



# Salmonella and shiga-toxin containing *E. coli* in coastal streams: Impact of land use\*

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## Motivation

Coastal streams that run through agricultural watersheds can be major non-point sources of fecal indicator bacteria (FIB) and zoonotic pathogens including *Salmonella* and *E. coli* O157:H7 to coastal waters. Understanding how agricultural activities influence concentrations of FIB and pathogens in the nation's waterways is a necessary step for protecting and enhancing the Nation's natural resource base and environment.

The present work aims to elucidate how watershed, physical, and biological factors control the presence and fate of FIB, salmonellae, *E. coli* O157:H7, host specific markers in *Bacteroidales*, and the shiga-toxin gene (a virulence factor of *E. coli* O157:H7) in coastal streams. Field studies are being carried out in 13 coastal streams draining watersheds along the central California coast with diverse land use characteristics to pin-point how agricultural land use impacts the prevalence of these organisms and their subsequent flux to the coastal ocean. Microcosm studies will explore how water chemistry and grazers impact the persistence of salmonellae in stream water.

## Study Site



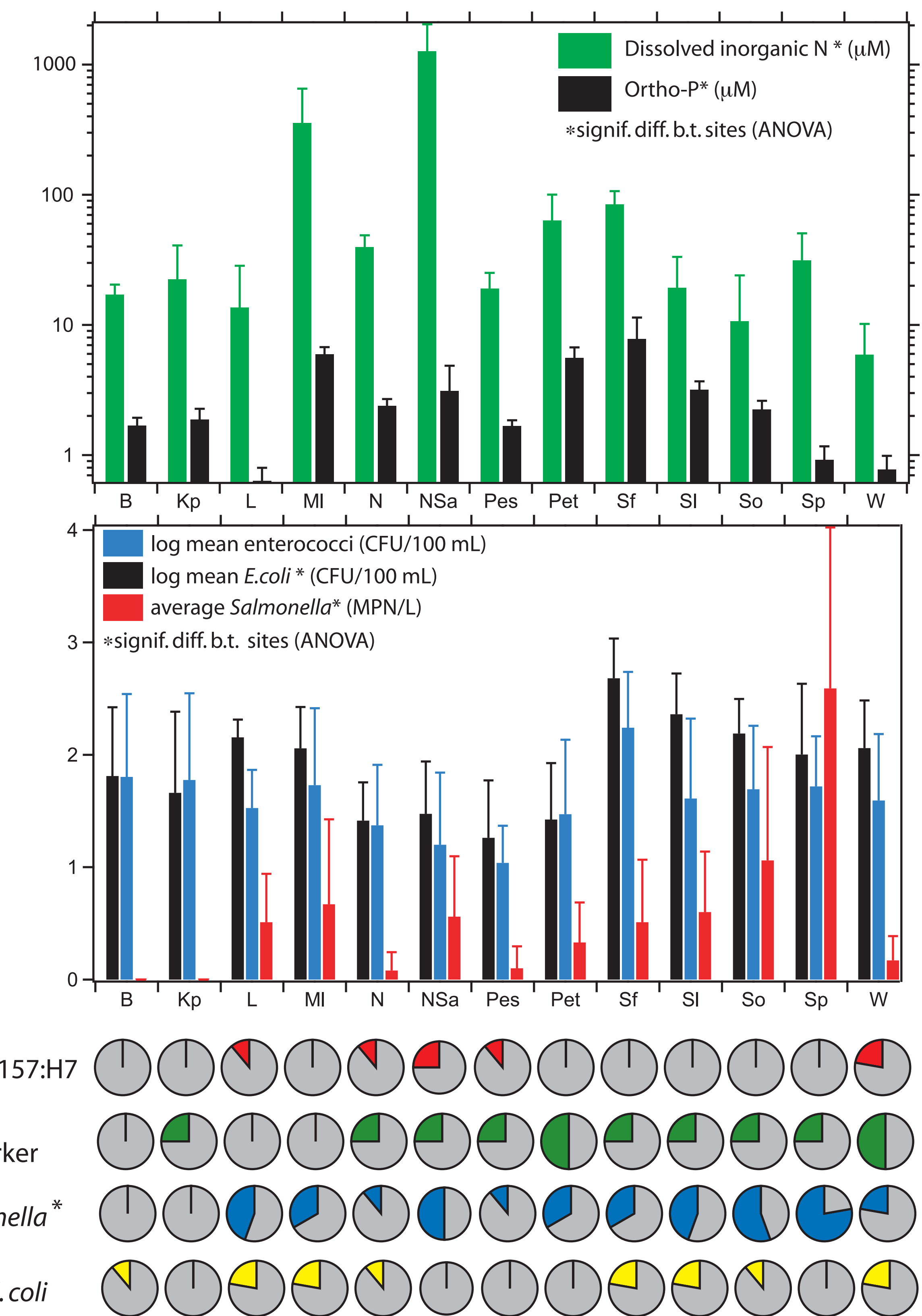
Water-body	Size (km <sup>2</sup> )	Urb%	Ag%	Fo%	ISC%	Pop#	Dominate land use
Bolinas Lagoon (B)	53.3	5.1	24.9	65.8	16.85	2340	Forested
Kirby Park (K)	30.6	4	55.3	9.9	19.46	1739	Agricultural
Lagunitas Creek (L)	34.8	1.9	38.1	55.3	13.68	1226	Agricultural
Moss Landing (MI)	30.6	4	55.3	9.9	19.46	1739	Agricultural
Napa River (N)	299	20.8	49.6	9.4	51.58	179977	Agricultural
Petaluma River (Pet)	105	12.4	61.8	4.3	50.51	42786	Agricultural
Pescadero Cr. (Pes)	nd	nd	nd	nd	nd	nd	Forested*
Salinas River (NSa)	276.6	9.3	80.9	4.4	36.44	85519	Agricultural
San Francisco Cr. (Sf)	31.5	59.4	20.2	16.8	37.03	171203	Urban
San Lorenzo River (SI)	25.9	72	13.5	10	46.7	61580	Urban
San Pedro Creek (Sp)	33.2	21.8	23.2	49.9	27.65	18493	Agricultural
Soquel Creek (So)	12.1	83.4	3.4	8	51.68	31079	Urban
Waddell Creek (W)	29.6	0.4	3	96.2	7.97	8	Forested

