

Spatial and Temporal Dynamics of Fecal Coliform and *Escherichia coli* Associated with Suspended Solids and Water within Five Northern California Estuaries

David J. Lewis,* Edward R. Atwill, Maria das Graças C. Pereira, and Ronald Bond

Fecal coliform and *Escherichia coli* associated with suspended solids (SS) and water in five northern California estuaries were studied to document process influences and water quality monitoring biases affecting indicator bacteria concentrations. We collected and analyzed 2371 samples during 10 sampling events for the five studied estuaries. Concentrations during wet-season stormflow conditions were greater than during wet-season base flow and dry-season base flow conditions. Results also document concentration gradients across the length of the studied estuaries and with depth of sample collection. Highest concentrations were associated with shallow samples collected furthest inland. Corresponding decreases occurred the deeper and closer to the estuary mouth a sample was collected. Results also identify direct relationships of wind speed and discharge velocity and indirect relationship of tide stage to indicator bacteria concentrations. Bacteria associated with suspended solids (SS), after conversion to the same units of measurement (mass), were three orders of magnitude greater than in the water fraction. However, the mean proportion contributed by SS to composite water sample concentrations was 8% (SE 0.3) for fecal coliform and 7% (SE 0.3) for *E. coli*. Bacteria from the SS proportion is related to seasonality, tide stage, and discharge velocity that are consistent with mechanisms for entrainment, transport of SS, and reduced particle settling. These results are important for both managing and monitoring these systems by improving sample spatial and temporal context and corresponding bacteria concentration values across the freshwater–saltwater interface.

ESTUARIES AND SURROUNDING WATERSHEDS are areas of intense development, recreational use, and agriculture and aquaculture production. There are increased environmental impacts in conjunction with these pressures, most notably impacts to water quality from microbial pollution. As a result, water quality authorities worldwide are developing and implementing regulations and policies. Programs such as the European Union's Water Framework Directive (CEC, 2000; CEC, 2006) and Australia's National Water Quality Management Strategy, including fresh and marine water quality guidelines (ANZECC, 2000), are setting water quality criteria and directing water body assessment and mitigation. In the United States, similar action is being taken through Total Maximum Daily Loads (TMDLs) (Kay et al., 2007).

Estuaries encompass the land–marine margin and corresponding subtidal, intertidal, and nontidal riverine zones. The result is a dynamic freshwater–saltwater interface that can confound efforts to measure and detect trends in microbial water quality. Exchanges and mixing of upland and near-shore microbial sources with ocean water through stream and river hydrology and tidal shifts are part of this dynamism (Pachepsky and Shelton, 2011). Precipitation in the form of rainfall generates flow paths that connect upland microbial pollution sources, including suspending particulates, to the estuary. Instances of increased bacteria values in relationship to rainfall and watershed storm hydrology are found in estuary studies in New South Wales, Australia (Shah et al., 2007), North Carolina, USA (Coulliette and Noble, 2008), western Portugal (Almeida et al., 2007), and southern France (Chu et al., 2011).

There is also the potential for river and estuary sediment to be a microbial reservoir that can influence water quality through resuspension. The concept and role of sediment as a reservoir of bacteria that influences water column bacteria concentrations was documented by McDonald et al. (1982), among others. Models predicting microbial concentrations in estuarine waters have been improved with the inclusion of sediment resuspension fac-

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*Corresponding author (djllewis@ucanr.edu).

D.J. Lewis, Univ. of California Cooperative Extension-Marin, 1682 Novato Blvd., Suite 150B, Novato, CA 94947; E.R. Atwill, Western Institute for Food Safety and Security, School of Veterinary Medicine, Univ. of California-Davis, One Shields Ave., Davis, CA 95616-8734; M.D.G.C. Pereira and R. Bond, School of Veterinary Medicine, Haring Hall, Univ. of California-Davis, One Shields Ave., Davis, CA 95616-8734. Assigned to Associate Editor Michelle Soupir.

Abbreviations: cfu, colony-forming unit; PBS, phosphate buffered saline; SS, suspended solids; TMDL, Total Maximum Daily Load; TSS, total suspended solids.

When upland-derived freshwater pulses arrive during storms to the estuary, there is mixing and stratification of fresh and saltwater. Almeida et al. (2007), for example, documented saline stratification from top to bottom and a saline gradient moving inland across the freshwater–saltwater interface with a corresponding inverse gradient of microbial concentration. Given this heterogeneity across the fresh-to-saltwater transition zone, microbial water quality monitoring programs can introduce biases in the measured values of microbial concentration depending on the frequency, timing, and location of water sample collection. Reliable access to water sampling locations of estuaries can be problematic when conducted on foot, often forcing a sampler to use nearby bridges or to restrict sampling to inland sites adjacent to roadways typically dominated by freshwater inflows, especially during winter storm-flow conditions. This presents the potential to inaccurately reflect estuarine water quality. Conversely, sampling estuaries subsequent to large tidal inflows will probably reflect local marine microbial water quality conditions. Hence, the timing, frequency, and location of sampling relative to such factors as wind, precipitation, stream velocity, and tidal stage can substantially influence the measured concentration of bacterial indicators and subsequent decisions regarding compliance with water quality standards.

The studied estuaries are uniquely mediterranean and therefore add to the existing literature through representation of the mediterranean climate, ecosystems, and estuaries. Additionally, our study design and sample size permitted us to conduct a robust statistical investigation of the dynamism in microbial levels for the two studied fractions. Our selection of indicator bacteria for



Materials and Methods

The five estuaries studied represent a variety of environmental conditions across the northern California coast. From north to south they are the Russian River; Salmon Creek; Estero Americano; Walker Creek; and Lagunitas Creek (Fig. 1, Table 1). In general, they have similar climate, precipitation, and hydrology. Specifically, this similarity is the result of the mediterranean climate in California, with cool wet winters and dry hot summers. As a result, all five estuaries experience an inflow of freshwater during the winter. The five estuaries differ in area of contributing watershed, as well as land use, including agriculture, urbanization, and recreation. Additionally, the Russian River, Salmon Creek, and Estero Americano are bar-built estuaries, whereas Walker and

Estuary	Drainage area	Population	Estuary and land use	Type
	km ²			
Russian River	3864	301,930	Urban and rural residential, contact/noncontact recreation, grazing livestock, wine grape production, minor timber	Bar-built/closed
Salmon Creek	90	1,385	Rural residential, noncontact recreation, grazing livestock	Bar-built/closed
Estero Americano	80	1,224	Rural residential, noncontact recreation, grazing livestock, dairy farming	Bar-built/closed
Walker Creek	196	204	Rural residential, contact and noncontact recreation, grazing livestock, dairy farming	Low-flow/open
Lagunitas Creek	241	3,028	Rural residential contact and non-contact recreation grazing livestock, dairy farming	Low-flow/open

Lagunitas Creeks are part of the low inflow system of Tomales Bay (Kimbrow et al., 2010). These differences and similarities provide a good context for understanding how these factors interact with bacteria movement in estuaries.

