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Quantifying environmental reservoirs of fecal indicator bacteria associated with sediment and submerged aquatic vegetation

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Summary

Elevated concentrations of fecal indicator bacteria (FIB) in aquatic sediments and vegetation have prompted concern that environmental reservoirs of FIB disrupt the correlation between indicator organisms, pathogens and human health risks. FIB numbers, however, are typically normalized to volume of water or mass of substrate. Because these reservoirs tend to differ greatly in magnitude within and between water bodies, direct comparison between water column and benthic population sizes can be problematic. Normalization to a set volume of water or mass of substrate, e.g. cfu (100 ml)$^{-1}$ or cfu (100 g)$^{-1}$, can give a false picture of the relative contributions of various reservoirs to FIB numbers across the ecosystem, and of the potential for FIBs to trigger health advisories as they pass from one reservoir to another. Here, we normalized enterococci concentrations from water, sediment and submerged aquatic vegetation (SAV) to land surface area (m^2) to compare their relative importance in the entire system. SAV-associated enterococci comprised only 0–18% of the entire population, even though they displayed the highest concentrations of enterococci per unit mass. The largest proportion of the enterococci population was in the water column (4–77%) or sediments (20–95%), depending on the volume of each substrate available at a site and FIB concentrations within them. Models indicated that large shifts in the relative size of FIB populations in each substrate can result from changes in per cent SAV cover, water depth and depth of sediment colonization. It follows that high concentrations of FIB in sediments or SAV do not necessarily signify large environmental reservoirs of FIB that can affect the water column. Comprehensive analyses that include FIB measurements from water, SAV and sediment normalized to land surface area offer a more balanced perspective on total FIB numbers contained in various matrices of an aquatic system.

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Introduction

Fecal indicator bacteria (FIB) serve as surrogates for enteric pathogens and are used to monitor the presence of fecal contamination of environmental waters worldwide. While measurements of various FIB have shown good correlation with risks of waterborne illness in certain studies (Wade *et al.*, 2003; Zmirou *et al.*, 2003), other studies have indicated little or no correlation with pathogens, including those on *Salmonella*, *Campylobacter*, *Cryptosporidium*, *Giardia* or enteroviruses (Lund, 1996; Bonadonna *et al.*, 2002; Lemarchand and Lebaron, 2003; Harwood *et al.*, 2005). A probable factor in this lack of correlation is the ability of FIB – including coliforms, *Escherichia coli*, and enterococci – to persist in both culturable and non-culturable forms for extended periods in a wide variety of environmental matrices after their initial introduction.

A considerable body of work has demonstrated the survival of FIB in secondary habitats, including matrices such as terrestrial soils (Topp *et al.*, 2003; Ishii *et al.*, 2006), aquatic sediments or beach sand (Byappanahalli and Fujioka, 1998; Solo-Gabriele *et al.*, 2000; Craig *et al.*, 2004; Whitman *et al.*, 2006) and submerged aquatic vegetation (SAV) (Byappanahalli *et al.*, 2003; Whitman *et al.*, 2003; Ksoll *et al.*, 2007; Englebert *et al.*, 2008). The potential for these environmental reservoirs (defined here as a specific environmental matrix and its associated FIB) to serve as sources of FIB in the water column is a major concern that has been raised repeatedly in the literature (Solo-Gabriele *et al.*, 2000; Grant *et al*, 2001; Whitman *et al.*, 2003; Anderson *et al.*, 2005; Brownell *et al.*, 2007; Ishii and Sadowsky, 2008; Badgley *et al.*, 2010a). However, much of this work has focused primarily on *E. coli*, even though enterococci are approved by the USEPA

as FIB in both fresh and salt water (USEPA, 1986; USEPA, 2002). In addition, given the recent development of new methods that quantify fecal contamination by targeting enterococci (Haugland *et al*., 2005; Scott *et al*., 2005), it is becoming increasingly important to improve our understanding of the dynamics of this group in the environment.

Unfortunately, it has been difficult to quantify the importance of FIB reservoirs in relation to elevated FIB concentrations in the water column or increased human health risk across different ecosystems. Recent epidemiological studies have shown that increased exposure to beach sand carries increased risk of disease (Bonilla *et al*., 2007; Heaney *et al*., 2009), but no quantitative correlations have been determined between health risks and concentrations of FIB in sediments or SAV. The importance of benthic reservoirs of FIB has been inferred from correlations between sediment and water column concentrations during natural (An *et al*., 2002; Nagels *et al*., 2002; Le Fevre and Lewis, 2003; Jamieson *et al*., 2005; Whitman *et al*., 2006; Yamahara *et al*., 2007), and experimentally manipulated (McDonald *et al*., 1982; Wilkinson *et al*., 1995; Nagels *et al*., 2002; Muirhead *et al*., 2004) resuspension events, and from modelling efforts that incorporate benthic reservoirs (Steets and Holden, 2003; Bai and Lung, 2005). However, no standard methods for the detection and quantification of the link between FIB reservoirs and health risk have been adopted (Boehm *et al*, 2009).

