Ambient monitoring of bacterial indicators and enteric pathogens (*Salmonella* & *E. coli* O157:H7) along California's central coastal watersheds

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Executive Summary

The purpose of this study was to monitor bacterial indicators in ambient water samples and to analyze their association with *Salmonella* and *E. coli* O157:H7 in both water and sediment for a suite of central coastal California watersheds. Specific objectives include:

- Evaluate water and sediment from 23 different rivers and streams along the central coast of California for the presence of *E. coli* O157:H7 and the concentration of *Salmonella*.
- Look for seasonal trends of fecal coliform concentration in comparison to presence of *E. coli* O157:H7 and *Salmonella*.
- Analyze data for use in a Preliminary Project Report for TMDL development
- Help determine possible sources of pathogenic bacteria associated with recent outbreaks

Twenty three rivers, creeks or their estuaries were selected from the Central Coast Watersheds, with each site listed below:

- 304APT - Aptos Creek at Spreckles Drive
- 304LOR - San Lorenzo Estuary at Laurel Street
- 304SCO - Scott Creek Lagoon at Highway 1
- 304SOK - Soquel Creek at Knob Hill
- 305THU - Pajaro River at Thurwachter Bridge
- 307CML - Carmel River at Highway 1
- 308BSR - Big Sur River at Andrew Molera foot bridge
- 309DAV - Salinas River at Davis Road
- 309TDW - Tembladero Slough at Monterey Dunes Way
- 310ARG - Arroyo Grande Creek at 22nd Street
- 310PIS - Pismo Creek above Highway 101
- 310SLB - San Luis Obispo Creek at San Luis Bay Drive
- 310SRO - Santa Rosa Creek at Moonstone Drive
- 310SSC - San Simeon Creek at State Park foot bridge
- 310TWB - Chorro Creek at South Bay Boulevard
- 312SMA - Santa Maria River above Estuary
- 313SAI - San Antonio Creek at San Antonio Road West
- 314SYN - Santa Ynez River at 13th Street
- 315ABU - Arroyo Burro Creek at Cliff Drive
- 315ATA - Atascadero Creek at Ward Drive
- 315CRP - Carpinteria Creek downstream of Carpinteria Ave
- 315MIS - Mission Creek at Montecito Street
- 315RIN - Rincon Creek at Bates Road, Highway 101

Over the course of a twelve month period from April 2009 to April 2010, the Central Coast Region Water Quality Board conducted 56 sampling events in the central coast watersheds which resulted in 251 water and sediment samples being collected for analysis. Sampling events occurred monthly at each of the selected sites to observe seasonal trends within the data. Field measurements included pH, conductivity, turbidity, dissolved oxygen, salinity, and chlorophyll a.

The central Coast Regional Water Board's contract laboratory (BC Laboratories, Inc.) evaluated indicator bacteria per 100 mL of water sample, including total coliform, fecal coliform, and *E. coli*, *Salmonella* and *E. coli* O157:H7 were analyzed by the Western Institute for Food Safety and Security and Dr. Atwill's Laboratory, Department of Population Health and Reproduction, School of Veterinary Medicine at University of California, Davis.

The annual arithmetic mean for fecal coliform varied in excess of 2-$\log_{10}$ from site to site, ranging from a low of 114 MPN/100 mL at the Carmel River at Highway 1 (307CML) to a high of 20,223 MPN/100 mL at the Salinas River at Davis Road (309DAV). Similar variability occurred for *E. coli*. Seasonal peaks occurred during January and August for the mean concentration of fecal coliform and indicator *E. coli*, the result of a subset of site locations experiencing high concentrations of these bacterial indicators during these two months. Based on calculating the geometric mean of fecal coliform for each site's water data, 16 of the 23 water bodies were above the REC1 standard of 200 MPN/100 mL (sites 304APT, 304LOR, 304SOK, 305THU, 309DAV, 309TDW, 310ARG, 310SLB, 310SRO, 310TWB, 312SMA, 314SYN, 315ABU, 315ATA, 315CRP, 315MIS) and 1 site (312SMA) was above the REC2 standard of 2000 MPN/100 mL.

Salmonella was detected in 31% of water samples, with an arithmetic mean of 1.28 MPN/100 mL for the subset of positive samples. Twenty of the 23 sampling sites had at least one water sample test positive for *Salmonella*, but Aptos Creek, Soquel Creek and Carpinteria Creek consistently tested negative. Many of the serotypes of *Salmonella* isolated from these locations appear to be pathogenic for humans; for example, *S.* Typhimurium and *S.* Newport were relatively common isolates from the coastal watersheds. Although the prevalence and the concentration of *Salmonella* from water bodies classified as above the REC 1 or REC 2 standards for fecal coliform were not significantly different from sites below these standards, the concentration of fecal coliform was significantly associated with the concentration of *Salmonella*. Lastly, *Salmonella* was detected in 23% of sediment samples, with an arithmetic mean of 19.1 MPN/100 g wet weight for the subset of positive samples. Twenty of the 23 sampling sites had at least one sediment sample test positive for *Salmonella*, but Scott Creek Lagoon, Soquel Creek, and Salinas River consistently tested negative. Preliminary statistical analysis found that the occurrence of *Salmonella* in the overlying water column was strongly associated with *Salmonella* in sediment: for each additional MPN/100 mL of waterborne *Salmonella* there was a 5.5 MPN/100 g increase in sediment-borne *Salmonella.*

Approximately 2.4% of water samples were positive for *E. coli* O157:H7, with four sites having one positive sample (304SOK, 305THU, 310SLB, 310TWB) and one location, 312SMA, having two water samples test positive. The odds of testing positive for *E. coli* O157:H7 from this site on the Santa Maria River was 13.1 times greater compared to water from elsewhere in the study. Moreover, the probability that a sample site tested positive on 2 out of 11 occasions for this pathogenic strain of *E. coli* by random chance, assuming a background prevalence of 2.4%, was very low (P=0.025). These data suggest that the occurrence of *E. coli* O157:H7 at this site on the Santa Maria River may be higher than background levels for central coastal California. This site also had the highest prevalence of *Salmonella* and the highest concentrations

of fecal coliform and indicator *E. coli*, suggesting that more intensive sampling may be warranted in order to clarify the cause(s) of these elevated bacterial concentrations.

There were no significant associations between the land use categories of urban, open, or agriculture and the bacterial levels at these sampling sites (*P*-value >0.05). In contrast, the surface area (km^2) of the catchment directly contributing to the sampling site was significantly associated (*P*-value = 0.047) with the odds of detecting *Salmonella* at the site (OR = 1.08, 95% CI 1.001, 1.17); for each additional square km in surface area, the odds of detecting *Salmonella* increased by about 8%.

The arithmetic mean of pH, temperature, conductivity, turbidity, dissolved oxygen, salinity, and chlorophyll a were 7.7, 14.37^{o}C, 2143.5 uS, 115.5 NTU, 9.58 ppm, 1.21, and 14.0 ug/L for the monitored watersheds. Seasonal shifts around these overall means followed predictable seasonal patterns for these coastal sites. For example, the typically reduced stream flows at these coastal confluent sites likely lead to increases in salinity and conductivity during summer and fall compared to winter, with the opposite trend occurring for turbidity and dissolved oxygen.

Introduction

Pathogenic bacteria are of major concern for food safety and water quality. According to the 2006 Summary Report produced by FoodNet and the Center for Disease Control and Prevention, 41% of reported human bacterial infections are caused from *Salmonella* and 4% are caused by *E. coli* O157:H7. Despite continual efforts to improve food safety and water quality, local and regional outbreaks still occur due to these two bacterial pathogens. There has been debate over the originating vertebrate source(s) of *E. coli* O157:H7 in recent outbreaks, for example it is unclear what the definitive vertebrate was for the outbreak of *E. coli* O157:H7 in California spinach in 2006. Domestic animals, wildlife, and human waste have all been identified as possible sources of foodborne and waterborne outbreaks during the past decade.