The Russian River Estuary experiences varying periods of being closed and open. Of the five estuaries studied, it has the most inconsistent status in terms of estuary type but is predominately bar-built. Land use includes dairy farming, livestock grazing, wine grape production, urban and rural development, and some timber harvesting. The Russian River is a drinking water source for approximately 600,000 people and is also popular for contact and noncontact water recreation.

The Salmon Creek watershed and estuary is a small terminal system approximately 2 km north of the village of Bodega Bay. The village of Salmon Creek, CA, is located on the southern shore of the estuary. This estuary is primarily rural and dominated by livestock grazing and rural residencies.

The Estero Americano is south and west of the town of Valley Ford, CA. It is the smallest of the five studied estuaries. Land use in the watershed has traditionally been livestock grazing and dairy farming, which continue today.

Walker Creek is one of the two major systems that drains into Tomales Bay. Similar to the Estero Americano, land use in the watershed is typified by livestock grazing and dairy farming. Tomales Bay, including the mouth of Walker Creek are popular for noncontact water recreation and are used for commercial and recreational shellfish harvesting. The small town of Tomales is located north and east of the estuary on Keyes Creek, a tributary within the watershed. Walker Creek enters the outer portion Tomales Bay, an area with greater tidal exchange and connection with the Pacific Ocean than the inner portion of the Bay (Kimbrow et al., 2010).

Lagunitas Creek is the other major system contributing to Tomales Bay. Land use in the watershed is a mix of recreational lands for hiking, livestock grazing ranches and dairy farms, and several small towns. The estuary has been popular for noncontact water recreation including swimming, canoeing, and kayaking. Shellfish harvesting is also conducted in the portion of Tomales Bay north of where Lagunitas Creek empties into it. As a result of being located within the inner portion of Tomales Bay, Lagunitas Creek experiences tidal exclusion and longer water residence time than in the outer portions such as around Walker Creek (Kimbrow et al., 2010).

Study Design, Sample Collection, and Environmental Data Collection

At each estuary, we established a three-dimensional sampling grid of 45 sample points composed of 5 transects \times 3 positions (L \times W) that were sampled at three depths that attempted to span the entire saltwater–freshwater transition zone. The five transects were numbered 1 through 5, with 1 being located at the mouth of the estuary (primarily saltwater) and 5 being the furthest upstream (primarily freshwater), typically at the first riffle. Respective lengths of the sampling grid for each estuary were 4.0, 1.3, 7.4, 3.3, and 5.3 km for the Russian River, Salmon Creek, Estero Americano, Walker Creek, and Lagunitas Creek.

Facing downstream during summer base flow conditions, we established three sampling positions at each transect at 25, 50, and 75% of the channel width moving left to right. Water

samples were collected at each position from each of the three depths below the surface using an adapted depth sampler. With the sampler, we placed a sterile sample bottle at the desired depth and opened it for collection of the sample. A 1-L water sample was collected at approximately 30 cm below the surface, at the middle of the water column, and approximately 30 cm above the bottom of the channel.

For each of the five studied estuaries, we sampled each grid once per month for 10 mo, beginning in August 2004 and ending June 2005. We collected 45 water samples during each sampling event and assigned each of these sampling events into one of three seasons and flow conditions: wet-season stormflow, wet-season base flow, and dry-season base flow (Table 1).

We complemented sample collection with field measurements of dissolved oxygen, temperature, and discharge. We used a Yellow Springs Instruments Model 550A to measure dissolved oxygen and temperature at each sample position. To calculate discharge at each site we measured instantaneous flow using a Global Waters flow meter (Global Waters Inc.) and the sample transect cross-sectional area. These measurements were used in area–velocity method (velocity \times channel width \times channel depth) to calculate flow volume (Mosley and McKercher, 1993).

In addition to these field measurements, we collected precipitation, wind, and tidal data from existing meteorological stations in the study area. Precipitation data came from stations operated by Marin County in Point Reyes Station, by University of California Bodega Marine Laboratory in Bodega, and California Department of Forestry and Fire Prevention in Santa Rosa. These data were used to compile 24-h, 5-d, and annual cumulative precipitation for each sampling event. Average daily wind speed for each sample event was based on data from these same stations, as well as data reported at www.IWINDSURF.com. Tidal stage data were recorded based on tide tables for Tomales Bay, Bodega Bay, and Jenner, CA, using the appropriate and approved time adjustments for sample locations. We also used the WXTIDE32 software to obtain and confirm tidal height at time of sample collection (<http://www.wx tide32.com>).

Sample Analysis

Bacteria

All samples were transported on ice to University of California, Davis on the same day of sampling and stored in the dark under refrigeration (4°C) until processed for bacterial enumeration. Each composite water sample was split into SS and a residual water fraction. Residual water and SS fractions were analyzed separately for indicator bacteria within 6 to 96 h after collection. Mean hour of analysis was 41 h, with the longer duration times due to replating samples that had too numerous colonies to count. Specifically, each 1-L sample was mixed by hand, partitioned into four 250-mL sterile bottles, and remixed on an automated wrist shaker (Burrell Scientific) for 5 min at setting 7. To determine the level of centrifugation needed to pellet the total SS without reducing the amount of unattached bacteria in the water fraction, we evaluated three relative centrifugal forces (1000, 1500, 2000 *g*) for 5 min on 15 raw water samples from the Estero Americano, Salmon River, and Walker River. There were no significant differences in the weight of the pellets or in the concentration of *E. coli* in the residual water for the three different *g* forces. In contrast, the two

higher g forces did reduce the concentration of fecal coliforms in the water fraction; hence, we used 1000 g for 5 min as the protocol. After the suspension was centrifuged (Thermo Electron Corp.) at 1000 g for 5 min, the supernatant (residual water) was removed using a pipette and analyzed for fecal coliforms and *E. coli* as described below. The residual pellet was resuspended in 0.5 mL of sterile deionized water, the four aliquots pooled into a preweighed 2.0-mL microcentrifuge tube, and each 2.0-mL tube centrifuged at 14,000 g for 10 min. Supernatant was decanted and the weight of the SS pellet determined. The pellet was gently resuspended in 1200 μ L of phosphate buffered saline (Sigma-Aldrich) (PBS) for 30 s using a sterile 1 mL disposable pipette, with 800 μ L and 400 μ L added to separate aliquots of 45 mL of sterile PBS and analyzed for *E. coli* and fecal coliforms, respectively.

