in the seawater microcosms. It is possible that freshwater microcosms had more active predators compared to seawater microcosms which had influence on the survival of FIB and sewage markers. However, no statistical significance was observed between freshwater and seawater microcosms (Table 2). This data suggests that the types of water did not have a major influence on the persistence of FIB and sewage markers under our experimental conditions.

Nonetheless, the inactivation time of the HF183 marker in relation to *E. coli* and enterococci in both freshwater and seawater microcosms suggest that this marker may be suitable for the identification of recent

This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

sewage pollution in freshwater and seawater. In this study, the numbers of the HF183 marker at day 0 were 3-4 orders of magnitude higher than the HAVs in both freshwater and seawater microcosms, which may provide increased sensitivity for detecting the HF183 marker in environmental waters over HAVs. It has to be noted that the sole presence of the HF183 markers in environmental water samples may not indicate the presence of sewage pollution since several studies reported the presence of the HF183 marker in small numbers of non-target animal faecal samples in certain geographical areas (Gawler *et al.* 2007; Gourmelon *et al.* 2007; McQuaig *et al.* 2009; Ahmed *et al.* 2013). One of the most important features of enteric viruses is that their presence (1-10 infectious units) indicate health risk (CDC 2002)

In conclusion, the results reported here provide important information about the relative potential persistence of FIB and sewage markers in freshwater and seawater. The shorter persistence of the HF183 marker in our experimental conditions relative to FIB suggests the suitability of the HF183 marker to identify and quantify recent sewage pollution in environmental waters. The HAVs persisted longer than FIB and the HF183 marker suggesting FIB and bacterial marker may not be sufficient enough to identify potential health risks posed by enteric viruses since some of these viruses can remain infective for lengthy periods. Based on our results, we suggest a bacterial marker and as well as a viral marker should be used in a tandem fashion for the accurate assessment of the source specific faecal pollution and associated human health risks.

Materials and methods

Microcosms

Microcosm (six 20 L rectangular polyethylene containers) experiments were conducted at the outdoor facility at Ecosciences Precinct, Dutton Park, Southeast Queensland, Australia. For the inactivation experiment, a freshwater (pH = 8.0, electrical conductivity = 507 µS) sample was collected from a drinking water reservoir located at Montville, Sunshine Coast. A seawater sample (pH = 7.6, electrical conductivity = 55,000 µS) was collected from the Caloundra Beach, Sunshine Coast, Australia. A raw sewage sample was collected from the inlet of a sewage treatment plant. The water and sewage samples were collected in sterile 20 L polypropylene carboys, transported to the laboratory, and kept at 4ºC in the dark until used. The numbers of FIB and sewage marker in the sewage sample were 1.4×10^6 CFU 100 ml^{-1} (*E. coli*), 3.1×10^6 100 ml^{-1} (enterococci), 5.4×10^8 GU 100 ml^{-1} (HF183) and 8.2×10^6 GU 100 ml^{-1} (HAVs), respectively.

Three containers were filled with freshwater and the remaining three containers were filled with seawater. Both types of water were not filtered or autoclaved to allow the inactivation of FIB and sewage markers to occur

This article is protected by copyright. All rights reserved.

Accepted Article

in the presence of background autochthonous microorganisms. To maintain the water temperature similar to the environmental water, all containers were kept in a large plastic container filled with 1,200 l of water. Continuous water flow in the large plastic container was achieved by a submerged pump. Each microcosm was spiked with 2 l of raw sewage (1:4 dilution). An airstone was placed in each microcosm, and air was pumped continuously for the duration of the experiment to maintain aerobic conditions. The microcosms were exposed to diurnal cycles of natural levels of solar insolation. Evaporation was allowed to occur over the course of the experiment and the volume of water lost was noted by observing the reduction of water level in each microcosm on each sampling day. The numbers of FIB and sewage markers in the microcosms were corrected for evaporation by adding freshwater and seawater in respective microcosms. Variables such as volume of water, water temperature, mixing, the quantity of sewage inoculums and ambient climatic conditions were similar for all microcosms. The type (Freshwater vs. seawater) of water was the only variable. Freshwater and seawater microcosms not spiked with sewage were used as controls.