Central Coast Regional Water Quality Control Board (CCRWQCB) staff documented *E. coli* O157:H7 in various streams in the Region during collaborative monitoring with the California Department of Public Health (CDPH) and the United States Department of Agriculture. This sampling was conducted in part as a preliminary evaluation for eventual TMDL development and as a follow-up to determine possible vertebrate sources associated with contaminated leafy green commodities in Monterey and San Benito Counties. In these studies, *E. coli* O157:H7 was detected in the upper Gabilan watershed and downstream at 6 additional sites including the Old Salinas River Estuary (Cooley et al., 2007). These findings suggest that sampling at lower ends of watersheds can be effective at detecting these pathogens from possible upstream sources.

The focus of this study was to characterize the seasonal occurrence of *E. coli* O157:H7, *Salmonella*, and various nutrients in ambient water and sediment samples from 23 water bodies (rivers, streams, estuaries) along the central coast of California and to determine their relationship with bacterial indicators used to monitor water quality. An additional goal was to compare reported pathogen concentrations to water quality objectives and guidelines including, but not limited to, the Central Coast Regional Water Board Water Quality Control Plan (Basin Plan, 2007), and USEPA Bacterial Water Quality Standards for Recreational Waters guidelines (USEPA Standards, 2003).

Materials and Methods

Sample locations

Sampling was done on a monthly basis over the course of a year in the lower reaches or coastal confluences of these 23 sampling sites located within the following 10 major watersheds of the California central coast: Santa Cruz, Pajaro, Carmel, Big Sur, Salinas, San Luis Obispo, Santa Maria, Santa Antonio, Santa Ynez, and Santa Barbara (Fig 1, Table 1). These long-term coastal integrator sites capture over 90% of the CCWQCB watershed area.

Twenty three sites were selected for sampling in the Central Coast watersheds (Table 1) based on experience and data of previous monitoring projects. For all sites, safety and all-

weather access are priorities for sampling activities. Sampling locations were distributed throughout the Central Coast Watersheds. All samples were collected as grab samples.

To address the objective of this project, the field measurement parameters selected included temperature, dissolved oxygen, pH, and specific conductivity; and parameters for laboratory analysis include fecal coliform, *E. coli*, *E. coli* O157:H7 and *Salmonella*. The *E. coli* analysis was conducted at the Central Coast Water Board's contract lab. The *E. coli* O157:H7 and *Salmonella* analysis were conducted in laboratory at the Western Institute for Food Safety and Security at UC Davis. Progress reports were submitted to the State Water Board after completion of laboratory analysis of samples on a quarterly basis.

Environmental sampling

Water samples were collected at the midpoint of a flowing stream; field staff walked out to the middle of the stream to collect samples. Samples were collected in autoclaved-sterilized Nalgene polycarbonate bottles. Water samples were collected facing upstream and inverting the sampling cup perpendicular to the creek surface. Once the midpoint between creek surface and creek bed was reached, the collection bottle was inverted 90^{o} to collect water. Sediment samples were collected facing upstream and inverting sampling cups perpendicular to the creek surface until the creek bed was reached. The sediment collection cups were then used to scoop up the sediment. Excess water was drained from the sediment collection cups. Samples were shipped overnight with ice packs and processed within 24 hours of collection.

Detection of *E. coli* O157:H7

We used a modification of the method described by Cooley et al. (2007) to enrich and isolate *E. coli* O157:H7 from environmental samples. Ten grams of sediment was aseptically weighed out and transferred into 100mL TSB. Vacuum filtration was used to filter 500mL water; the membrane was then added to 100mL TSB. Both, sediment and water, samples were incubated for 2 hours at 25^{o}C with shaking at 150 RMP, then 8 hours at 42^{o}C, and finally held static overnight at 6^{o}C. Immuno-Magnetic Separation (IMS) was run using an automated IMS Dynal BeadRetriever (Invitrogen Carlsbad, California USA), according to manufacturer's instructions. Briefly, 500µL Phosphate Buffered Saline-Tween-20 (PBS) was aseptically added to wells 1 and 2 of Dynal Tube strip, 1000µL PBS was added to wells 3 and 4, and 100µL PBS was added to well 5. 500µL of the cultured TSB was added to wells 1 and 2 followed with 10µL Dynabeads anti-*E. coli* O157:H7 (Invitrogen Carlsbad, California USA). After incubation and washing, the beads were re-suspended in well 5 (see figure 1). 50µL of the re-suspended beads-PBS complex were plated and streaked for isolation onto Rainbow agar (Biolog, Hayward, CA) with novobiocin (20 mg/L MP Biomedicals, LLC Solon, Ohio USA) and tellurite (0.8 mg/L MP Biomedicals, LLC Solon, Ohio USA) (NT-Rainbow). The remaining 50µL was plated and streaked for isolation on Sorbitol MacConkey Agar (BD Sparks, MD USA) with cefixime (0.05 mg/L USP Rockville, MD USA) and tellurite (2.5 mg/L) (CT-SMAC). The plates were

incubated overnight for 18-24 hours at 37°C. Two presumptive positive colonies per plate were picked and streaked onto LB (BD Franklin Lakes, NJ) for extraction and cryogenic storage.

DNA was extracted using simple boiling method. Briefly, a 10µL loopful of bacteria was swirled into 100µL DNase free water in a microcentrifuge tube and incubated at 100°C for 20 minutes. After incubation, the microcentrifuge tubes were centrifuged for 10 minutes at 5000 rpm. The supernatant was transferred to a sterile microcentrifuge tube. The two suspect colonies per positive sample were PCR-confirmed using primers from Paton and Paton (2003). Each reaction contained 48.5µL master mixture and 1.5µL DNA. The master mixture was composed of: 1x Buffer, 0.4µM forward and reverse primers, 200µM dNTPs, 1.5mM MgCl2, and the remaining volume was adjusted with DNase free water to a final volume of 48.5µL per reaction. The PCR assays was performed using an Eppendorf thermocycler (Eppendorf Hauppauge, NY USA) with an initial denaturation at 95˚C for 1min, then followed with 30 cycles of 94˚C for 15 seconds, 55˚C for 15 seconds, and 72˚C for 1 minute, and with a final extension of at 72˚C for 1 minute. The samples were held at 4˚C until removed from the thermocycler. PCR products were visualized on an EtBr stained 2% agarose gel and UV Transillumination and measured with Invitrogen Low Mass Ladder (Invitrogen Carlsbad, California USA).

Detection of *Salmonella*

The samples were processed immediately upon receipt using an in house protocol for *Salmonella* enrichment. Briefly, sediment was weighed out in reps of four by three different weights to acquire the MPN; weights included 0.1gram, 1.0gram, and 10 grams. The sediment was enriched in Buffered Peptone Water (BPW) (BD Franklin Lakes, NJ) at 37°C for 20 hours. After incubation 10µL was transferred to 1000µL Rappaport Vassiliadis (RV) (BD Franklin Lakes, NJ) and incubated for 24-48 hours at 42°C, then channel-streaked onto Xylose Lysine Desoxycholate agar (XLD) (BD Franklin Lakes, NJ). After further isolation onto XLD agar, all suspect *Salmonella* colonies were biochemically confirmed using Lysine (EMD Gibbstown, NJ USA), Triple Sugar Iron (Remel Lenexa, KS), Citrate (Remel Lenexa, KS USA), and Urea (BD Franklin Lakes, NJ USA). Water samples were processed using the same method as the sediment samples. The water was filtered using vacuum membrane filtration in reps of four by three different volumes; the volumes included 5mL, 25mL, and 75mL. The waster was enriched in BPW following the same protocol listed above. Isolates of *Salmonella* were serotyped at the California Animal Health and Food Safety Laboratory at Riverside, California.