Escherichia coli and fecal coliform enumerations for the residual water and SS were conducted using direct membrane filtration (Clesceri et al., 1998). For residual water, aliquots of 5 to 400 mL were filtered through a sterile membrane (47 mm diam., 0.45 μ m pore, Fisherbrand) using a sterile stainless steel manifold (Hydro-lab). For *E. coli*, the membrane was placed onto CHROMagar EC (DRG International) and incubated at 35°C for a 2-h resuscitation period, transferred to 44.5°C for another 23 h (\pm 2h), then colonies enumerated. For fecal coliforms, the membrane was placed onto mFC agar (Difco Laboratories) and incubated at 44.5°C for 24 h, then colonies enumerated and adjusted to colony-forming unit (cfu) per 100 mL. For SS, 45 mL of sample suspension were processed as for residual water, described above.

Total Suspended Solids and Salinity

In addition to measuring indicator bacterial, a 100-mL subsample of each composite water sample was analyzed for total suspended solids (TSS) and salinity. Analysis for TSS was made using a 0.45- μ m pore filter in accordance with American Public Health Association protocols (Clesceri et al., 1998). Salinity analysis was conducted using a refractometer with automatic temperature compensation (Fisher Scientific Catalog Number 12-946-27).

Statistical Analysis

Descriptive statistics for fecal coliforms and *E. coli* concentrations in the residual water and SS fractions were calculated using S-Plus 2000 software (MathSoft, Inc.). Bacterial concentration in composite water was then generated by adding the bacterial counts from the SS and residual water fraction that comprised the original composite water sample. Linear mixed effects regression (Pinheiro and Bates, 2000) was used to model the association between indicator bacteria and the various estuarine, climate, and hydrological factors that can influence or bias bacterial counts in these systems. \log_{10} (concentration + 1) of each bacterial indicator was used as the outcome variable; estuarine, climate, and hydrological factors were set as fixed effects; each sample position (estuary, transect, position, and depth) was set as a group effect to adjust the P values for repeated sampling ($n = 10$) at the same site. A forward stepping algorithm was used to develop the multivariate regression model, with $P \leq 0.05$ based on a likelihood ratio test or conditional t -test set as the criterion for inclusion of the variable in the final model, including any significant quadratic or cubic terms. Finally, similar linear mixed effects regression models were developed for the proportion of total bacteria in composite water contributed by SS (SS bacteria counts/total bacteria counts), except that an arcsine trans-

formation (arcsin the square root of the proportion) was used for the outcome variable given that it was a proportion bounded by 0 and 1. To avoid any affect from collinearity in the final regression models, we identified and used only one variable where any variables demonstrated collinearity. When collinearity was encountered, we gave priority to variables that are informative about factors relative to monitoring estuary systems, such as sample location and depth instead of salinity.

Adjusting Concentration Values

Differences in the number of hours needed to sample each estuary resulted in different intervals of time between sample collection and processing at the analytical laboratory. To adjust bacterial indicator (fecal coliforms, *E. coli*) enumerations to a single time-duration standard of 24 h, we conducted a time-dependent decay analysis for fecal coliforms and *E. coli*. For each estuary site ($n = 5$) and for each season ($n = 3$, dry baseflow, wet baseflow, wet stormflow), two water samples were collected on different dates ($n = 2$). For each of these 30 sampling dates, fecal coliforms and *E. coli* were enumerated in the SS and residual water using membrane filtration as described above at approximately 4, 8, 24, 30, 48, and 54 h postcollection to generate the necessary raw data for statistical modeling of the decay coefficients in our source water. Salinity may also influence the decay rate for bacterial indicators, but given the already complex statistical model we refrained from adding this variable into the hold time model.

A linear mixed effects regression model (Pinheiro and Bates, 2000) was used to estimate the magnitude and significance of the time-dependent decay coefficients for fecal coliforms and *E. coli* in our source water. The \log_{10} concentration of each bacterial indicator was used as the outcome variable, site, season, and time (duration in hours between water collection in the field and bacterial enumeration in the laboratory) were set as fixed effects, and water sample ID set as a repeated measure (group random effect) to control for potential lack of independence of bacterial concentration. Level of significance for the various terms was set at P value < 0.05 , based on either a likelihood ratio test or a conditional t -test.

To adjust the bacterial indicator (BI) concentration in each sample tested x hours ($t = x$) after initial time of collection ($t = 0$) to a single 24-h standard ($t = 24$), we first assumed the following basic model:

$$\log_{10}(\text{BI}_{t=x} + 1) = \log_{10}(\text{BI}_{t=0} + 1) + \beta(t = x) \quad [1]$$

where $\log_{10}(\text{BI}_{t=x} + 1)$ is the observed \log_{10} (concentration + 1) of the bacterial indicator determined x hours ($t = x$) after initial time of collection, $\log_{10}(\text{BI}_{t=0} + 1)$ is the modeled \log_{10} (concentration + 1) of the bacterial indicator at the initial time of collection ($t = 0$), and $\beta(t = x)$ is the decay coefficient generated by the fitted linear mixed effects model described above. The decay process is for samples held under refrigeration at approximately 4°C. Once $\beta(t = x)$ is obtained, Eq. [2] is used to adjust each sample to a single 24-hour standard ($t = 24$), which is derived as follows:

$$\begin{aligned} \log_{10}(\text{BI}_{t=24} + 1) &= \log_{10}(\text{BI}_{t=0} + 1) + \beta(24) \\ \text{using Eq. [1], } \log_{10}(\text{BI}_{t=24} + 1) &= \log_{10}(\text{BI}_{t=x} + 1) - \beta(x) + \beta(24) \\ \log_{10}(\text{BI}_{t=24} + 1) &= \log_{10}(\text{BI}_{t=x} + 1) + \beta(24 - x) \\ \text{BI}_{t=24} &= (\text{BI}_{t=x}) 10^{\beta(24-x)} + 10^{\beta(24-x)} - 1 \end{aligned} \quad [2]$$

where $BI_{t=24}$ is the fitted or expected concentration of the bacterial indicator at a 24-h standard, $BI_{t=x}$ is the observed concentration of the bacterial indicator determined x hours ($t = x$) after initial time of collection, $10^{3(24-x)}$ is the expected decay coefficient adjustment factor raised to the power of 10 which allows us to model concentrations of the bacterial indicator directly instead of \log_{10} concentration values, and we subtract 1 to remove the 1 cfu added to the original \log_{10} transformation of cfu data.