One of the major impediments to our ability to readily interpret the importance of benthic reservoirs of FIB is that colony-forming units (cfu) or other measurements have typically been normalized to values that do not represent the contribution of each substrate type to total population numbers within a system. Concentrations in the water column are reported by normalizing to volume [e.g. cfu $(100\ ml)^{-1}$], while sediment and SAV measurements are normalized to mass (e.g. cfu g^{-1}) (Byappanahalli and Fujioka, 1998; Solo-Gabriele *et al*., 2000; Topp *et al*., 2003; Whitman *et al*., 2003; Anderson *et al*., 2005; Jeng *et al*., 2005; Ishii *et al*., 2006). From a public health or risk of illness perspective, this normalization strategy is appropriate for the water column, because concentration is the value most directly related to human health risk. However, when modelling FIB transport or figuring total maximum daily loads for a water body, a more appropriate perspective is to consider the aquatic system as a whole. In these cases, normalization to mass of substrate does not allow for direct comparison of the relative contribution of each substrate type to the overall bacterial load within an environment and does not allow for a direct comparison of the importance of different reservoirs.

It is possible, however, to look at the problem from the perspective of land surface area and ask how many bacteria are associated with each substrate type within a system by normalizing to a common parameter, which incorporates the relative amount of each substrate in the system. For example, an aquatic habitat is characterized by overlying water, a sediment substrate and, in some cases, SAV. The water depth, the amount of SAV present and the depth to which bacteria are living within the sediment influence the relative importance of each matrix. Therefore, it is possible to examine the relative contribution of these different reservoirs to the entire bacterial population by normalizing bacterial numbers (cfu) to the total amount of water, sediment and SAV harbouring FIB on a land area basis (e.g. per square meter) in a system. This approach allows a direct comparison of bacterial population sizes in water column and benthic samples (Weiskel *et al*., 1996; Muirhead *et al*., 2004; Jamieson *et al*., 2005), and the relative contribution of each to the total population in the entire system. The utility of this method was previously shown in estimating stocks of fecal coliforms in Buttermilk Bay, Massachusetts, USA (Valiela *et al*., 1991).

In this study we revisited several sites in the Tampa Bay watershed that were described and sampled in a previous study (Badgley *et al*., 2010b) and collected new data to better characterize the habitats. These data, combined with the previously published FIB densities, were then used to conduct a new analysis on the relative population sizes of the enterococci in the water, sediment and SAV at each of the sites. The study had two specific objectives: (i) identify and quantify key habitat characteristics that would allow the normalization of enterococci concentrations to land surface area and allow direct comparison of the population sizes in water, sediment and SAV at each site and (ii) develop a simple model that can be used to estimate shifts in the relative population sizes at a given site that result from important habitat changes such as variation in bacterial concentrations, water depth, SAV cover and sediment resuspension.

Results

Habitat characterization and land surface area normalization

Water depths at the sites ranged from < 50 cm at the stream sites, < 1 m with 0.5–1.0 m tides at the bay sites, and up to 2–3 m at the river and lake sites. Based upon sediment grain size analyses (data not shown) sediments were found to consist primarily of sand and the densities were highly consistent at all of the sites (~ 1.9 gww cm^{-3}) except for the two stream sites, which included a higher proportion of organic material and were slightly less dense (~ 1.6 gww cm^{-3}) (Table 1). Depth profiles of enterococci concentrations in sediments at the six sites

Table 1. Habitat characteristics measured at each of the sites, which were used to convert enterococci concentrations in each substrate from volume-normalized mass-normalized values to area-normalized values.

	Water depth (cm)	Sediment depth[a] (cm)	Sediment density (gww cm^{-3})	SAV density[b] (kg m^{-2})	SAV % cover[c]	Enterococci concentrations (cfu m^{-2})			
						Water	Sediment	SAV	Total
Small stream	36	3	1.63	2.9	80	1.84×10^6	1.65×10^6	2.52×10^5	3.74×10^6
Small stream (high)	98	3	1.63	2.9	80	4.95×10^6	1.65×10^6	2.52×10^5	6.85×10^6
Large stream	17	12	1.66	10.5	60	6.90×10^5	2.92×10^6	3.66×10^5	3.97×10^6
Large stream (high)	140	12	1.66	10.5	60	5.58×10^6	2.92×10^6	3.66×10^5	8.86×10^6
River	190	6	1.92	5.4	10	3.70×10^6	1.06×10^6	2.37×10^4	4.79×10^6
River (shore only)	36	6	1.92	5.4	95	6.74×10^5	1.06×10^6	2.37×10^4	1.43×10^6
Lake	320	6	1.90	5.4	95	2.97×10^6	7.61×10^5	1.62×10^5	3.89×10^6
Lake (shore only)	53	6	1.90	5.4	90	5.22×10^5	7.61×10^5	1.62×10^5	1.44×10^6
Upper bay	80	9	1.91	2.4	40	2.72×10^5	1.61×10^6	6.40×10^3	1.84×10^6
Upper bay (high tide)	30	9	1.91	2.4	40	4.42×10^5	1.61×10^6	6.40×10^3	2.06×10^6
Upper bay (low tide)	130	9	1.91	2.4	40	1.02×10^5	1.61×10^6	6.40×10^3	1.72×10^6
Lower bay	75	15	1.92	2.6	80	2.01×10^5	1.45×10^6	3.24×10^3	1.66×10^6
Lower bay (high tide)	125	15	1.92	2.6	80	6.7×10^5	1.45×10^6	3.24×10^3	1.79×10^6
Lower bay (low tide)	25	15	1.92	2.6	80	3.35×10^5	1.45×10^6	3.24×10^3	1.52×10^6

a. Sediment depth is the depth to which enterococci concentrations were found to be within one order of magnitude of surface concentrations.
b. SAV density is the biomass density of SAV within a vegetated patch.
c. SAV % cover is the total percentage of aquatic bottom covered by vegetated patches as opposed to bare sand.