Detection of fecal coliforms and indicator *E. coli*

Standard Methods 9221 E and 9223 B were used to enumerate fecal coliform and *E. coli*, respectively.

Statistical analyses

The geometric mean for each site was calculated by taking the natural logarithm of each water or sediment sample, generating an arithmetic mean for each site from these log-transformed values, then untransforming the mean using an exponential function (exp(arithmetic

mean of log-transformed values)). McNemar's Test was used to determine the significance of disagreement between the two different methods of determining compliance with REC 1 and REC 2 recreational standards, i.e., the geometric mean from 5 samples compared to the >10% rule.

The prevalence and concentration of *Salmonella* and *E. coli* O157:H7 (prevalence only) from water bodies classified as above or below REC 1 or REC 2 standards were compared using either mixed effects linear, negative binomial, or logistic regression, with site ID set as the group variable to control for potential correlated data due to repeated sampling of each site over the course of a year (Stata Version 9). In addition, to supplement the analyses from these mixed models, a Fisher's Exact Test and 95% exact confidence interval for the odds ratio was calculated for the occurrence of *E. coli* O157:H7 for water bodies above and below the REC 1 and REC 2 standards. Exact tests do not control for the repeated sampling and correlated data concerns, but are appropriate models in the presence of sparse data in contingency tables that can violate large sample size assumptions (StatXact Version 4.0) (Table 6).

The association between fecal coliform and *Salmonella* in water was assessed by using a mixed effects linear regression model, with fecal coliform (MPN/100 mL) set as the covariate, *Salmonella* (MPN/100 mL) or its $\log_{10}$ transformed value set as the dependent variable, and site ID set as the group effect due to repeated sampling of sites over the course of year (Stata Version 9). Similarly, a mixed effects linear or negative binomial regression was used to determine the significance between the mean concentration of bacterial contaminants for sites listed for a TMDL and sites not listed for a TMDL, with site ID set as the group effect due to repeated sampling of sites over the course of year (Stata Version 9).

Results and Discussion

Bacterial indicators in water

The annual arithmetic mean for fecal coliform varied in excess of 2-$\log_{10}$ from site to site, ranging from a low of 114 MPN/100 mL at the Carmel River at Highway 1 (307CML) to a high of 20,223 MPN/100 mL at the Salinas River at Davis Road (309DAV). This high annual mean value for the Salinas River was the result of a single sample in August 2009 having >160,000 MPN/100 mL fecal coliform, the highest value observed in the year-long study (Fig 2). Similar variability occurred for *E. coli* (data not shown) given that the estimated concentration of fecal coliform and indicator *E. coli* were correlated for most water samples (Fig 3), as might be expected given that this bacterium is a subset of the fecal coliform group. The diagonal line shown in Figure 3 is for a perfect 1:1 fit between these two bacterial indicators; the raw data show that in general there are fewer *E. coli* than fecal coliform in each water sample given that the majority of data points fall below the 1:1 relationship.

Seasonal peaks occurred during January and August for the arithmetic mean concentration of fecal coliform and indicator *E. coli* (Fig 4), which is in part driven by a subset of site locations experiencing high concentrations of these bacterial indicators during these two months. As indicated above, the Salinas River at Davis Road had a water sample taken August 6, 2009 that

measured >160,000 fecal coliform/100 mL and >240,000 *E. coli*/100 mL. Usage of the geometric mean would reduce the appearance of these high mean values but still result in numerous monitored water bodies exceeding the water quality standards for the intended beneficial use, as shown in Table 3 below. The mechanism(s) driving these higher values are likely different for January compared to August, with winter storm-based flows typically mobilizing terrestrial sources of bacterial indicators (e.g., feces) combined with resuspension of sediments along these stream and irrigation canal/ditch corridors that contain attached bacteria. In contrast, summer spikes of indicator bacteria can result from in-stream or in-channel defecation due to watering behavior of domestic and wild animals or summer irrigation tail-water return flows subsequent to irrigation for agriculturally active areas in this region of California. The summer climate in combination with warmer surface water in the presence of sufficient nutrients might support the growth of indicator bacteria in these irrigation ditch sediments.

Recreation standard 1 (REC1) and standard 2 (REC2) have maximum geometric means of 200 and 2000 fecal coliform per 100 mL, respectively, for not less than a minimum of 5 samples taken within 30 days. Our monthly sampling from each site did not comply with this standard method of 5 samples within 30 days, but if we calculate a geometric mean for each site from its entire set of samples taken over the course of the annual study (7 to 12 samples per site), then 16 of the 23 water bodies were above the REC1 standard (sites 304APT, 304LOR, 304SOK, 305THU, 309DAV, 309TDW, 310ARG, 310SLB, 310SRO, 310TWB, 312SMA, 314SYN, 315ABU, 315ATA, 315CRP, 315MIS) and 1 site (312SMA) was above the REC2 standard (Table 3). There was relatively good agreement (*P*-value = 0.25, i.e., not significantly different) between the REC 1 standard based on the geometric mean compared to the standard based on the 10% rule, with 70% (geomean) and 83% (10% rule) of sample sites exceeding each standard, respectively (Table 4). In contrast, there was significant disagreement between these two methods for the REC 2 standard (*P*-value = 0.004), given that only 4% (geomean) compared to 43% (10% rule) of sample sites exceeded each standard, respectively.

Waterborne pathogens and their relationship to bacterial indicators and recreational standards

Salmonella was detected in approximately a third of all water samples collected (31%), with an arithmetic mean of 0.39 and 1.28 MPN/100 mL for all samples and the subset of positive samples, respectively. Twenty of the 23 sampling sites had at least one water sample test positive for *Salmonella*, but Aptos Creek, Soquel Creek and Carpinteria Creek consistently tested negative for this pathogen. Approximately 2.4% of water samples were positive for *E. coli* O157:H7, with four sites having just one positive water sample (304SOK, 305THU, 310SLB, 310TWB) and one location, 312SMA, having two water samples test positive on June 17, 2009 and October 22, 2009 (Table 3). Based on sparse data, the odds of testing positive for *E. coli* O157:H7 from this site on the Santa Maria estuary was 13.1 times greater compared to water from elsewhere in the study (OR=13.1, 95% exact CI 1.03, 103.5). Moreover, the probability that a sample site tests positive on two occasions for this pathogenic strain of *E. coli* by random chance, assuming a background prevalence of 2.4%, is very low (*P*=0.025). This suggests that the occurrence of *E. coli* O157:H7 at this site on the Santa Maria River may be higher than background levels for Central Coastal California.

Neither the prevalence nor the concentration of waterborne *Salmonella* from water bodies classified as above or below REC 1 or REC 2 standards were significantly different (*P*-value >0.05) when adjusted for repeated sampling at sites (Table 5). Similarly, the prevalence of *E. coli* O157:H7 for water samples from sites above compared to below the REC 1 standard was also not significantly different (*P*=0.20), but in contrast there was a significant (*P*=0.05) association between the occurrence of this pathogenic strain of bacteria and water from sites classified as above or below the REC 2 standard. Specifically, the odds of testing positive for waterborne *E. coli* O157:H7 was 13 times larger for water from site 312SMA that is above the REC 2 standard (>2000 MPN/100 mL fecal coliforms) compared to all the other water samples that were from sites below the REC 2 standard (OR=13.1; 95% exact CI 1.03-103.5) (Table 6). It is important to note that site 312SMA (Santa Maria River at its estuary) was the only location above the REC 2 standard, so this significant association is based on data from just one location. Nonetheless, this site also had the highest prevalence of *Salmonella* and the highest observed concentrations of fecal coliform and indicator *E. coli* (Table 3), suggesting that more spatially-dense sampling (e.g., above and below) may be warranted in order to identify land use practices causing these elevated bacterial concentrations.