Results and Discussion

Decay Coefficients for Indicator Bacteria

Linear mixed effects regression modeling determined that *E. coli* in residual water exhibited significant decay coefficients, with significant interactions with season and estuary site (Table 2), but that significant decay coefficients were not found for *E. coli* in SS or for fecal coliforms in either residual water or SS ($P > 0.05$). The decay coefficient for baseline referent conditions (Russian River, season being wet stormflow conditions) based on transformed data, $\log_{10}(\text{concentration}+1)$, was $\beta = -0.00021$ per hour of refrigerated storage. This baseline decay function is then modified depending on season and location, as shown in Table 2. An example interpretation of the model in Table 2 is such: for samples taken from the Russian River during wet stormflow conditions, every additional 10 h that a residual water sample was held under refrigeration was associated with a mean reduction in *E. coli* concentration (cfu/100 mL) of $\sim 0.5\%$ ($10^{-0.00021 \times 10} = 0.995$). The 24-h adjustment functions as follows: using the same baseline conditions and assuming we observed 50 cfu/100 mL of *E. coli* for a sample tested 14 h after collection, the expected 24-h concentration would be 49.75 cfu ($50 \times 0.995 = 49.75$ cfu/100 mL).

Residual Water and Suspended Solids

A total of 2371 water samples were collected from August 2004 to June 2005. In general, the number of wet-season stormflow sample events was lower than that for the other two seasons in each of the five studied estuaries (Fig. 2). The timing and number of storm events during the field season constrained our ability to evenly distribute wet-season stormflow sampling events across all five studied estuaries. Review of *E. coli* concentrations

Table 2. Estimated time-dependent decay coefficients for adjusting fecal coliforms and *Escherichia coli* to a 24-h duration standard, stratified by matrix (residual water and suspended solids) and indicator bacteria (fecal coliforms, *E. coli*).

Factors	Coefficient†	P value†
Duration (h)	−0.00021	0.92
Duration × season interaction		
wet-storm‡	0.0	–
wet-base	−0.0045	0.02
dry-base	−0.0078	0.0001
Duration × site interaction		
Russian River‡	0.0	–
Walker Creek	0.0023	0.37
Salmon Creek	−0.00034	0.90
Estero Americano	0.0072	0.005
Lagunitas Creek	0.00063	0.82

† Adjusted for potential lack of independence due to repeated sampling of estuaries.

‡ Referent condition for the categorical variable.

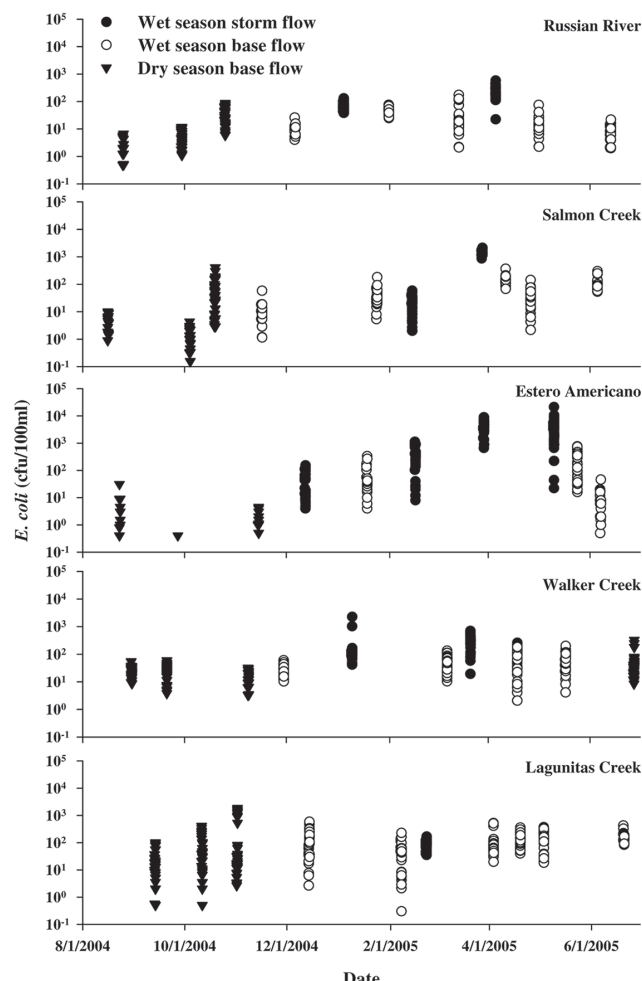


Fig. 2. Concentration for *Escherichia coli* in composite water samples collected during 10 sampling events in the Russian River, Salmon Creek, Estero Americano, Walker Creek, and Lagunitas Creek estuaries (top to bottom) from August 2004 to June 2005. Sample events are differentiated by wet-season stormflow (filled circles), wet-season base flow (open circles), and dry-season base flow (filled triangles). cfu = colony-forming unit.

for residual water fractions across the 10 sampling events within each estuary illustrates the season variability for indicator bacteria in these systems (Fig. 2). For example, examining the values for the Russian River, from the first sampling event on 25 Aug. 2004 to the 10th event on 13 June 2005, concentrations rise and fall for both fractions. This seasonality corresponds with winter storm season hydrologic responses in river discharge. It is further evidenced by the highest concentrations occurring during the two wet-season stormflow events on 1 Jan. 2005 (fifth event) and 5 Apr. 2005 (eighth event). This pattern is consistent for SS-associated indicator bacteria, as well.