generally showed a decline in concentration with increasing sediment depth (Fig. 1). Cutoff depths (the depth beyond which at least an order of magnitude decline in concentrations was observed) were highly variable, ranging from 3 cm at the small stream site to 15 cm at the lower bay site (Table 1, Fig. 1). SAV biomass was higher at the freshwater sites, with values of about 2.5 kg m^{-2} at the bay sites and 2.9–10.5 kg m^{-2} at the freshwater sites. SAV cover over the entire bottom was also highly variable, ranging from 10% at the river site to almost complete cover at the lake site (Table 1).

Total enterococci concentrations at all of the sites, when normalized to land surface area, were surprisingly consistent, on the order of 10^6 cfu m^{-2} (Table 1). Generally, sites with lower sediment enterococci numbers normalized to mass (e.g. bay sites) were compensated by having a relatively deeper depth of colonization in the sediment. This resulted in total population sizes that were comparable to sites with higher sediment enterococci numbers per mass, but shallower depths of colonization (e.g. stream sites).

When comparing matrices, the area-normalized numbers at each site were highest in water and sediments, ranging from 10^5–10^6 cfu m^{-2}, while the concentrations in SAV were consistently lower, ranging from 10^5 cfu m^{-2} at the stream sites to 10^3 cfu m^{-2} at the bay sites (Table 1). When the numbers of enterococci on each substrate were re-examined as relative proportions of the total population of enterococci at a given site, the results differed between the freshwater and estuarine sites (Fig. 2). At the freshwater sites, water depth seemed to be the major factor in determining relative population sizes of enterococci in each substrate. In situations with shallow water depths (such as during periods of low stream flow, or when considering only the shoreline at the lake or river site), the sediment population was similar to or even greater than the waterborne population. However, during periods of high water in the streams, or when accounting for the entire volume of water at the lake or river site, the waterborne population became the dominant portion (Fig. 2A). Conversely, at the estuarine sites, the populations in the sediments were consistently dominant, typically accounting for 80–90% of the total enterococci load regardless of changing water levels during the tidal cycle (Fig. 2B). SAV was consistently found to harbour small to negligible fractions of the total enterococci (per square meter), ranging from a maximum of 9.2% at the large stream site down to 0.2% at the lower bay site (Fig. 2).

Modelling theoretical habitat changes

After examining the relative population sizes of enterococci in each substrate at each site, individual terms in the equations presented in the *Calculations and modelling* section (see *Experimental procedures*, below) were manipulated to simulate changes in key habitat characteristics such as water depth, SAV coverage, depth of sediment colonization and FIB concentration. Varying these values within a realistic continuum provided an estimate of how the total numbers of enterococci in each reservoir would potentially change because of habitat variability. For example, to investigate the potential for SAV to serve as an important reservoir, we first chose to model the large stream site, which had the highest proportion of total enterococci associated with SAV. Figure 3 shows how the relative population sizes of enterococci in

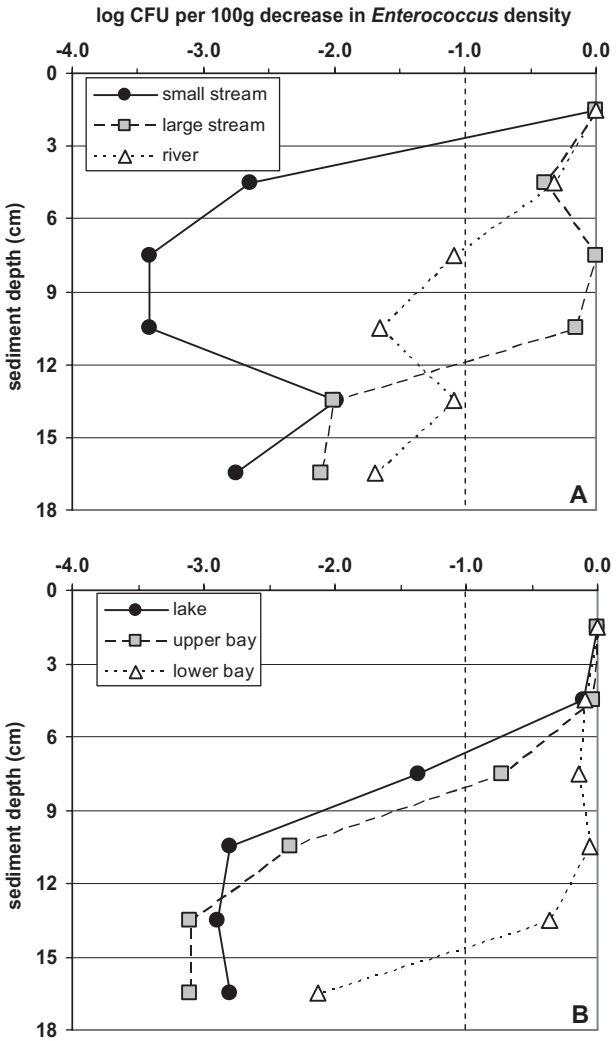

Fig. 1. Depth profiles of mean enterococci concentrations (presented as decrease in log cfu (100 g)$^{-1}$ from the shallowest depth) in from sediment cores at the (A) small stream, large stream and river sites; and the (B) lake, upper bay and lower bay sites ($n = 3$ at each depth). The vertical dashed line represents a one order of magnitude decrease from surface levels, which was used as the cutoff depth for the calculations of sediment depth in the models.