Although the concentration of *Salmonella* was not significantly higher at sites classified as exceeding REC 1 or REC 2 recreational standards, the concentration of fecal coliform was significantly associated with the concentration of *Salmonella* (Table 7). Two different mixed effects linear regression models were fitted to the data, the first used fecal coliform concentration as MPN/100 mL (model A) and the other used log10-transformed values for fecal coliform concentration. Neither model could accurately predict the occurrence the *Salmonella* given this pathogen's large variability across the range of fecal coliform concentrations (i.e., R^2 values were ≤5.0%) (Fig 5). In our follow up analyses for a peer reviewed report we will determine if additional covariates can improve the model's fit to the data.

Waterborne pathogens and their relationship to watershed-scale land use categories

The surface area for the entire watershed and the contributing area of the smaller catchment directly influencing the sampling site are listed in Table 2. In addition, for each of these respective surface areas (entire watershed or catchment) the percent of land usage defined as open, urban, or agricultural was obtained from the 1992 National Land Cover Datasheet (NLCD) for each sampling site. Using mixed effects negative binomial regression for bacterial concentrations or logistic regression for the presence/absence of *Salmonella*, there were no significant associations (*P*-value >0.05) between any of these surface area or land use parameters and the bacterial levels at these sampling sites, with one exception. The surface area (km^2) of the catchment was significantly associated (*P*-value = 0.047) with the odds of detecting *Salmonella* at the site (OR = 1.08, 95% CI 1.001, 1.17). For example, for each additional square km in area, the odds of detecting *Salmonella* increased by about 8%.

Comparing bacterial concentrations for sites requiring or not requiring a TMDL

Of the 23 rivers and creeks tested for this project, 17 are currently listed for a TMDL for the Central Coast Regional Water Quality Control Board (Table 3), with 12 of these TMDL listed sites due to excessive fecal coliform levels. Although the arithmetic mean concentration of fecal coliform, indicator *E. coli* and *Salmonella* appear to be higher for water samples collected from TMDL- listed sites compared to not listed sites, these means were not significantly different (*P*-value >0.05) when evaluated using a mixed effects linear or negative binomial regression, most likely due to the high standard deviations surrounding these means (Table 8). If water samples were taken from the reaches of each water body that lead to a pathogen TMDL requirement, this sampling design may have resulted in a larger mean difference between listed and not listed locations.

Occurrence of *Salmonella* and *E. coli* O157:H7 in sediments

Salmonella was detected in approximately a quarter of all sediment samples (23%), with an arithmetic mean of 4.41 and 19.1 MPN/100 g wet weight for all samples and those that tested positive, respectively. Twenty of the 23 sampling sites had at least one sediment sample test positive for *Salmonella*, but Scott Creek Lagoon, Soquel Creek, and Salinas River consistently tested negative. Over the course of this project, Soquel Creek never tested positive for *Salmonella* in either water or sediment samples. Even though fewer sediment samples tested positive for *Salmonella* compared to water (23% versus 31%), the arithmetic mean concentration for positive sediment was about 15 times larger (4.41/1.28 = 14.9) than the arithmetic mean concentration for positive water if we standardize these concentrations on a per 100 gram basis (assume 1 mL water = 1 g sediment). Similar to this study, large bacterial reservoirs in the upper layer of sediment have been documented for several northern California estuaries for a similarly conducted project on indicator *E. coli* (Atwill et al., 2007). None of the sediment samples had detectable *E. coli* O157:H7.

Preliminary statistical analysis shows that the occurrence of *Salmonella* in the overlying water column was strongly associated with finding *Salmonella* in sediment, such that for each additional MPN/100 mL of waterborne *Salmonella* there was an associated 5.5 MPN/100 g increase in sediment-borne *Salmonella* (Fig 6). Nevertheless, this significant (*P*<0.001) association does not indicate which matrix serves as the reservoir for the other (i.e., causal direction), or if both water and sediment are seeded with *Salmonella* from a third common source (e.g., terrestrial). More detailed analysis of the DNA fingerprints of *Salmonella* isolated from the same site on the same day would help clarify whether both water and sediment share a common bacterial source (DNA fingerprints match) or if each matrix has strains of *Salmonella* that are unique from each other (DNA fingerprints dissimilar).

Serotypes of *Salmonella* from water and sediment

Numerous serotypes of *Salmonella* were isolated from water and sediment that have a history of causing human illness (e.g., *S. Typhimurium*, *S. Newport*) suggesting that these strains may be

pathogenic for humans and also animals if ingested at appropriate doses (Table 9). According to the *Salmonella* Annual Summary Report 2006 compiled by the Centers for Disease Control and Prevention, the majority of isolates found in this study have been reported to cause human illness during the previous decade. Table 9 lists the frequency of isolation for the various serotypes found during the project compared to each serotypes frequency of isolation from human cases from 1996-2006. For example, *S. Typhimurium* was isolated 8 times during this project; this isolate accounts for 19% of all reported human clinical cases in the US from 1996-2006 and 17% of human cases in 2006 alone.

Seasonal patterns of chemical and physical parameters in water

Seasonal shifts in chemical and physical parameters of water followed predictable seasonal patterns for these stream, rivers, and estuarine sites along central coastal California (Table 10, Fig 7 and 8). For example, the typically reduced stream flows at these coastal confluent sites can lead to increases in salinity and conductivity during summer and fall compared to winter (Jan-Mar). Similarly, turbidity and dissolved oxygen were considerably lower in summer and fall compared to winter.

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Table 1. Sample locations (n=23) along Central Coastal California

Waterbody	SiteTag	Site Description	Lat	Long
Santa Cruz Watershed				
Aptos Creek	304APT	304APT-Aptos Creek at Spreckles Drive	36.97391526	-121.9027384
San Lorenzo River	304LOR	304LOR-San Lorenzo Estuary at Laurel Street	36.96973998	-122.022029
Scott Creek	304SCO	304SCO-Scott Creek Lagoon at Highway 1	37.04052698	-122.22769
Soquel Creek	304SOK	304SOK-Soquel Creek at Knob Hill	36.98014342	-121.9562397
Pajaro Watershed				
Pajaro River	305THU	305THU-Pajaro River at Thurwachter Bridge	36.87977498	-121.791946
Carmel Watershed				
Carmel River	307CML	307CML-Carmel River at Highway 1	36.53637598	-121.911678
Big Sur Watershed				
Big Sur River	308BSR	308BSR-Big Sur River at Andrew Molera foot bridge	36.28659298	-121.843048
Salinas Watershed				
Salinas River (Lower)	309DAV	309DAV-Salinas River at Davis Road	36.64680598	-121.701385
Tembladero Slough	309TDW	309TDW-Tembladero Slough at Monterey Dunes Way	36.772182	-121.786597
San Luis Obispo Watershed				
Arroyo Grande Creek(below res)	310ARG	310ARG-Arroyo Grande Creek at 22nd Street	35.09521298	-120.60625
Pismo Creek	310PIS	310PIS-Pismo Creek above Highway 101	35.14034698	-120.634501
San Luis Obispo Creek (below Osos Street)	310SLB	310SLB-San Luis Obispo Creek at San Luis Bay Drive	35.18832298	-120.717918
Santa Rosa Creek (310)	310SRO	310SRO-Santa Rosa Creek at Moonstone Drive	35.56798798	-121.103195
San Simeon Creek	310SSC	310SSC-San Simeon Creek at State Park foot bridge	35.59453898	-121.121021
Chorro Creek	310TWB	310TWB-Chorro Creek at South Bay Boulevard	35.35422598	-120.826942
Santa Maria Watershed				
Santa Maria River	312SMA	312SMA-Santa Maria River above Estuary	34.963774	-120.641796
San Antonio Watershed				
San Antonio Creek (313)	313SAI	313SAI-San Antonio Creek at San Antonio Road West	34.78235698	-120.529225
Santa Ynez Watershed				
Santa Ynez River	314SYN	314-SYN-Santa Ynez River at 13th Street	34.676773	-120.554422