There are differences between the five studied estuaries in the concentrations of fecal coliforms and *E. coli* for both fractions (Table 3, Fig. 2). Overall mean concentrations were highest in the Estero Americano; however, when results are compared by season and flow conditions, Estero Americano had the highest concentrations for both fecal coliform and *E. coli* in water during wet-season stormflow and also the lowest concentrations in dry-season base flow (Fig. 2). This result is probably related to the relationship between bacterial concentrations, salinity, and season in sampled water (Fig. 3). Both fecal coliform and *E. coli*

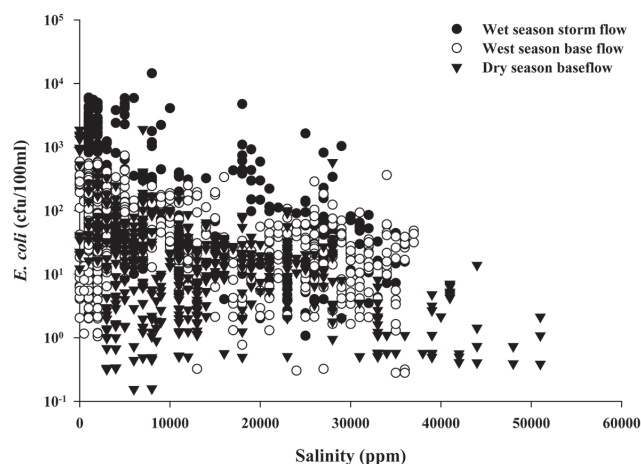


Fig. 3. Comparison of season and storm group *Escherichia coli* concentrations as a function of salinity in composite water samples collected from the Estero Americano.

were inversely related to salinity values. In addition, concentrations in residual water and SS fractions were greatest in samples collected during wet-season stormflow, followed by wet-season base flow, followed by dry-season base flow. The Estero Americano, a bar built estuary with relatively fewer freshwater inputs in the dry-season base flow period, became hypersaline with salinity values above 32,000 ppm during this season. Comparatively, Lagunitas had the lowest mean fecal coliforms and *E. coli* residual water concentrations during wet-season base flow and highest mean values during the dry-season low flow conditions. Lagunitas is a low-inflow estuary with corresponding freshwater inputs during the summer. Additionally, its position in the rear of Tomales Bay contributes to relatively longer residence time for freshwater inputs across the three seasons (Largier et al., 1997).

Summary statistics also demonstrate the relative differences for both fecal coliforms and *E. coli* in the two fractions (Table 3). To

compare bacterial concentrations between water (measured by volume) and SS (measured by mass), we needed to standardize the values to the same units. Our first step was to adjust water concentrations to be colony-forming units per gram (cfu/g), assuming water has a specific gravity of 1.0 g/cm³. Using these standardized values, the mean concentrations for all residual water and SS samples are 3 and 2684 cfu/g for fecal coliforms and 3 and 1900 cfu/g for *E. coli*, respectively. This supports our assertion that suspended sediment on a per gram basis exhibits high amounts of indicator bacteria and is consistent with recent quantification of the microbial community diversity (Lyons et al., 2010) and presence of pathogens (Lyons et al., 2007) on such marine aggregates.

The total quantity of SS in any given water sample directly influences the contribution of SS bacteria to the composite water sample bacteria value. The concentration in a composite water sample was on average 23 mg/L (SE 0.50), ranging from 4 to 321 mg/L. This mean concentration corresponds with a mean percent contribution of SS to the overall bacteria load of 8% (SE 0.3) for fecal coliforms and 7% (SE 0.3) for *E. coli*. This suggests that the majority of indicator bacteria contained in the water column of these estuaries will not readily settle out given their near-neutral buoyancy and presumably low settling velocities. These results are consistent with previous work showing the majority of indicator bacteria in a water column are unbound to suspended particles (Ferguson et al., 2003). The seasonality and relationship to drivers such as tide stage on the proportion of SS-associated indicator bacteria has implications for transport dynamics and sources of microbial pollution. In the case of *E. coli*, this proportion was greatest in wet-season stormflow (Fig. 4), when freshwater inputs likely dominated. This is in comparison with the other two seasons in which mixing of freshwater inputs and saline ocean waters was relatively more complete. There is an interesting bimodality to this proportion during the model, suggesting that saltwater inflows via tidal exchange can explain this effect, as was observed in previous work

Table 3. Mean fecal coliforms and *Escherichia coli* concentrations values from untransformed data for suspended solids (SS) (colony-forming unit [cfu]/g), and residual and composite water (cfu/100 mL) from five northern California estuaries.

Estuary (n)	Fecal coliforms				<i>E. coli</i>			
	Mean	SE	Min.†	Max.	Mean	SE	Min.†	Max.
SS (cfu/g)								
Russian (463)	1780	166	0	28,294	1237	112	0	16,208
Salmon (466)	2853	348	0	52,962	2212	261	0	33,849
Americano (464)	5588	893	0	181,508	3303	412	0	78,184
Walker (468)	1288	108	0	16,985	932	70	0	9,189
Lagunitas (468)	2012	336	0	150,150	1837	362	0	134,932
Residual Water (cfu/100 mL)								
Russian (477)	61	4	0	667	47	4	0	585
Salmon (474)	185	21	0	2,689	171	18	0	2,116
Americano (476)	953	99	0	21,110	576	60	0	14,413
Walker (472)	115	10	0	2,355	85	7	0	2,277
Lagunitas (472)	111	5	0	933	136	10	0	1,894
Composite water (cfu/100 mL)								
Russian (477)	71	5	0	709	54	4	0	620
Salmon (474)	206	23	0	3,037	189	20	0	2,279
Americano (476)	1033	106	0	21,846	601	62	0	14,730
Walker (472)	120	10	0	2,439	88	7	0	2,313
Lagunitas (472)	115	5	0	943	142	10	0	1,927

† Values for indicator bacteria were below detection limit, resulting in a concentration value of zero.

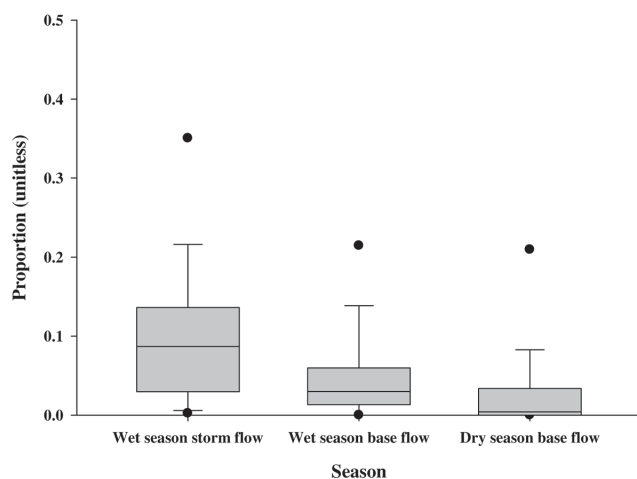


Fig. 4. Proportion of total suspended solids-associated *Escherichia coli* in composite water grouped by season and storm group. Box represents mean and 25 and 75% deviation from the mean. Whiskers represent 5 and 95% deviation from the mean.

(e.g., Almeida et al., 2007). This suggests that monitoring protocols that attempt to compare microbial water quality between different estuaries will need to have comparable locations along the freshwater–saltwater interface, perhaps adjusting measured bacterial concentrations based in part on salinity values similar to the method

Table 4. Linear mixed effects regression model for the associations of composite water sample fecal coliforms concentration with sampling, climatic, and estuary characteristics in five northern California estuaries. Coefficients are $\log_{10} + 1$ values due to data transformation to account for zero values in fecal coliforms concentration.