Three replicate samples (40 ml) were collected from each microcosm on days 0, 3, 5, 7, 10 and 14. The numbers of FIB and sewage markers were determined at each sampling day. Data loggers (HOBO devices; Onset Computer Corporation, Pocasset, Mass.) were placed in each freshwater and seawater microcosm to record the water temperature for the duration of the experiment.

Isolation and enumeration of FIB

The spread plate method was used for the isolation and enumeration of FIB from the microcosms. In brief, appropriate serial dilutions were made for each replicate sample and 100 µl of each serially diluted sample was spread on Chromocult™ coliform agar (Merck, Darmstadt, Germany) and Chromocult™ enterococci agar (Merck) plates for the isolation of *E. coli* and enterococci, respectively. Agar plates were incubated overnight at 37°C for 24 and 48 h. Plates with 2-200 colony forming units (CFU) were enumerated.

Concentration of water samples and DNA extraction

Water sample from each microcosm was concentrated with Amicon® Ultra centrifugal filters (Ultracel - 50K) (Millipore, Billerica, MA). 40 ml of sample was passed through Amicon® columns by centrifuging at 3,000 *g* for 10 min to obtain a final volume of 200 µl concentrated sample. For qPCR analysis of HF183 and HAVs, DNA sample was extracted from each 200 µl of concentrated sample using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). Extracted DNA samples were resuspended in 200 µl AE buffer and stored at -80ºC

This article is protected by copyright. All rights reserved.

Accepted Article

until use. The concentration of all extracted DNA samples was quantified using a Nanodrop spectrophotometer (ND-1000, NanoDrop Technology, Wilmington, DE).

qPCR assays

Standards for qPCR assays were prepared from the plasmid DNA of HF183 and HAVs as described elsewhere (Ahmed *et al.* 2010; Sidhu *et al.* 2013). The numbers of gene copies were calculated, and ten-fold serial dilution ranging from 10^6 to 10^0 copies μl^{-1} of DNA extract was prepared, and stored at –20°C.

For sewage markers, previously published primers, probes, and cycling parameters were used (Heim *et al.* 2003; Seurinck *et al.* 2005). qPCR of HF183 and HAVs were performed in 20-µl reaction mixtures using Sso Fast™ EvaGreen® Supermix (Bio-Rad Laboratories, CA, USA). The qPCR mixture contained 10 µl of Supermix, 300 nmol l^{-1} each primer (for HF183), 100 nmol l^{-1} of each primer (for HAVs) DNase- and RNase-free deionized water, and 3 µl of template DNA. For each PCR experiment, a negative control (sterile water) was included. Each qPCR reaction was run in triplicates. The qPCR assays were performed using the Bio-Rad iQ5 thermal cycler (Bio-Rad Laboratories, Richmond, California, USA).

Climatic data

Ambient temperature, rainfall, evaporation, relative humidity, wind speed and solar exposure data were collected from the Australian Bureau of Meteorology (BOM) web site during each of the inactivation experiments. Temperature data loggers (HOBO devices; Onset Computer Corporation, Pocasset, Mass.) were placed in each microcosm to record the temperature at 1 h intervals for the duration of the experiment.

T_{90} inactivation calculations and statistical analysis

For each FIB and sewage marker, all determined numbers in each replicate at each sampling day were normalized to $\log_{10}$ values and plotted over time. One $\log_{10}$ inactivation time (T_{90}) in days for each FIB and sewage marker was determined from each plot using the following equation (Gordon and Toze 2003).

$T_{90} = -t/(\log 10\ C_t/C_0)$

Where C_0 is the number (CFU or GU 100 ml^{-1}) at day 0, C_t is the final number (CFU or GU 100 ml^{-1}) at day *t*. The average T_{90} was calculated from the replicates of each FIB and sewage marker. Only data points above the assay limit of detection were used in the T_{90} calculations. An analysis of variance (ANOVA) was performed on the T_{90} inactivation times of FIB and sewage markers within and between the freshwater and seawater microcosms. The critical P-value for the test was set at 0.05.