Santa Barbara Watershed				
Arroyo Burro Creek	315ABU	315ABU-Arroyo Burro Creek at Cliff Drive	34.40505098	-119.739115
Atascadero Creek(315)	315ATA	315ATA-Atascadero Creek at Ward Drive	34.42345198	-119.819287
Carpinteria Creek	315CRP	315CRP-Carpinteria Creek down stream Carpenteria Ave	34.39350898	-119.511814
Mission Creek (Santa Barbara County)	315MIS	315MIS-Mission Creek at Montecito Street	34.41303698	-119.694007
Rincon Creek	315RIN	315RIN-Rincon Creek at Bates Road, u/s Highway 101	34.37686498	-119.476931

Table 2. Summary of land usage in the total watershed and in the immediate catchment area surrounding each site sampled during April 2009-April 2010 along the Central Coast Region.

	Total Watershed				Total Catchment			
Site Code	**Area (km^2)[a]**	**Open[b]**	**Urban**	**Agriculture**	**Area (km^2)**	**Open**	**Urban**	**Agriculture**
304APT	63.4	88.5	10.9	0.6	0.6	15.3	83.0	1.3
304LOR	298	94.1	4.8	0.0	1.9	86.0	13.5	0.0
304SCO	77.0	98.7	0.6	0.5	0.4	52.3	0.6	41.4
304SOK	107	91.9	7.5	0.5	3.4	18.4	81.6	0.0
305THU	141	93.5	0.1	0.4	0.3	94.5	0.0	0.0
307CML	648	95.7	1.8	1.6	1.3	49.3	17.4	32.7
308BSR	149	99.2	0.2	0.0	1.9	99.2	0.3	0.0
309DAV	9772	83.0	1.0	12.5	0.5	49.3	3.0	43.2
309TDW	382	47.9	12.3	37.8	0.1	2.8	0.0	96.3
310ARG	421	78.9	4.6	13.5	0.02	4.2	41.7	0.0
310PIS	114	83.2	5.1	9.9	21.9	71.9	24.6	0.2
310SLB	212	79.0	9.7	10.3	2.4	83.7	3.1	12.3
310SRO	123	92.7	2.0	5.2	5.8	78.0	21.5	0.3
310SSC	76	99.4	0.1	0.3	0.2	88.1	10.4	0.0
310TWB	115	89.5	3.2	6.0	2.5	70.0	6.5	0.1
312SMA	4455	85.2	1.3	8.8	1.1	73.5	0.3	1.0
313SAI	354	83.6	0.8	13.9	3.4	93.6	1.2	4.0
314SYN	2202	89.5	1.4	7.4	13.6	77.3	1.6	14.8
315ABU	23.4	70.6	28.1	0.4	2.8	57.2	41.6	0.1
315ATA	51.2	68.2	25.1	4.8	1.9	28.7	30.0	34.7
315CRP	43.3	79.2	3.1	16.3	9.3	29.7	13.9	52.8
315MIS	31.4	63.6	35.6	0.1	15.9	32.3	66.6	0.1
315RIN	38.1	82.0	1.0	15.9	2.2	40.5	6.6	52.5

[a] Size of the catchment area listed in square kilometer.
[b] Land usage classified by the National Land Cover Datasheet (NLCD) as of 1992 (percent x 100).

Table 3. Summary results for each site sampled from April 2009 to April 2010 along Central Coastal California

		Concentration (MPN/100 mL)			Prevalence (%)			
Site Code	N	Fecal coliform[a]	*E. coli*[a]	*Salmonella*[b]	*Salmonella*[c]	*E. coli* O157:H7[c]	REC1/ REC2[d]	TMDL[e]
304APT	12	696	349	0.00	0.00	0.00	1/0	Yes
304LOR	12	539	313	0.07	0.167	0.00	1/0	Yes
304SCO	11	145	102	0.02	0.091	0.00	0/0	No
304SOK	12	384	151	0.00	0.00	0.083	1/0	Yes
305THU	12	491	370	0.18	0.25	0.083	1/0	Yes
307CML	10	85	44	0.03	0.10	0.00	0/0	No
308BSR	12	67	30	0.04	0.167	0.00	0/0	No
309DAV	11	1444	939	0.14	0.273	0.00	1/0	Yes
309TDW	12	907	473	0.12	0.167	0.00	1/0	Yes
310ARG	7	1790	360	0.20	0.571	0.00	1/0	Yes
310PIS	12	168	128	0.85	0.75	0.00	0/0	Yes
310SLB	12	464	267	0.17	0.50	0.083	1/0	Yes
310SRO	9	449	312	0.44	0.22	0.00	1/0	Yes
310SSC	12	167	104	0.20	0.25	0.00	0/0	Yes
310TWB	10	262	161	0.71	0.50	0.10	1/0	No
312SMA	11	3488	1071	0.80	0.73	0.182	1/1	Yes
313SAI	11	123	49	1.50	0.273	0.00	0/0	Yes
314SYN	10	326	136	0.74	0.40	0.00	1/0	Yes
315ABU	11	1352	390	1.33	0.55	0.00	1/0	Yes
315ATA	11	378	244	0.29	0.273	0.00	1/0	Yes
315CRP	9	447	133	0.00	0.00	0.00	1/0	No
315MIS	11	1536	1027	1.09	0.55	0.00	1/0	No
315RIN	11	170	109	0.21	0.364	0.00	0/0	Yes

[a] Geometric mean (MPN/100 mL) for each site for the duration of the project (4/2009-4/2010).
[b] Arithmetic mean (MPN/100 mL) for each site for the duration of the project (4/2009-4/2010).
[c] Percent positive samples when a positive=1 and negative=0.
[d] (A/B) A and B are the site's results for the REC 1 (>200 MPN/100 mL) and REC2 (>2000 MPN/100 mL) standards, respectively: 1 indicates the site exceeds the bacterial standard; 0 indicates the site is below the bacterial standard.
[e] Yes indicates that the site is listed for a TMDL; No indicates that the site is not listed.

Table 4. Comparison of the two methods for determining compliance with the recreational standards for contact vs. non-contact recreation used by the Central Coast Regional Water Quality Board for 23 water bodies (stream, rivers, estuaries)

		REC 1 Geomean[a]	
		>200	**≤ 200**
10% of samples[b]	**>400**	16	3
	≤ 400	0	4

		REC 2 Geomean[a]	
		>2000	**≤ 2000**
10% of samples[b]	**>4000**	1	9
	≤ 4000	0	13

[a] REC 1 and REC 2 standard is exceeded when the geometric mean is >200 and >2000 fecal coliform (MPN/100 mL) for 5 samples taken within 30 days for a site, respectively.
[b] No more than 10% of samples from a site can have fecal coliform levels above 400 and 4000 MPN/100mL within 30 days for REC 1 and REC 2, respectively.