Factor	Coefficient	95% CI†	P value†
Intercept	2.4	(2.18, 2.62)	<0.0001
Transect‡			
1	0.00	–	–
2	0.06	(–0.06, 0.18)	0.3173
3	0.11	(–0.008, 0.24)	0.0666
4	0.17	(0.05, 0.30)	0.0055
5	0.20	(0.08, 0.32)	0.0016
Depth sample collected (m)	–0.13	(–0.18, –0.07)	<0.0001
Season‡			
Wet-season stormflow	0.00	–	–
Wet-season base flow	–0.76	(–0.87, –0.65)	<0.0001
Dry-season base flow	–1.54	(–1.66, –1.43)	<0.0001
Tides			
Tide height	–2.08	(–2.69, –1.47)	<0.0001
Tide height ²	2.65	(1.94, 3.35)	<0.0001
Tide height ³	–0.93	(–1.17, –0.70)	<0.0001
Precipitation			
24-h cumulative (mm)	0.08	(0.05, 0.11)	<0.0001
24-h cumulative ² (mm)	–0.005	(–0.007, –0.003)	<0.0001
5-d cumulative (mm)	0.03	(0.02, 0.03)	<0.0001
5-d cumulative ² (mm)	–0.0007	(–0.0008, –0.0006)	<0.0001
Annual cumulative (mm)	0.0002	(0.00007, 0.0003)	0.0005
Wind speed (km/h)	0.01	(0.009, 0.014)	<0.0001
Discharge velocity (m/s)	1.08	(0.79, 1.38)	<0.0001

† Adjusted for potential lack of independence due to repeated sampling of estuaries.

‡ Reference condition is transect 1 closest to the estuary mouth and wet-season stormflow, respectively.

we used for adjusting bacterial concentrations to a standard 24-h hold time (Eq. [1] and [2]).

Similar to transect location, sampling depth imparted a negative bias on the measured concentration of either bacterial indicator (Tables 4 and 5, Fig. 5). For every additional meter in depth that a water sample was taken, there was 25 to 27% reduction in the measured concentration of indicator bacteria (e.g., for *E. coli*, $10^{-0.137} = 0.73$). Again, this can be explained by saltwater stratification as a function of depth, such that water samples taken near the bottom had salinity values that averaged 12,000 ppm compared with 9,000 ppm for samples taken near the surface (data not shown). This demonstrates another important way in which a sampling protocol can introduce bias through sample collection depth. It is important to note, however, that water column position, defined as sampling positions at 25, 50, and 75% of the channel width moving left to right, did not have a significant association ($P > 0.05$) with concentration for either indicator bacteria.

As in previous work, season is significantly associated with the concentration of both indicator bacteria. Compared with the wet-season stormflow referent condition, there is an approximately 83% reduction during wet-season base flow and a 97% reduction during dry-season base flow for concentrations of both fecal coliforms and *E. coli* (Tables 4 and 5, Fig. 6). Valid comparisons of microbial water quality between different estuaries would need to be matched on the proportion of samples taken from these three different seasons.

Table 5. Linear mixed effects regression model for the associations of composite water sample *Escherichia coli* concentration with sampling, climatic, and estuary characteristics in five northern California estuaries. Coefficients are $\log_{10} + 1$ values due to data transformation to account for zero values in *E. coli* concentration.

Factor	Coefficient	95% CI†	P value†
Intercept	2.37	(2.16, 2.58)	<0.0001
Transect‡			
1	0.00	–	–
2	0.09	(–0.03, 0.21)	0.1756
3	0.15	(0.02, 0.27)	0.0237
4	0.19	(0.06, 0.31)	0.0032
5	0.24	(0.11, 0.436)	0.0002
Depth sample collected (m)	–0.14	(–0.19, –0.08)	<0.0001
Season‡			
Wet-season stormflow	0.00	–	–
Wet-season base flow	–0.79	(–0.90, –0.68)	<0.0001
Dry-season base flow	–1.39	(–1.50, –1.28)	<0.0001
Tides			
Tide height	–1.69	(–2.31, –1.08)	<0.0001
Tide height ²	2.13	(1.43, 2.84)	<0.0001
Tide height ³	–0.74	(–0.97, –0.50)	<0.0001
Precipitation			
24-h cumulative (mm)	0.07	(0.04, 0.10)	0.0037
24-h cumulative ² (mm)	–0.004	(–0.006, –0.003)	<0.0001
5-d cumulative (mm)	0.022	(0.016, 0.027)	<0.0001
5-d cumulative ² (mm)	–0.0006	(–0.0007, –0.0005)	<0.0001
Wind speed (km/h)	0.01	(0.009, 0.015)	<0.0001
Discharge velocity (m/s)	1.02	(0.72, 1.31)	<0.0001

† Adjusted for potential lack of independence due to repeated sampling of estuaries.

‡ Reference condition is transect 1 closest to the estuary mouth and wet-season stormflow, respectively.

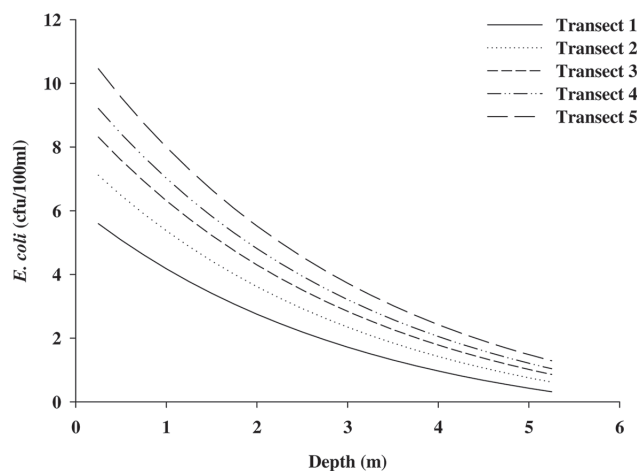


Fig. 5. Modeled concentration of *Escherichia coli* in water as a function of sample collection depth, based on model in Table 3 and comparing study transects values (1 = closest to estuary mouth; 5 = furthest inland). Modeled conditions were set at dry-season base flow, 24-h and 5-d precipitation of zero, wind speed of 11.7 km/h, discharge velocity of 0.08 m/s, and tide stage at sampling of 0.93 m. cfu = colony-forming unit.