This article is protected by copyright. All rights reserved.

Accepted Article

Acknowledgements

This research presented in this paper was funded by the CSIRO Water for a Healthy Country Flagship Program.

Conflict of Interest

No conflict of interest declared.

References

Ahmed, W., Wan, C., Goonetilleke, A. and Gardner, T. (2010) Evaluating sewage-associated JCV and BKV polyomaviruses for sourcing human faecal pollution in a coastal river in Southeast Queensland, Australia. *J Environ Qual* **39,** 1743-1750.

Ahmed, W., Yusuf, R., Hasan, I., Goonetilleke, A. and Gardner, T. (2010) Quantitative PCR assay of sewage-associated *Bacteroides* markers to assess sewage pollution in an urban lake in Dhaka, Bangladesh. *Can J Microbiol* **56,** 838-845.

Ahmed, W., Sidhu, J.P.S. and Toze, S. (2012) Evaluation of the *nifH* gene marker of *Methanobrevibacter smithii* for the detection of sewage pollution in environmental waters. *Environ Sci Technol* **46,** 543-550.

Ahmed, W., Masters, N. and Toze, S. (2013) Consistency in the host specificity and host sensitivity of the *Bacteroides* HF183 marker for sewage pollution tracking. *Lett Appl Microbiol* **55,** 283-289.

Aslan-Yilmaz, A., Okus, E. and Ovez, S. (2004) Bacteriological indicators of anthropogenic impact prior to and during the recovery of water quality in an extremely polluted estuary, Golden Horn Turkey. *Mar Pollut Bull* **49,** 951-958.

Bae, S. and Wuertz, S. (2011) Survival of host-associated *Bacteroidales* cells and their relationship with *Enterococcus* spp., *Campylobacter jejuni*, *Salmonella enteric* serovar Typhimurium, and adenoviruses in freshwater microcosms as measured by propidium mono-azide-quantitative PCR. *Appl Environ Microbiol* **78,** 922-932.

Centres for Disease Control and Prevention. (2003) Norovirus activity-United States, 2002. *MMWR,* **52,** 41-45

Desmarais, T.R., Solo-Gabriele, H.M. and Sadowsky, M.J. (2002) Influence of soil on faecal indicator organisms in a tidally influenced subtropical environment. *Appl Environ Microbiol* **68,** 1165-1172.

Dick, L.K., Stelzer, E.A., Bertke, E.E., Fong, D.L. and Stoeckel, D.M. (2010) Relative decay of *Bacteroidales* microbial source tracking markers and cultivated *Escherichia coli* in freshwater microcosms. *Appl Environ Microbiol* **76,** 3255-3262.

Fong, T.T., Griffin, D.W. and Lipp, E.K. (2005) Molecular assays for targeting human and bovine enteric viruses in coastal waters and their application for library-independent source tracking. *Appl Environ Microbiol* **71,** 2070-2078.

Gawler, A.H., Beecher, J.E., Branda˜, J., Carroll, N.M., Falca˜o, L., Gourmelon, M., Masterson, B., Nunes, B., et al. (2007) Validation of host-specific *Bacteriodales* 16S rRNA genes as markers to determine the origin of faecal pollution in Atlantic Rim countries of the European Union. *Water Res* **41,** 3780–3784.

Gordon, C. and Toze S. (2003) Influence of groundwater characteristics on the survival of enteric viruses. *J Appl Microbiol* 95, 536-544.

Gourmelon, M., Caprais, M.P., Se´qura, R., Le Mennec, C., Lozach, S., Piriou, J.Y. and Rince´, A. (2007) Evaluation of two library-independent microbial source tracking methods to identify sources of faecal contamination in French Estuaries. *Appl Environ Microbiol* **73,** 4857–4866.

Green, H.C., Shanks, O.C., Sivaganesan, M., Haugland, R.A. and Field, K.G. (2011) Differential decay of human faecal *Bacteroides* in marine and freshwater. *Environ Microbiol* **13,** 3235-3249.

Heim, A., Ebnet, C., Harste, G. and Pring-Akerblom, P. (2003) Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol.* **70,** 228-239.