Table 5. The occurrence of waterborne *Salmonella* and *E. coli* O157:H7 at sites exceeding REC 1 or REC 2 recreational standards for Central Coastal California, April 2009 to April 2010

Recreational standards for fecal coliform[a]	*Salmonella* prevalence (%)	*Salmonella* concentration[b]	*E. coli* O157:H7 prevalence (%)
REC 1			
≤200	23/79 (29%)	0.41	0/79 (0%)
>200	54/172 (31%)	0.39	6/172 (3.5%)
REC 2			
≤2000	69/240 (29%)	0.37	4/240 (1.7%)
>2000	8/11 (73%)	0.80	2/11 (18%)

a. The geometric mean of fecal coliform was calculated for each site and classified relative to the REC 1 and REC 2 standards in the Water Quality Control Plan Report for the Central. Coastal Basin, adopted by the Regional Board in 1974.
b. Arithmetic mean of *Salmonella* (MPN/100 mL).

Table 6. The association between a site exceeding REC 1 or REC 2 recreational standards and the presence of *E. coli* O157:H7 for 23 water bodies (stream, rivers, estuaries) in Central Coastal California, April 2009 to April 2010

		REC 1 Geomean[a]	
		>200	**≤ 200**
E. coli O157:H7[b]	**pos**	6	0
	neg	166	79

		REC 2 Geomean[a]	
		>2000	**≤ 2000**
E. coli O157:H7[b]	**pos**	2	4
	neg	9	236

[a] REC 1 and REC 2 standard is exceeded when the geometric mean is >200 and >2000 fecal coliform (MPN/100 mL) for 5 samples taken within 30 days for a site, respectively.
[b] Presence or absence of detectable *E. coli* O157:H7 in 500 mL water samples.

Table 7. Significant association between the concentration of fecal coliform and the concentration of *Salmonella* for 23 water bodies along central coastal California, April 2009 to April 2010

Mixed effects linear regression model	Regression coefficient	*P*-value	95% CI
Model A (R^2=2.4%)			
Intercept	0.342	--	--
Conc. of fecal coliform[a]	1.61×10^{-5}	0.014	3.3×10^{-6}, 2.9×10^{-5}
Model B (R^2=5.0%)			
Intercept	-0.584	--	--
Log(conc. of fecal coliform)[b]	0.377	<0.001	0.17, 0.58

[a] Fecal coliform concentration as MPN/100 mL.
[b] Log_{10} transformation of fecal coliform concentration: log(MPN/100 mL).

Table 8. Concentration of bacterial indicators and pathogens for water samples from 23 sites, some of which are listed for a Total Maximum Daily Load (TMDL) in the Central Coast Basin Plan as of 2008, California 303(d) list of water quality limited segments

Bacteria	N	Arithmetic mean or %	sd
Fecal Coliform (MPN/100 mL)[a]			
TMDL not listed[b]	61	1818	7356
TMDL listed[b]	185	4278	14,598
E. coli (MPN/100 mL)[a]			
TMDL not listed[b]	62	1665	7943
TMDL listed[b]	185	3547	20,981
Salmonella (MPN/100 mL)[c]			
TMDL not listed	63	0.32	1.02
TMDL listed	188	0.42	1.46
E. coli O157:H7 (present/absent)			
TMDL not listed[b]	63	1.6 %	--
TMDL listed[b]	188	2.7 %	--

[a] Fecal coliform and *E. coli* measured according to Standard Methods 9221 E and 9223 B, respectively.
[b] Water body (river, stream, estuary) that contains the sampling site is listed for a TMDL.
[c] *Salmonella* measured using a multiple tube method to find the most probable number.

Table 9. Serotypes of *Salmonella* from water and sediment from waterbodies along Central Coastal California, contrasted against the human disease burden caused by each serotype from 1996-2006 as reported by the CDC

Salmonella serotype	No. of isolations of each serotype from water or sediment	Reported human cases across US, 1996-2006	% of all reported human cases, 1996-2006
S. Typhimurium	8	75058	19.2
S. Newport	11	32955	8.4
S. Heidelberg	2	20473	5.2
Salmonella untypeable	20	16411	4.2
S. Montevideo	1	9459	2.4
S. Muenchen	14	7960	2.0
S. Infantis	1	6031	1.5
S. Braenderup	1	5833	1.5
S. I 4,5,12:i:-	1	4698	1.2
S. Mbandaka	4	2048	0.5
S. Senftenberg	1	1457	0.4
S. Give	10	1059	0.3

Table 10. Seasonal differences in physical and chemical water parameters for 23 sites along central coastal California, April 2009 to April 2010

Mixed effects linear regression model	Regression coefficient	*P*-value
pH		
Jan-Mar[a]	7.64	--
Apr-Jun	0.22	<0.001
Jul-Sep	0.17	0.003
Oct-Dec	-0.11	0.04
Water temp (C)		
Jan-Mar[a]	12.2	--
Apr-Jun	3.7	<0.001
Jul-Sep	6.8	<0.001
Oct-Dec	-0.6	0.22
Dissolved oxygen (ppm)		
Jan-Mar[a]	11.0	--
Apr-Jun	-1.7	<0.001
Jul-Sep	-2.8	<0.001
Oct-Dec	-1.4	0.002
Turbidity (ntu)		
Jan-Mar[a]	233	--
Apr-Jun	-133	0.09
Jul-Sep	-203	0.02
Oct-Dec	-180	0.02
Conductivity (uS)		
Jan-Mar[a]	1124	--
Apr-Jun	177	0.74
Jul-Sep	3071	<0.001
Oct-Dec	1282	0.02
Salinity		
Jan-Mar[a]	0.60	--
Apr-Jun	0.08	0.82
Jul-Sep	1.80	<0.001
Oct-Dec	0.84	0.02
Chlorophyll A (ug/L)		
Jan-Mar[a]	13.8	--
Apr-Jun	-2.8	0.45
Jul-Sep	9.5	0.02
Oct-Dec	-3.4	0.33

[a] Intercept term, i.e., the referent season to which the other seasonal means are compared. For example, the calculated mean turbidity in spring (Apr-Jun) compared to winter (Jan-Mar) would be 100 ntu (233-133=100) compared to 233 ntu in winter.