The relationship between tide stage and concentration of fecal coliforms and *E. coli* is described by negative first-order, positive second-order, and negative third-order coefficients (Tables 4 and 5). The combined result of these three coefficients is a decrease in indicator bacteria concentrations with increases in tide stage by more than 3 logs (Fig. 6). There is a window of tidal stage between 0.5 and 1.5 m through which concentrations do not significantly change, suggestive of a relative steady state of mixing of higher bacterial-laden freshwater and lower bacterial-laden saltwater. Above 1.5 m of tidal stage, the sample location is presumably dominated by saltwater leading to lower bacterial concentrations. Conversely, the portion of the tidal swing below 0.5 m is characterized by maximum inflows of freshwater leading to higher bacterial concentrations and shallow water columns with greater relative opportunity for sediment resuspension. Monitoring programs that are not standardized as to tidal stage can impart substantial bias on measured bacterial concentrations when comparing sites, evaluating land use impacts, or attempting to monitor water quality improvements subsequent to remediation efforts.

The relationship of precipitation to indicator bacterial concentration consists of a combination of 24-h, 5-d, and annual cumulative precipitation, demonstrating the role of antecedent rainfall and bacterial flushing on daily, weekly, and annual time steps. Concentrations of fecal coliform and *E. coli* increase with each additional millimeter of rainfall in the 24 h before sampling, as indicated by the positive first-order coefficient term in the models. This is indicative of storm flushing and the role that additional rainfall has in generating runoff and stream discharge, hydrologically connecting an estuary to the surrounding upland watershed. This increase in flushing in response to 24-h cumulative precipitation has a threshold of 10 mm as indicated by the negative coefficient for the second order term in the models. Once 24-h cumulative precipitation exceeds 10 mm, bacterial concentrations begin to decline with each additional millimeter of rainfall, indicating that bacterial sources become limited relative to rainfall amounts and corresponding runoff volumes. This effect was previously observed in upland runoff, where fecal coli-

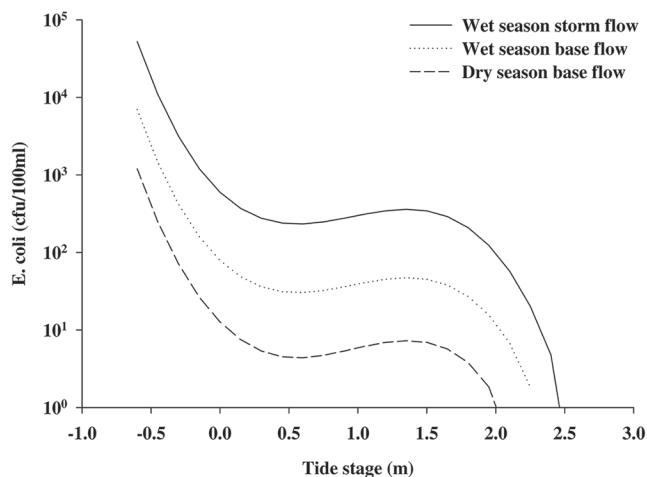


Fig. 6. Modeled concentration of *Escherichia coli* in water as a function of tide stage at sampling, based on model in Table 3 and comparing season and flow groups. Model conditions used transect 1 or estuary mouth, depth of sample collection of 0.3 m, 24-h cumulative precipitation of 1.3 mm for wet-season stormflow and zero for the other two seasons, 5-d cumulative precipitation of 10.6 mm for both wet-season flow categories and zero for dry season, wind speed of 11.7 km/h, and discharge velocity of 0.08 m/s. cfu = colony-forming unit.

forms (Lewis et al., 2009, 2010), *Cryptosporidium* oocysts (Miller et al., 2008), and *Giardia duodenalis* cysts (Miller et al., 2007) all exhibited source limitations once 24-h cumulative precipitation exceeded a certain threshold.

Similarly, bacterial concentrations also increase with incremental increases in 5-d cumulative precipitation until a threshold of 18 to 20 mm is reached, as indicated by the first-order positive and second-order negative coefficients for these models. The implication is that above this threshold, source limitations appear to occur, leading to reductions in bacterial concentrations. This observation is consistent with the role that 5-d antecedent rainfall has in saturating watershed soils, in effect priming the watershed, so that subsequent storms generate runoff. In contrast, annual cumulative precipitation is significantly associated only with fecal coliform concentration and exhibits a positive correlation with precipitation totals. The positive coefficient predicts a minor 0.05% increase in bacterial concentration for every additional millimeter of annual rainfall before sampling ($10^{0.0002} = 1.0005$).

Wind speed is significantly associated with the concentration of fecal coliforms and *E. coli*, such that for every additional kilometer per hour increase, there is an associated increase of 2.6 to 2.7% in bacterial concentration. This result is consistent with other studies confirming the role of wind in resuspending sediment and bacteria reservoirs within that sediment (Roslev et al., 2008; Wu et al., 2009). Admittedly, this analysis does not include wind direction (Ufnar et al., 2006), which may be different for each of the studied estuaries.

The coefficient for discharge velocity in both models is positive, indicating that increases in bacteria concentrations are associated with increases in river flow. For example, for every additional 0.1 m/s increase, there is an associated increase of 26 to 28% in bacterial concentration (e.g., *E. coli*, $10^{1.02 \times 0.1} = 1.26$). Higher velocity is representative of increased watershed hydrologic connectivity and therein flow paths for transport of bacteria. Additionally,

increases in velocity contribute to conditions that impede the influx of low bacteria saltwater through tidal exchange.

The five studied estuaries are influenced by mediterranean climate with the transport and delivery of bacteria from upland sources during winter storms. A flushing of bacteria at the daily, weekly, and annual time steps also points to sources of bacteria in the surrounding watersheds that are not constant. Tidal swings influence bacteria concentrations through timing and distribution of saltwater, water with relatively low bacteria levels, across each estuary. Similarly, discharge velocity influences bacteria concentration through the timing and distribution of freshwater, water with relatively high bacteria levels, across each estuary. The documented relationship that wind has with bacteria concentrations points to the potential for sediment bacteria reservoirs to be a source of bacteria through resuspension under certain conditions.

Suspended Solid–Associated Bacteria

The intercept coefficients for the proportion contributed by SS-associated bacteria to the composite water sample concentration, when untransformed, are 0.09 (9%) and 0.13 (13%) for fecal coliform and *E. coli*, respectively (Tables 6 and 7). Although the suspended solids fraction, on a standardized basis, is microbiologically rich relative to the water fraction, it generally comprises less than 15% of the total concentration of bacteria in a water sample.