Hörman, A., Rimhanen-Finne, R., Maunula, L., von Bonsdorff, C.H., Torvela, N., Heikinheimo, A. and Hänninen, M.L. (2004) *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., noroviruses, and indicator organisms in surface water in Southwestern Finland, 2000-2001. *Appl Environ Microbiol* **70,** 87-95.

Jeanneau, L., Solecki, O., Wéry, N., Jardé, E., Gourmelon, M., Communal, P.-Y., Jadas-Hécart, A., Caparis, M.P., Gruau, G. and Proucher, A.-M. (2012) Relative decay of faecal indicator bacteria and human-associated markers: A microcosm study simulating wastewater input into seawater and freshwater. *Environ Sci Technol* **46,** 2375-2382.

McQuaig, S.M., Scott, T.M., Lukasik, J.O., Paul, J.H. and Harwood, V.J. (2009) Quantification of human polyomaviruses JC virus and BK virus by Taqman quantitative PCR and comparison to other water quality indicators in water and faecal samples. *Appl Environ Microbiol* **75,** 3379-3388.

This article is protected by copyright. All rights reserved.

Noble, R.T., Lee, I.M. and Schiff, K.C. (2004) Inactivation of indicator microorganisms from various sources of faecal contamination in seawater and freshwater. *J Appl Microbiol* **96,** 464-572.
Okabe, S. and Shimazu, Y. (2007) Persistence of host-specific *Bacteroides-Prevotella* 16S rRNA genetic markers in environmental waters: effects of temperature and salinity. *Appl Microbiol Biotechnol* **76,** 935-944.
Power, M.L., Littlefield-Wyer, J., Gordon, D.M., Veal, D.A. and Slade, M.B. (2005) Phenotypic and genotypic characterization of encapsulated *Escherichia coli* isolated from blooms in two Australian Lakes. *Environ Microbiol* **7,** 631-640.
Schulz, C.J. and Childers, G.W. (2011) Faecal *Bacteroidales* diversity and decay in response variations in temperature and salinity. *Appl Environ Microbiol* **77,** 2563-2572.
Seurinck, S., Defoirdt, T., Verstraete, W. and Siciliano, S.D. (2005) Detection and quantification of the human-specific HF183 *Bacteroides* 16S rRNA genetic marker with real-time PCR assessment of human faecal pollution in freshwater. *Environ Microbiol* **7,** 249-259.
Sidhu, J.P.S., Hodgers, L., Ahmed, W., Chong, M. and Toze, S. (2012) Prevalence of human pathogens and indicators in stormwater run-off in Brisbane, Australia. *Water Res* **20,** 6652-6600.
Sidhu, J.P.S., Ahmed, W. and Toze S. (2013) Sensitive detection of human adenovirus from small volume of primary wastewater samples by quantitative PCR. *J Virol Methods* **187,** 395-400.
Sinton, L.W., Hall, C.H., Lynch, P.A. and Davies-Colley, R.J. (2002) Sunlight inactivation of faecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. *Appl Environ Microbiol* 68, 1122-1131.
Solecki, O., Jeanneau, L., Jardé, E., Gourmelon, M., Marin, C. and Pourcher, A.M (2011) Persistence of microbial and chemical pig manure markers as compared to faecal indicator bacteria survival in freshwater and seawater microcosms. *Water Res* **45,** 4623-4633.
Sokolova, E., Aström, J., Patterson, T.J.R., Bergstedt, O. and Hermansson, M. (2012) Decay of *Bacteroidales* genetic markers in relation to traditional faecal indicators for water quality modelling of drinking water sources. *Environ Sci Technol* **46,** 892-900.
Stoeckel, D.M. and Harwood, V.J. (2007) Performance, design, and analysis in microbial source tracking studies. *Appl Environ Microbiol* **73,** 2405–2415.
Stricker, A.R., Wilhartitz, I., Farnleitner, A.H. and Mach, R.L. (2008) Development of a sensitive quantification of *Bacteroides* sp. ribosomal DNA from human and cattle origin and evaluation in spring water matrices. *Microbiol Res* **163,** 140-147.
Tambalo, D.D., Fremaux, B., Boa, T. and Yost, C.K. (2012) Persistence of host-associated *Bacteroidales* gene markers and their quantitative detection in an urban and agricultural mixed prairie watershed. *Water Res* **46,** 2891-2904.
Toze, S., Hodgers, L., Palmer, A., Sidhu, J., Page, D., Williams, M., Kookana, R., Bartkow, M., Sedlak, D., Stratton, H., Ahmed, W., Schroeder, S. and Christie, M. (2012) Natural attenuation of pathogens and trace contaminants in South East Queensland waterways. Urban Water Security Research Allianace Technical Report No. 87.
Ufnar, J.A., Wang S.Y., Christiansen, J.M., Yampara-Iquise, H., Carson, C.A. and Ellender, R.D (2006) Detection of the *nifH* gene of *Methanobrevibacter smithii*: a potential tool to identify sewage pollution in recreational waters. *J Appl Microbiol* **101,** 44–52.
Walters, S.P., Yamahara, K.Y. and Boehm, A.B. (2009) Persistence of nucleic acid markers of health-relevant organisms in seawater microcosms: implications for their use in assessing risk in recreational waters. *Water Res* **43,** 4929-4939.