the system would change in response to theoretically varying water depth (0–1 m) and SAV coverage (0–100%). The relative percentage of total enterococci on SAV was predicted to increase from 0% to 18% as water depth decreased and SAV increased. The percentage in the water column was predicted to decrease from 58% to 0% along the same gradient. So at the shallow depths sometimes found in this stream (15–20 cm), the proportion of enterococci found in SAV was predicted to exceed that found in the water if the SAV coverage were to approach 100%. However, as a result of high concentrations of enterococci in the sediment, SAV was never predicted to harbour a dominant proportion of total enterococci under any conditions. At all levels of water depth and SAV coverage, the SAV-associated population was always considerably smaller than the size of the sediment-associated population, even though the mass-normalized concentrations in SAV at this site averaged nearly 10^4 cfu (100 g)$^{-1}$.

Another interesting result of the modelling exercise was the potential for large changes in relative population sizes over relatively short time scales. Even though, on average, the vast majority of total enterococci were found in the sediments at the bay sites, in some individual samples the proportions of enterococci in water and sediment were relatively equal. In these cases, using the model to investigate the effects of tidal fluctuations in water depth (0.3–1.3 m) resulted in predictions of rapid changes in the relative reservoir sizes. Figure 4 represents one example (April 2008 at the upper bay site) where this was the case. Tidal fluctuations were predicted to actually cause shifts between states where the water

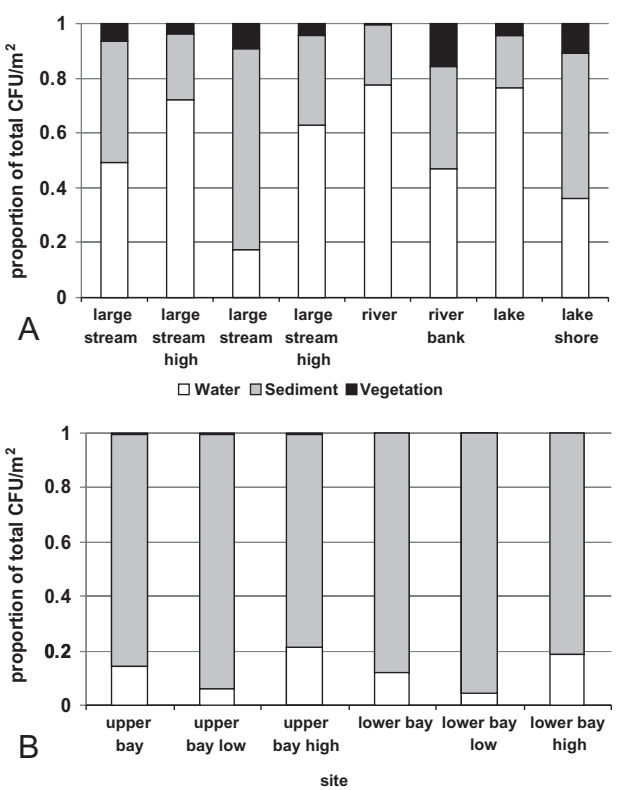

Fig. 2. Mean proportion of total number of enterococci per square meter area found in water, sediment and SAV samples sites around the Tampa Bay watershed obtained from monthly samples between May 2007 and April 2008.
A. The two columns for each stream site represent normal and extreme high water depths. The two columns for the river and lake sites represent values for the entire water body versus those if consideration is constrained to the nearshore banks only.
B. The three columns for each site represent changing proportions for varying water depths at mid-, low and high tidal levels respectively.

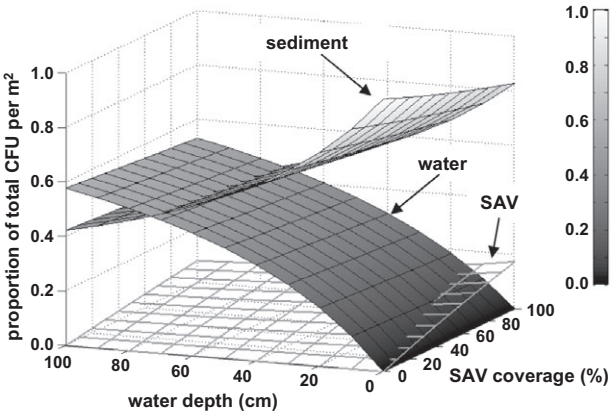

Fig. 3. The relative proportion of total enterococci found in water, sediment and SAV in response to theoretically varying values for water depth and SAV bottom cover at the large stream site. Shades of gray and associated sidebar indicate proportion value on the *z*-axis for water and sediment fractions. Model constants: waterborne FIB = 4.0×10^2 cfu (100 ml)$^{-1}$; sediment FIB = 1.5×10^3 cfu (100 g)$^{-1}$; sediment depth = 12 cm; sediment density = 1.66 g cm^{-3}; SAV bacteria = 5.8×10^3 cfu (100 ml)$^{-1}$; SAV biomass = 10.5 kg m^{-2}.

and sediment alternated as the dominant proportion of total enterococci. SAV, meanwhile, consistently harboured a very small fraction of the total enterococci.