The response and fit values for these two models had correlation coefficients of adjusted $r^2 = 0.11$ and $r^2 = 0.17$, respectively. It may be possible to improve these model fits by the inclusion of linear partitioning approaches and a resuspension term (Wu et al., 2009) but likely only minimally (Russo et al., 2011). Proportional value increases are associated with increases in depth of sample collection and discharge velocity as indicated by the positive coefficients. Decreases in proportional values are associated with wet-season base flow and dry-season base flow categories and tide stage. Relationships with tide stage at time of sampling are represented by a negative first-order and positive second-order coefficient (Fig. 7). Combined, these models attribute changes in the portion of SS-associated bacteria to changes in transport capability. For example, increases in discharge velocity are consistent with flow conditions

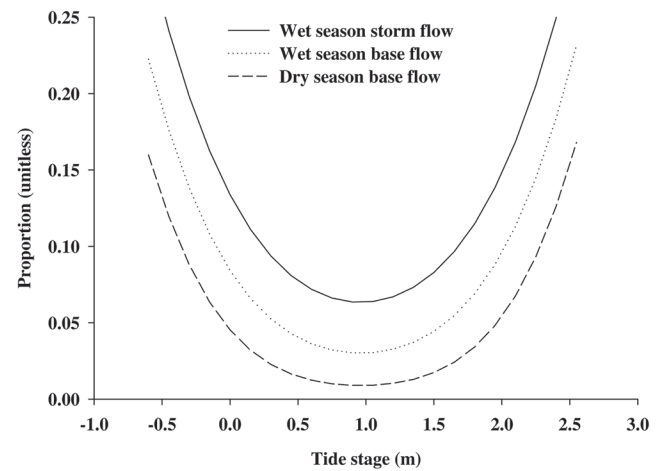


Fig. 7. Modeled proportion of suspended solids–associated *Escherichia coli* to *E. coli* concentration of a composite water samples as a function of tide stage at time of sampling. Based on the model in Table 6 and comparing season values. Modeled conditions used a depth of sample collection of 0.3 m, wind speed of 11.7 km/h, and discharge velocity of 0.08 m/s.

that can keep particles and attached bacteria in suspension. This is true for the model values across the three seasons with decreasing hydrologic connectivity and transport capacity in the two base flow seasons relative to the stormflow season. Similarly, across the tidal swing, there is a transition from high transport capacity above 1.5 m to the tidal window of low transport capacity between 0.5 to 1.5 m to high transport capacity again below 0.5 m. Note that transect location was not significantly related to the proportional values, indicating that location across the freshwater saltwater interface is not a factor associated with the proportion of total waterborne bacteria attributed to SS in the water column.

Increases and decreases in this portion are associated with transport processes, including differences between the three seasons and corresponding storm and base flow conditions, and tidal stages with the greatest exchange of water. That the proportion provided by SS-associated bacteria increases with depth is indicative of these solids being concentrated through settling or their

Table 6. Linear mixed effects regression model for the associations of the percent contribution of TSS bacteria to the composite water sample fecal coliform concentration with sampling, climatic, and estuary characteristics in five northern California estuaries. Coefficients are arcsine transformed values due to data transformation to account for distribution of proportional values.

Factor	Coefficient	95% CI†	P value‡
Intercept	0.31	(0.27, 0.36)	<0.0001
Depth sample collected (m)	0.009	(0.007, 0.043)	0.0055
Season‡			
Wet-season stormflow	0.00	–	–
Wet-season base flow	–0.07	(–0.10, –0.05)	<0.0001
Dry-season base flow	–0.07	(–0.11, –0.04)	<0.0001
Tides			
Tide height	–0.15	(–0.24, –0.06)	0.0008
Tide height ²	0.09	(0.05, 0.14)	<0.0001
Wind speed (km/h)	–0.002	(–0.003, –0.001)	0.0003
Discharge velocity (m/s)	0.17	(0.07, 0.26)	0.0007

† Adjusted for potential lack of independence due to repeated sampling of estuaries.

‡ Reference condition is wet-season stormflow.

Table 7. Linear mixed effects regression model for the associations of the percent contribution of TSS bacteria to the composite water sample *Escherichia coli* concentration with sampling, climatic, and estuary characteristics in five northern California estuaries. Coefficients are arcsine transformed values due to data transformation to account for distribution of proportional values.

Factor	Coefficient	95% CI†	P value‡
Intercept	0.37	(0.33, 0.41)	<0.0001
Depth sample collected (m)	0.03	(0.02, 0.04)	<0.0001
Season‡			
Wet-season stormflow	0.00	–	–
Wet-season base flow	–0.08	(–0.11, –0.06)	<0.0001
Dry-season base flow	–0.16	(–0.19, –0.13)	<0.0001
Tides			
Tide height	–0.25	(–0.32, –0.18)	<0.0001
Tide height ²	0.13	(0.10, 0.17)	<0.0001
Wind speed (km/h)	–0.001	(–0.002, –0.0003)	0.0080
Discharge velocity (m/s)	0.09	(0.003, 0.17)	0.0413

† Adjusted for potential lack of independence due to repeated sampling of estuaries.

‡ Reference condition is wet-season stormflow.

resuspension from the estuary bottom by discharge and wind-driven wave action or both. That these contributions are small but appreciable is consistent with the findings of Wu et al. (2009) and Russo et al. (2011), wherein their models were improved by the inclusion of a resuspension term to account for partitioning of free water and suspended solid bacterial fractions. In the five study estuaries, the finest grain material was in the Estero Americano, which corresponded to higher bacterial concentrations in the sediment. This is consistent with the indirect relationship of bacteria and particle size documented by Wu et al. (2009), suggesting that resuspension of sediment bacteria has greater potential to influence water quality in that estuary than in the other four.

Conclusions

Differences exist between the studied estuaries in the bacteria concentrations for both the water and SS fractions. Variations in land use and bacteria sources probably contribute to these differences. The differences also result from the processes and dynamic exchanges of freshwater and saltwater across these systems.

Water quality monitoring programs are limited with regard to sample site selection because of access and changes in conditions across seasons and across years. Biases introduced by this limitation can be reduced by putting samples into the spatial and temporal context of the saltwater–freshwater interface. Documentation of tidal stage, 24-h, 5-d, and annual cumulative precipitation, and discharge velocity at sampling is important for explaining a sample's hydrologic connectivity with the watershed and position within tidal swings. Measuring salinity and TSS will also be useful for contextualizing bacteria concentration results. Salinity measurements combined with consistent sample collection at the same site and depth across time will help to reduce and explain biases that can be introduced.

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