Accepted Article

This article is protected by copyright. All rights reserved.

Accepted Article

Table 1 Average T_{90} decay times of faecal indicator bacteria (FIB) and sewage markers in freshwater and seawater microcosms

Faecal indicators/sewage markers	Freshwater microcosms			Seawater microcosms		
	Inactivation rate (-*k*) ± SD	T_{90} (days) ± SD	R^2 ± SD	Inactivation rate (-*k*) ± SD	T_{90} (days) ± SD	R^2 ± SD
E. coli	-0.45 ± 0.01	2.2 ± 0.05	0.93 ± 0.03	-0.58 ± 0.02	1.7 ± 0.05	0.94 ± 0.02
Enterococci	-0.53 ± 0.01	1.9 ± 0.04	0.97 ± 0.02	-0.53 ± 0.01	1.9 ± 0.02	0.97 ± 0.02
HF183	-0.29 ± 0.02	3.5 ± 0.19	0.88 ± 0.07	-0.37 ± 0.06	2.7 ± 0.40	0.98 ± 0.01
HAVs	-0.08 ± 0.03	13 ± 3.84	0.78 ± 0.03	-0.11 ± 0.04	9.4 ± 2.93	0.83 ± 0.03

k : Inactivation rate per day
T_{90}: The number of days needed to produce 90% inactivation
R^2: Coefficients of determination
SD: Standard deviation

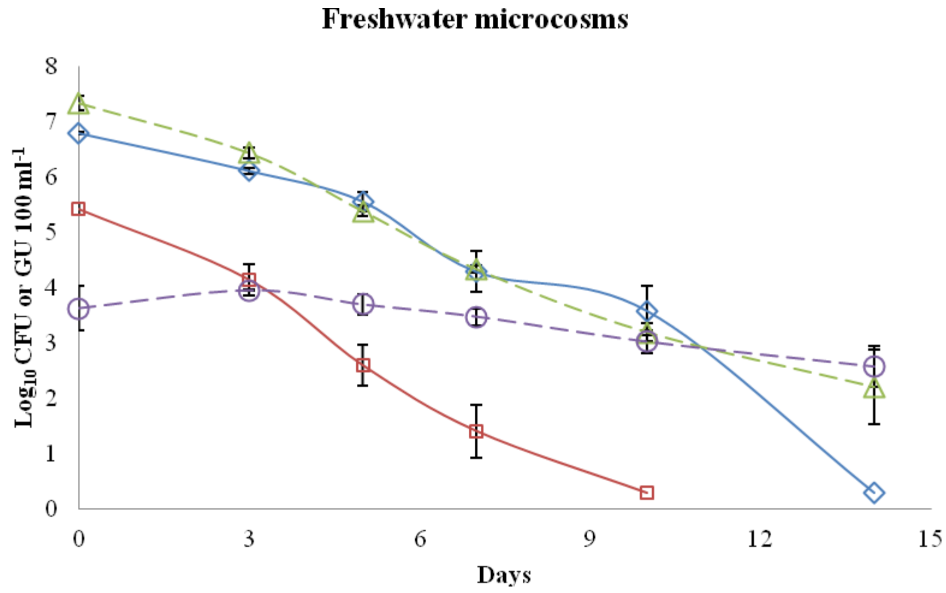
Table 2 ANOVA results on the T_{90} inactivation times of faecal indicator bacteria (FIB) and sewage markers in freshwater and seawater microcosms