Finally, the idea of sediment resuspension at the upper bay site was explored more thoroughly in Fig. 5. Increases in waterborne enterococci concentrations that would result from the resuspension of various amounts of sediment enterococci concentrations [10^2–10^4 cfu (100 g)$^{-1}$]. At low sediment concentrations [10^2 cfu (100 g)$^{-1}$] and with the model limited to 5 cm of total sediment depth, too few enterococci were resuspended to ever exceed 104 cfu (100 ml)$^{-1}$, which is the USEPA recommended single sample maximum for marine beaches. At moderate sediment concentrations [10^3 cfu (100 g)$^{-1}$], resuspension of about 3 cm of sediment was required to exceed the regulatory limit, while at relatively high concentrations [10^4 cfu (100 g)$^{-1}$], an exceedance was predicted after resuspension of approximately 0.5 cm of sediment.

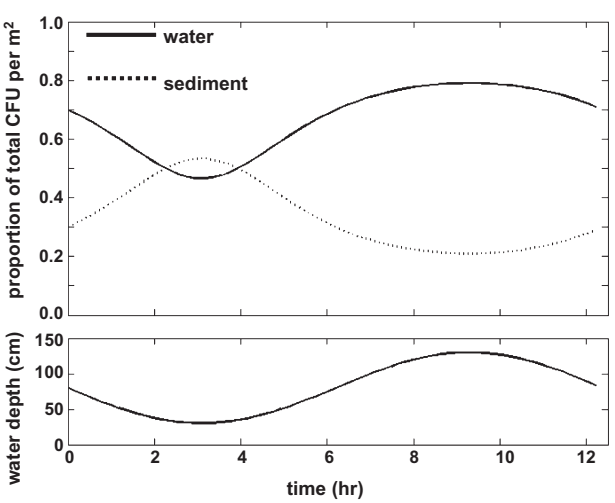

Fig. 4. The relative proportion of total enterococci found in water and sediment in response to theoretically varying water depth as a result of tidal fluctuations at the upper bay site during the April 2008 sampling event. Model constants: waterborne FIB = 1.1×10^2 cfu (100 ml)$^{-1}$; sediment FIB = 2.2×10^2 cfu (100 g)$^{-1}$; sediment depth = 9 cm; sediment density = 1.92 g cm^{-3}; SAV FIB = 1.2×10^3 cfu (100 g)$^{-1}$; SAV biomass = 2.4 kg m^{-2}; SAV cover = 40%.

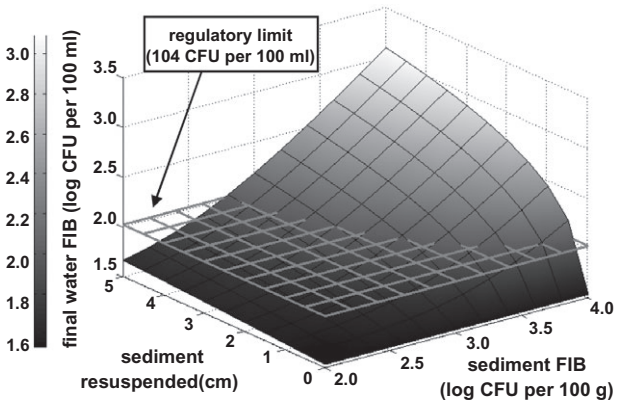

Fig. 5. Predicted increases in waterborne enterococci concentrations at the upper bay site resulting from theoretically varying values for sediment enterococci concentrations and the amount of sediment resuspended. (SAV negligible and not shown.) Model constants: initial waterborne FIB = 3.2×10^1 cfu (100 ml)$^{-1}$; water depth = 80 cm; sediment density = 1.92 g cm^{-3}; regulatory limit = 1.04×10^2 cfu (100 ml)$^{-1}$.

Discussion

Conventional approaches to normalizing and reporting data on numbers of FIB in water, sediment and SAV have made the analysis of their impact on water quality difficult. By quantifying key habitat characteristics at our sites, we were able to normalize numbers to land surface area and directly compare the relative size of benthic reservoirs of enterococci in the Tampa Bay watershed, which resulted in several key findings. First, total FIB concentrations, with all matrices combined, were very similar across all of the sites. Second, SAV always harboured the smallest percentage (between 0% and 18%) of the total number of enterococci in the system, despite the high FIB concentrations per unit SAV biomass at these sites (Badgley *et al.*, 2010b). Instead, the dominant proportion of the total enterococci population was always found in either the water or the sediment, depending on site characteristics such as water depth, FIB concentrations and the depth of sediment containing FIB at each site. Finally, a simple modelling exercise illustrates the potential for the relative size of FIB populations in different matrices to shift

dramatically in space and time, as well as how these calculations can be used to investigate the importance of benthic reservoir sizes at a given site.