Figure 1. Sample locations along central coastal California

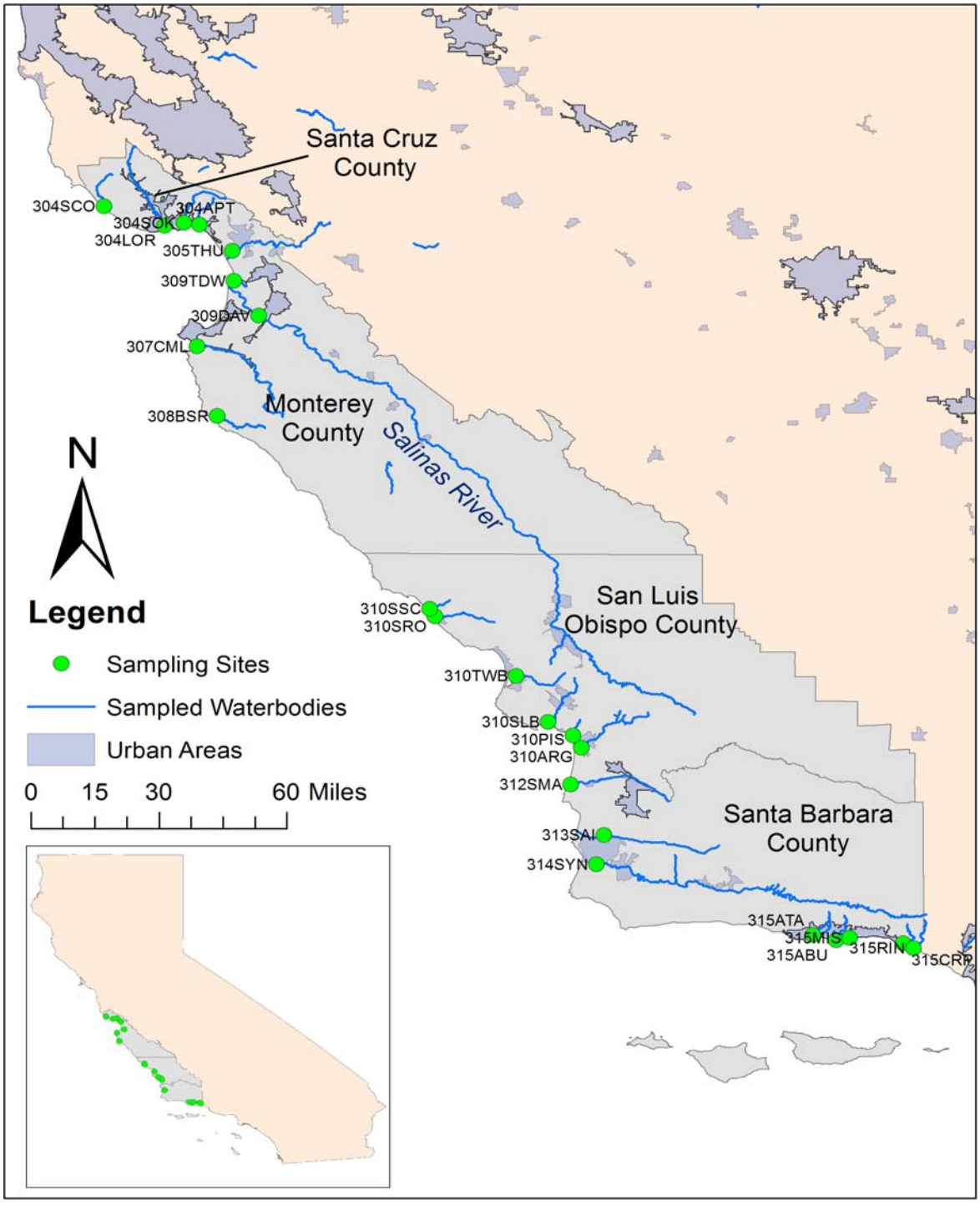

Figure 2. Single sample and annual arithmetic mean of waterborne fecal coliforms for each sample site

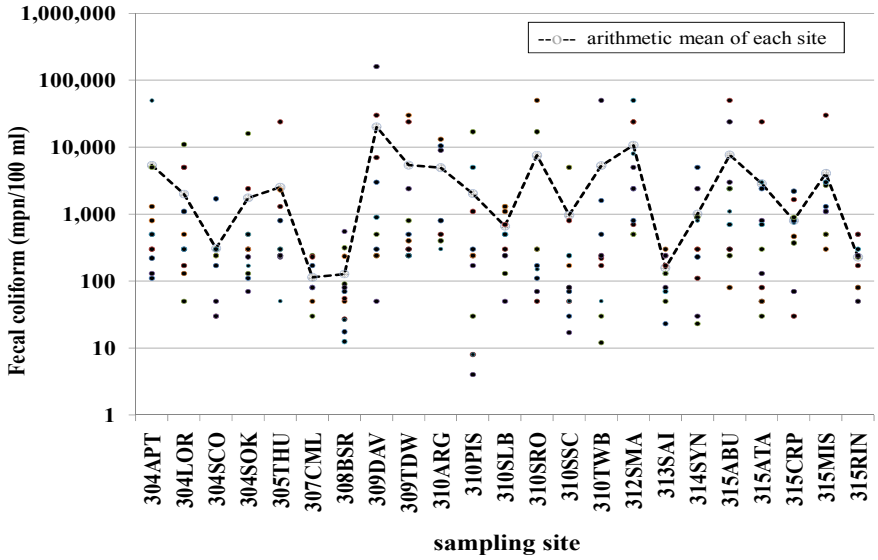

Figure 3. Correlation between fecal coliform and indicator *E. coli*

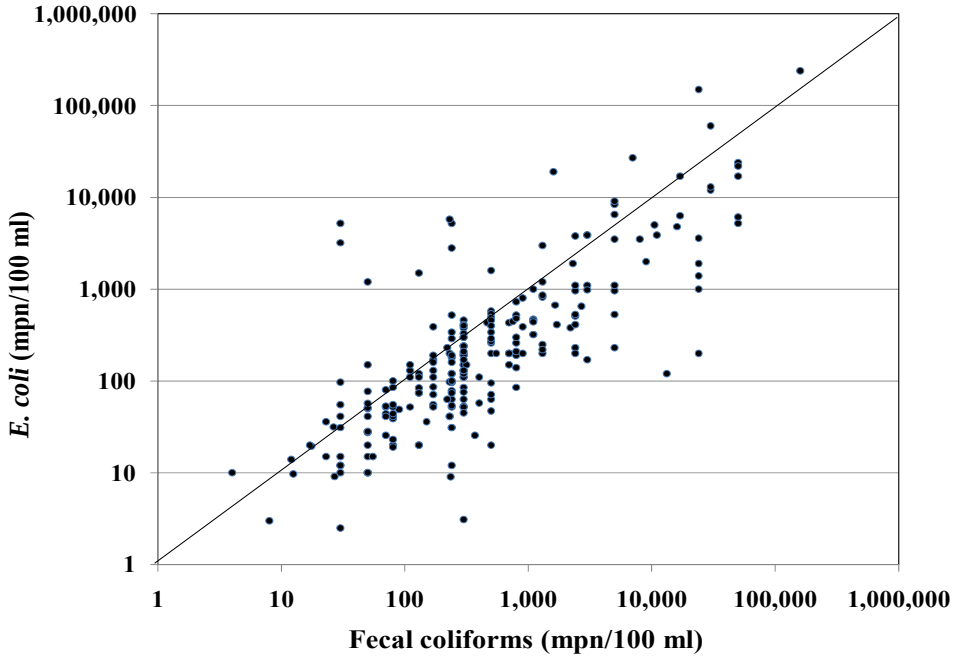

Figure 4. Monthly concentration of fecal coliform and indicator *E. coli* for all sites combined

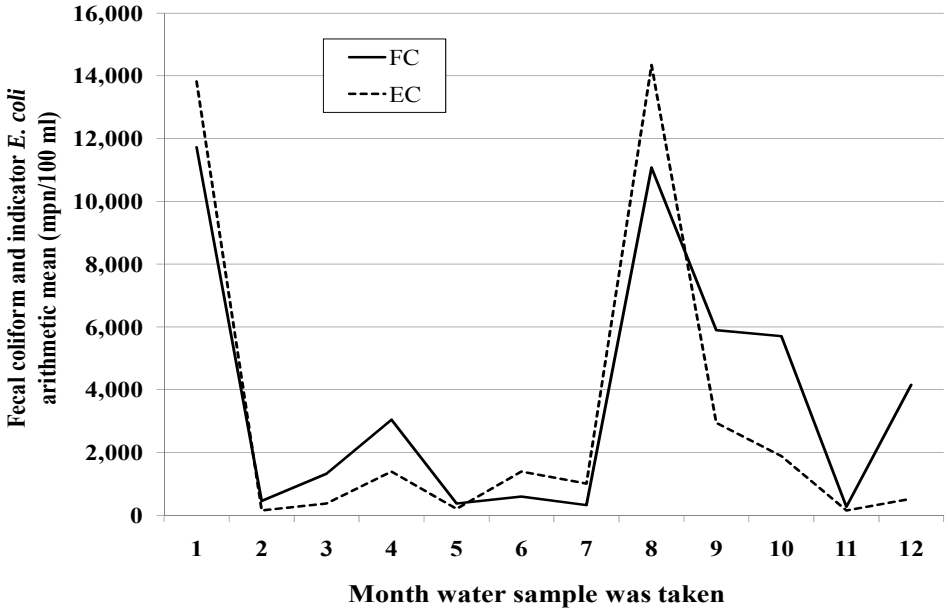

Figure 5. The association between fecal coliform and waterborne *Salmonella*, with two mixed effects regression models fitted to the data, for 23 sampling sites along central coastal California, April 2009 to April 2010