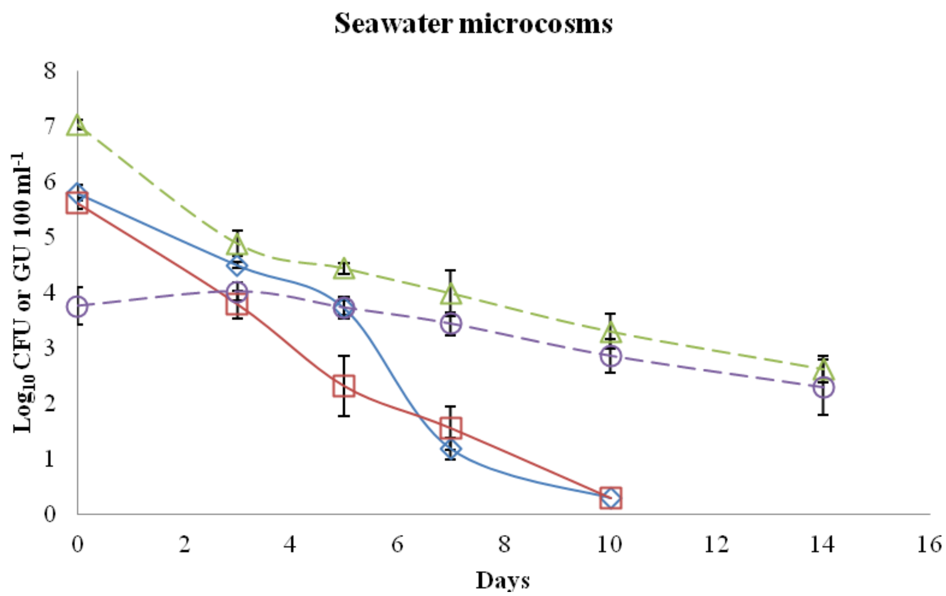
Faecal indicators/sewage markers	*E. coli* freshwater	*E. coli* seawater	Enterococci freshwater	Enterococci seawater	HF183 freshwater	HF183 seawater	HAVs freshwater
E. coli seawater	$P > 0.05$						
Enterococci freshwater	$P > 0.05$	$P > 0.05$					
Enterococci seawater	$P > 0.05$	$P > 0.05$	$P > 0.05$				
HF183 freshwater	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$			
HF183 seawater	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$		
HAVs freshwater	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	
HAVs seawater	$\mathbf{P < 0.01}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.01}$	$\mathbf{P < 0.01}$	$\mathbf{P < 0.01}$	$\mathbf{P < 0.05}$	$P > 0.05$

Bold faced values significantly differed from each other

This article is protected by copyright. All rights reserved.

Accepted Article





This article is protected by copyright. All rights reserved.

Accepted Article

Figure 1 Inactivation curves of *Escherichia coli* (◊), enterococci (□), HF183 (Δ), HAVs (○) in freshwater microcosms. In some cases, the error bars were too small to illustrate.

Figure 2 Inactivation curves of *Escherichia coli* (◊), enterococci (□), HF183 (Δ), HAVs (○) in seawater microcosms. In some cases, the error bars were too small to illustrate.

Supplementary Figure 1. Natural solar exposure and average temperature of the microcosms during the inactivation experiments (obtained from BOM).

Supplementary Table 1. Average (± SD) climatic parameters during the freshwater and seawater microcosms inactivation experiments

This article is protected by copyright. All rights reserved.