The similarity in the total mean enterococci populations (water + sediment + SAV) at all of the sites was highly surprising, and differs from our earlier findings based upon mass-normalized numbers. In our previous study (Badgley *et al*., 2010b), we found a significant increase in enterococci cfu/mass for water, SAV and sediment at sites that were situated farther up the watershed, with the average concentration at the small stream site approximately 20 times higher than at the lower bay site. When normalized to land surface area, however, that relationship disappears, and all of the sites and scenarios outlined in Table 1 vary by only a factor of six in total cfu m^{-2}. After examination of the data, it appears that the higher densities present in the upper reaches of the watershed are offset by the fact that water depths and sediment depths are considerably smaller than in the downstream sites, which tended to have deeper water and deeper colonization of the sediments by enterococci. These trends suggest that the differences in FIB concentrations seen along the watershed are more a result of different volumes of substrate available for colonization at each site, rather than major differences in total numbers of FIB at each site. This finding agrees with the previous suggestion that water volume and dilution potential have an important impact in buffering FIB concentrations (Badgley *et al*., 2010b).

SAV as a reservoir

The relatively small proportion of total enterococci that were found in SAV was also surprising, given that the sites in this study were specifically chosen for their high SAV biomass and that high FIB concentrations in SAV on a per gram basis were previously reported at these sites (Badgley *et al*., 2010b) and others (Whitman *et al*., 2003; Olapade *et al*., 2006). This discrepancy illustrates why it is critical to properly consider how FIB numbers are normalized before judging the relative importance of environmental reservoirs of FIB. Even though the traditional mass unit (1 g or 100 g) of SAV often contains orders of magnitude greater concentrations of enterococci than the traditional volume unit (100 ml) of water, our results show that this can easily be outweighed by the fact that there are typically orders of magnitude more volume units of water than mass units of SAV in a square meter of aquatic habitat.

It is important to note, however, that we are discussing *numerical* importance – the potential for a large number of FIB associated with one substrate to significantly affect the concentration in another substrate (e.g. bacteria being released from SAV into the water and affecting water column concentrations). We are *not* implying that SAV is unimportant as a refuge for FIB. Some sites may have enough SAV biomass to create a significant reservoir, and, as others have suggested, SAV may also serve an important role as a substrate for FIB growth, not just persistence (Byappanahalli *et al*., 2003; Ksoll *et al*., 2007). If the growth rates are sufficiently high, relative to rates of FIB mixing and dilution within the water column, SAV could have significant effects on FIB concentrations in the water column, even though it represents a relatively small reservoir at any given time.

Sediment as a reservoir

The relative proportion of total enterococci at a site that were contained within the sediments was highly variable, but the most important conclusion is that the concentrations of FIB in sediment and water are not sufficient data on their own to determine the reservoir size associated with each matrix, without also factoring in the total volume of each matrix present in the system. For example, at the freshwater sites, water depth played an important role, with the dominant proportion of FIB often contained within the sediments when depths were below approximately 1 m. At greater depths, the large volume of water reduced the relative importance of the sediment reservoir of FIB compared with the water column. Meanwhile, at the estuarine sites, sediments were more consistently found to contain the dominant proportion of FIB, at least up to maximum water depths observed in this study (~ 1.5 m). These sites displayed much deeper colonization of FIB within the sediments, 9–15 cm before a log unit decay in concentration occurred, and much lower enterococci concentrations in the water column. Valiela and colleagues (1991) came to similar conclusions about sediment reservoirs of FIB after a basin-wide analysis of Buttermilk Bay, Massachusetts, USA.

The importance of FIB-populated matrix volume to determining the sizes of sediment reservoirs highlights the need for improved understanding of how FIB populations in these matrices, and how the physical characteristics of the matrices themselves, change over time. Very few studies examine the depth of FIB colonization in sediments, but a wide range has been reported, from 1 cm in marsh sediments (Grant *et al*, 2001) to approximately 30 cm in freshwater beach sands (Whitman *et al*., 2006). In contrast, data on changes in the volume of water at a site, due to factors such as rainfall, tidal fluctuations and flood management, are often abundant. However, the changes in water depth that occur through these processes may also correlate with changes in other habitat characteristics such as FIB concentrations, SAV biomass and depth of colonization in the sediments, depending on the time scale. For example, FIB concentrations in the

water tend to increase after a rain event as a result of runoff from non-point sources on land or stormwater systems (Reeves *et al*., 2004; Ahn *et al*., 2005; Brownell *et al*., 2007), but may quickly decline again once the system has been initially flushed (McDonald *et al*., 1982; Nagels *et al*., 2002; Muirhead *et al*., 2004; Jamieson *et al*., 2005). Furthermore, as illustrated by the example in Fig. 4, some changes in matrices may cause the relative sizes of FIB reservoirs at a site to shift over very short time scales. Because short-term variability in FIB densities per mass of matrix is already known to occur (Boehm *et al*., 2002; Desmarais *et al*., 2002; Whitman and Nevers, 2004; Badgley *et al*., 2010a), any rapid changes that are also occurring in the matrix volume will potentially compound the effect and complicate the population dynamics (and attempts to estimate human health effects) even further. More research into these processes is greatly needed to improve attempts to model the fate and transport of FIB in the environment, but we believe the approach presented here gives a good first order approximation of the importance of sediments as FIB reservoirs.