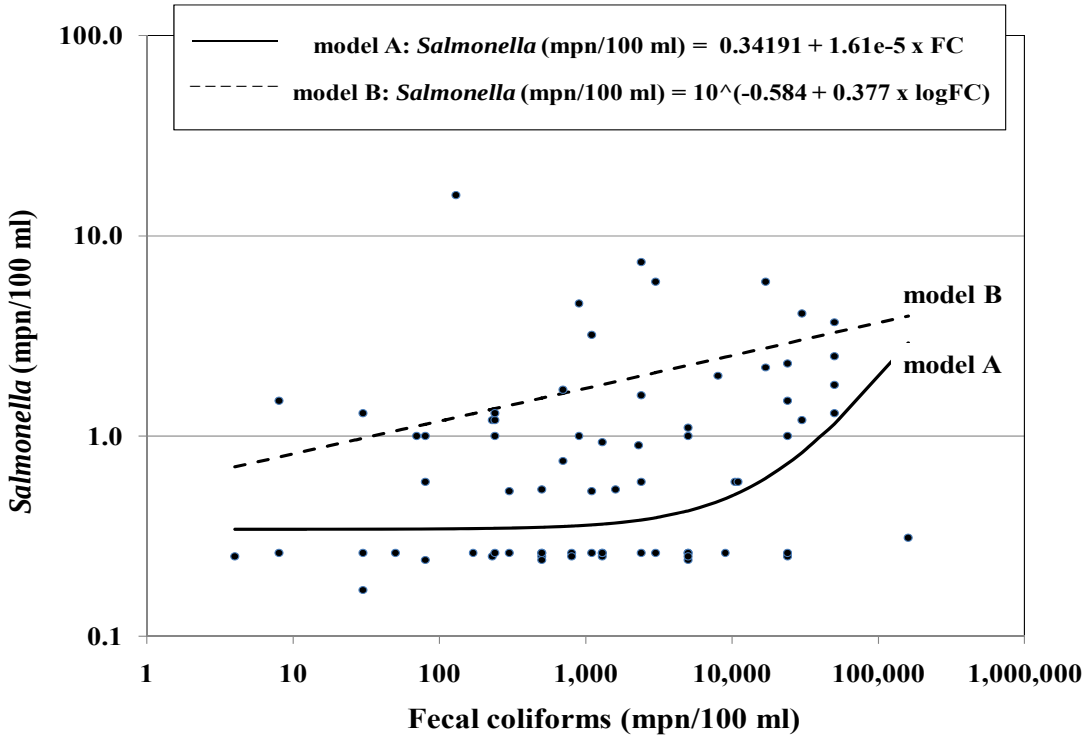

Figure 6. The association between *Salmonella* in water and in sediment, central coastal California, April 2009 to April 2010

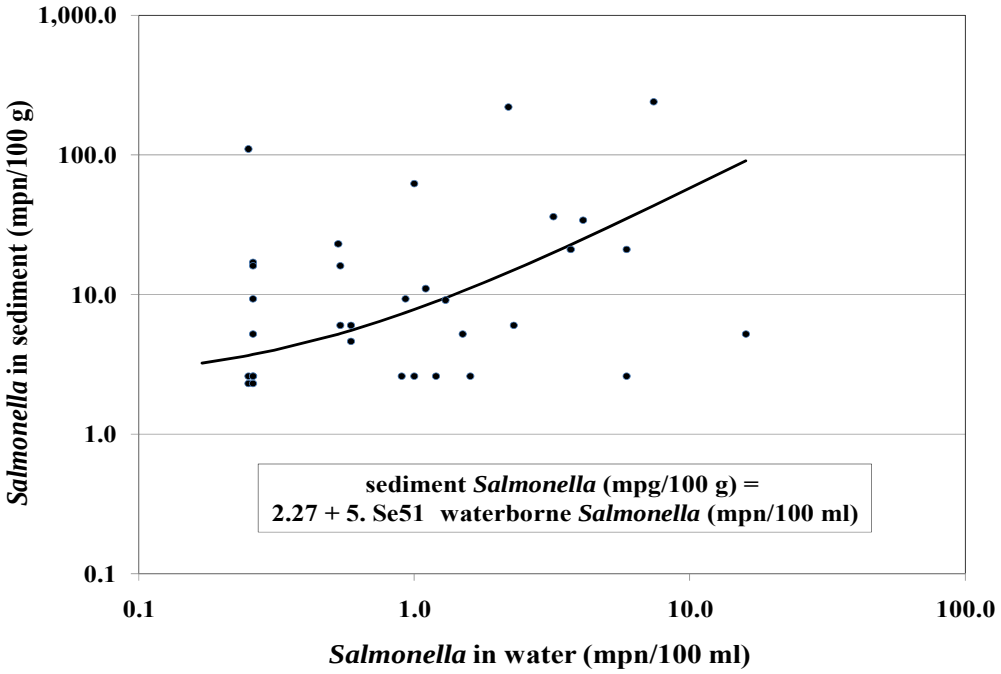

Figure 7. Monthly trends in water quality parameters from 23 sites along central coastal California, April 2009 to April 1020

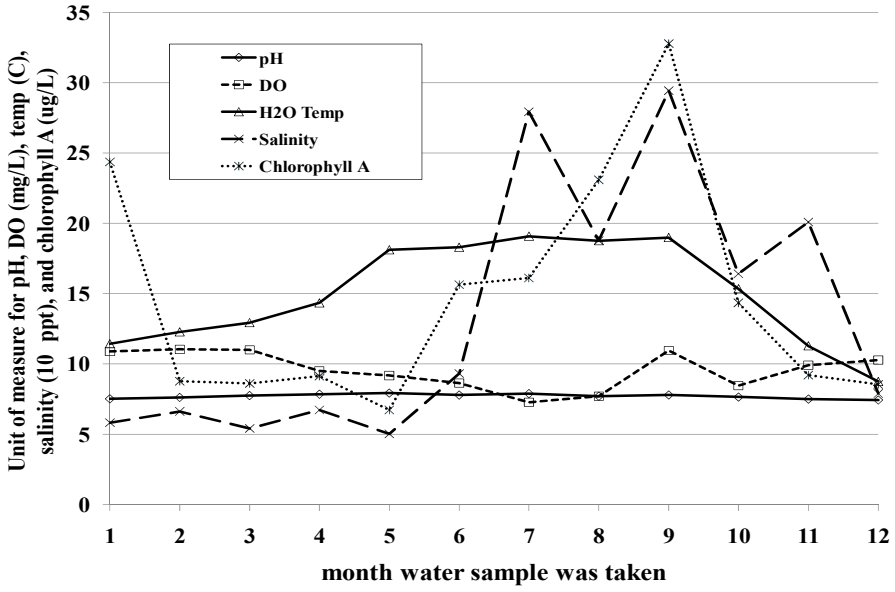

Figure 8. Monthly trends in conductivity and turbidity from 23 sites along central coastal California, April 2009 to April 1020