**TABLE & FIGURE 1.** The 13 waterbodies included in our study. In the figure, the site names are color coded according to dominate land use (agricultural, urban, and forested). The percent land use given in the table was determined using 1992 land use data, and the impervious surface coverage (ISC) from 2001 data. We are currently updating all the land use information with the newest land use data available. 'nd' indicates no data are available yet for this watershed. \* This is our best guess for Pescadero at the present time. Land use data are forthcoming.

## Measurements

- Samples are collected every 6 weeks from the outlet of the streams and rivers. 4 liters are collected from each site in acid washed, triple rinsed containers. Ancillary measurements (salinity, temperature, etc) are measured with a YSI water quality sonde. Discharge from streams without USGS stream gauges is measured using a flow meter.
- To measure **nutrients**, 30 mL of water is filtered through a 0.2  $\mu$ m pore size syringe filter for analysis with a nutrient autoanalyzer.
- Waters are analyzed immediately upon returning to the lab.
- **Enterococci and *E. coli*** are enumerated using membrane filtration onto mEI and MI agars following EPA methods.
- ***stx* in *E. coli*** is detected as follows. The membrane from MI agar after incubation is enriched in TSB for 4-5 h. The enrichment is pelleted and the DNA is extracted using a Qiagen kit. The DNA is then screened for *stx* using PCR.
- **Salmonellae** are enumerated using a modification of EPA method 1682 (an MPN assay). Confirmation is achieved using the genus-specific *invA* primers. The detection limit is 0.75 MPN/L.
- ***E. coli* O157:H7** presence is determined as follows. 10 mL of water is first enriched in buffered peptone water and then enrichment in TSB with novobiocin. *E. coli* O157:H7 is separated using magnetic beads with anti-O157:H7 antibodies. The pellet is suspended and then plated on CT-SMAC solid media. Putative *E. coli* are picked and screened for *stx*. Detection limit is 1 bacterium per 10 mL.
- **Human-specific, cow-specific, and pig-specific markers in *Bacteroidales*** are detected as follows. 100 mL of water is filtered through HA 0.2  $\mu$ m pore size filters. DNA is extracted from the filter using GITC buffer and Qiagen DNeasy kits. DNA is analyzed for the human-specific, cow-specific, and pig-specific markers in *Bacteroidales*.
- **Turbidity** is analyzed using a bench top turbidity meter.

## Preliminary Results



**FIGURE 2.** Average nutrients (top), log-mean indicators (middle), average salmonellae (middle), and presence (colored) and absence (grey) of *E. coli* O157:H7, the human marker in *Bacteroides* (HF marker), *Salmonella*, and *stx* in *E. coli* at the 13 sites. n=9 for each site except for NSa where n=8. Site abbreviations are given in Table 1. \* indicates significant difference in occurrence between sites.