Broader applicability

In this study, we chose to normalize enterococci concentrations in the Tampa Bay watershed to land surface area via a strategy that includes populations in the water, in sediments and on SAV, but there is nothing about our methods that is specific to a given taxon or site. Once the important reservoirs are identified and measured, this approach could be used in any habitat to determine the relative population sizes of various FIBs, pathogens or other organisms. While it is true that the interaction of different organisms with various substrates is likely to result in very different rates of processes such as survival, growth and sedimentation, inclusion of those dynamics was beyond the scope of the current work. Such processes have been included in other predictive models (Steets and Holden, 2003; Bai and Lung, 2005) and additional research into these dynamics is likely to greatly improve future attempts. However, they do not affect the primary goal of this study, which was to conduct a more accurate assessment of the enterococci population sizes at these sites than can be gleaned from the traditionally reported concentrations normalized to mass. Therefore, while the specific trends that were evident in our results may not hold true for other FIB or at other sites, we believe that habitat characterization and calculation of area-based population sizes are important tools that are valid for any organism at any site.

The comprehensive analysis presented here requires additional sampling and analytical effort compared with conventional water quality studies, and we are not advocating that such lengths be taken for all water quality assessments. However, these results highlight the pitfalls in relying only on FIB concentrations normalized to mass in determining the magnitude of FIB reservoirs. In such cases where these values might be important, such as determination of potential FIB sources or establishment of TMDL programs, the extra effort of analysing the various matrices from the perspective of land surface area may be very small compared with the scope of the entire project, and therefore easily justified. In this study, we chose to characterize our habitats at only one point in time, which represented a maximum level for certain values such as SAV biomass and water depth. In studies concerning very important areas such as busy beaches, even better characterization would be obtained from multiple measurements throughout the year, which would give a better idea of how these values, along with FIB concentrations, vary temporally.

Given that predictive modelling of FIB fate and transport is still very difficult and uncertain, the relatively simple analysis presented here can be particularly helpful in determining ranges of conditions under which benthic reservoirs are large enough to potentially affect water quality monitoring at a given site. For example, at the large stream site, we were able to estimate that, on average, SAV-associated populations of FIB would only be present in great enough numbers to significantly affect water column concentrations when the water was exceedingly shallow (< 15 cm), at which point both populations are dwarfed by that found in the sediments. SAV can therefore be essentially ruled out as a dominant reservoir of FIB at this site. Likewise, the model proved valuable for determining ranges of conditions under which the resuspension of bacteria-laden sediments might be a concern at a given site, thereby making it possible to rule out sediment resuspension as a primary concern in affecting water quality when conditions are outside these ranges. Our ability to characterize selected water bodies in more detail will generate a better understanding of the circumstances in which reservoirs other than the water column need to be considered in monitoring, modelling and mitigation efforts. Ensuring that we employ directly comparable normalization techniques is an important first step towards this end.

Experimental procedures

Environmental sampling

Data for bacterial concentrations are from Badgley *et al*. (2010b). Briefly, water, sediment and SAV at four freshwater and two estuarine sites in the Tampa Bay watershed were sampled monthly from May 2007 to April 2008. The sites included a small stream, large stream, riverbank, lakeshore, upper bay shoreline and lower bay shoreline (see Badgley *et al*., 2010b, for map and additional details). Samples were

placed on ice immediately after collection and processed in the laboratory the same day. Water samples were vacuum filtered directly onto 0.45 μm pore size nitrocellulose membranes and cultured at 41°C for 24 h on mEI agar (Difco Laboratories) supplemented with nalidixic acid (USEPA, 2002). Sediment and SAV samples were diluted 1:10 (w/v) in phosphate-buffered water and sonicated at 19 watts for 30 s to dislodge and resuspend attached cells (Anderson *et al*., 2005). Aliquots of the water were then filtered and cultured by standard membrane filtration methods (USEPA, 2002). Final concentrations are presented as cfu (100 ml)$^{-1}$ water or cfu (100 g)$^{-1}$ wet weight substrate.

Habitat characterization

In order to convert the FIB concentrations measured in our previous study to a land surface perspective, the same sites from Badgley *et al*. (2010b) were revisited for this study in July 2008 and the following habitat characteristics were measured: water depth, sediment density (g cm^{-3}), the depth of sediment containing FIB, the biomass density of SAV in a vegetated bed (g m^{-2}) and the per cent coverage of SAV beds over the entire aquatic bottom. It is important to remember that many of these values vary over seasonal cycles, and the ideal situation would be to collect these data at each sampling. However, the effort required for this approach is prohibitive at any sites that are not being intensively studied or modelled. For this reason, we chose to characterize these sites once during midsummer, which typically represents the period with the most rainfall and the highest amount of SAV growth in the Tampa Bay watershed, under the assumption that they would represent relatively maximal values for SAV biomass and water depth over an annual cycle. In addition, water depth data were obtained at the stream sites again in July 2009 during a period of exceptionally heavy rainfall and high water levels, and are presented as an extreme case for comparison. We believe this provides a good first order approximation of the population sizes and offers valuable insights into the relative amounts of enterococci contained in each matrix. The modelling efforts described below were then pursued to predict how deviations from these values might affect the observed results.

Water depth at each site was determined as the mean of 10 random measurements. At stream and river sites, these 10 values were obtained from transects across the channel to characterize the entire water body at the location of sampling. At the lake site, 10 locations were selected randomly on a bathymetric map averaged to get a mean depth for the entire lake. Finally, at the bay sites, only the local depth was used, which was characterized by 10 random measurements that were taken in the vicinity of the sampling location, halfway between high and low tide. At the river and lake sites, estimates of the local nearshore depth were also obtained in the same manner in an attempt to characterize the immediate sampling area as a contrast to using the entire water body.

To determine sediment density, five sediment samples from each site were collected in 50 ml centrifuge tubes. The sediment was allowed to settle in each tube during transport, and upon return to the laboratory, the overlying water was poured off prior to analysis. The volume and mass of the remaining sediment in each sample was recorded and the wet density was calculated, as this was the unit of normalization used previously (Badgley *et al*., 2010b). Next, each sediment sample was dried at 80°C for 24 h, and the dry mass was measured for each sample so that a dry density could be obtained as well. To determine the depth to which FIBs were present in significant concentrations in the sediments, three replicate sediment cores of approximately 2.5 cm in diameter and 20 cm in length were taken at each site. Upon removal, each core was longitudinally divided into six sections (3 cm long – the deepest 2 cm was discarded because of disturbance from sampling), which were homogenized and analysed for enterococci concentrations. The samples were analysed using the same methods as those described above. The enterococci concentration in each sediment sample was calculated [cfu (100 g)$^{-1}$ wet weight], and the cutoff depth was determined to be the last depth at which enterococci concentrations were within one order of magnitude of those found at the surface of the core (e.g. see Fig. 1). Concentrations at deeper depths, which were beyond an order of magnitude lower than surface concentrations, were assumed to be numerically insignificant in terms of total population size. This cutoff depth was then used in conjunction with the sediment concentrations observed in the original study (Badgley *et al*., 2010b) to estimate the total populations sizes present in the sediments (see *Calculations and modelling* section below).

To determine the amount of SAV biomass in a vegetated bed, five quadrats (0.0625 m^2) were thrown haphazardly into SAV patches at each site. The emergent portion of all SAV within the quadrat was removed down to bare sand and all excess water was allowed to drain for approximately 30 s. Next, the mass was immediately measured and the mean of all replicates for each site was used as the typical wet biomass of SAV. Finally, the per cent coverage of SAV beds (as opposed to bare sediment) over the entire bottom was determined via visual estimation at each site, which is commonly used to document changes in SAV cover in aquatic habitats (Fourqurean *et al*., 2001; Bell *et al*., 2008).

Calculations and modelling

The values obtained from the habitat characterizations, along with the mean enterococci concentrations for each substrate at each site observed in the previous study (Badgley *et al*., 2010b), were used in the following formulas to recalculate enterococci concentrations based upon land surface area. For waterborne bacterial concentrations, cfu m^{-2} was calculated as a function of cfu (100 ml)$^{-1}$ and water depth:

$$C_{WA} = 10^4 * C_{WV} * d_W \quad (1)$$

where C_{WA} = waterborne bacterial concentration normalized to area (cfu m^{-2}); C_{WV} = waterborne bacterial concentration normalized to volume [cfu (100 ml)$^{-1}$]; and d_W = water depth (m).

For sediment-associated bacterial concentrations, cfu m^{-2} was calculated as a function of cfu (100 g)$^{-1}$, sediment density and sediment depth:

$$C_{SA} = 10^4 * C_{SM} * D_S * d_S \quad (2)$$

where C_{SA} = bacterial concentration in sediment normalized to area (cfu m^{-2}); C_{SM} = bacterial concentration in sediment

normalized to sediment mass [cfu (100 g)$^{-1}$]; D_S = sediment density (g wet weight cm^{-3}); and d_S = sediment depth (m).

For SAV-associated bacterial concentrations, cfu m^{-2} was calculated as a function of cfu (100 g)$^{-1}$, SAV biomass in vegetated patches and proportion of bottom with SAV cover:

$$C_{VA} = 10 * C_{VM} * B_V * P_V \quad (3)$$

where C_{VA} = bacterial concentration in SAV normalized to area (cfu m^{-2}); C_{VM} = bacterial concentration in SAV normalized to mass [cfu (100 g)$^{-1}$]; B_V = SAV biomass in a vegetated patch (kg m^{-2}); and P_V = proportion of SAV cover over entire bottom. Finally, the total cfu m^{-2} for the entire system at each site was simply calculated as the sum of each substrate (Eqs 1–3):

$$C_{TL} = C_{WL} + C_{SL} + C_{VL} \quad (4)$$

where C_{TL} = total bacterial concentration in the system, normalized to land surface area. After the calculation of total concentrations, the relative population sizes for each substrate were calculated by dividing that substrate's population size by the total population size.

The above calculations were then used to theoretically manipulate different parameters and investigate how changes in these parameters might affect the distribution of enterococci among the matrices. It is important to note that these models are not intended to be predictive in a forecasting sense, but are simply intended to create a range of possible alternatives that would result from changes in different values for the habitat characterization measurements that were completed. For a given scenario, a MATLAB (The Mathworks, Massachussetts, USA) script was written that generated a range of values along a realistic continuum for the variables that were being altered, and then used mean values for those that were kept constant. Changing one or two variables at a time would then create a one- or two-dimensional matrix of solutions that illustrated the range of possible outcomes for each scenario.

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