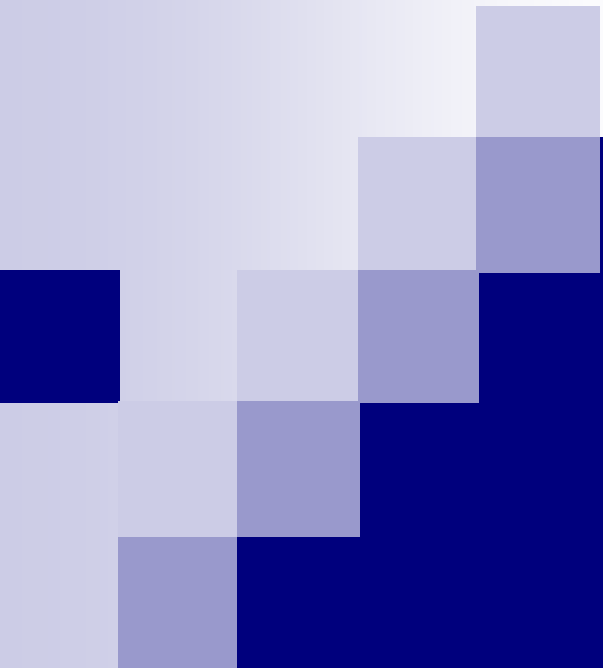




Does *Enterococcus* Indicate Fecal Contamination? Presence of Plant-Associated *Enterococcus* in Southern California Recreational Waters

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Paul Hannah, Martin Getrich, and
Charles McGee

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Enterococcus as a Fecal Indicator

- EPA epidemiology studies in 70's and 80's show good correlation between enterococcus concentration and bather illness in sewage-impacted water.
- 1986 – EPA recommends enterococcus as preferred salt water indicator.
- 1999 - AB411, new California law requires testing recreational waters for Enterococcus as well as Total and Fecal Coliforms
- Southern California experience: Use of enterococcus standard causes a 5 fold increase in number of beach failures, 60% are enterococcus failures only (Noble and Moore 2003).

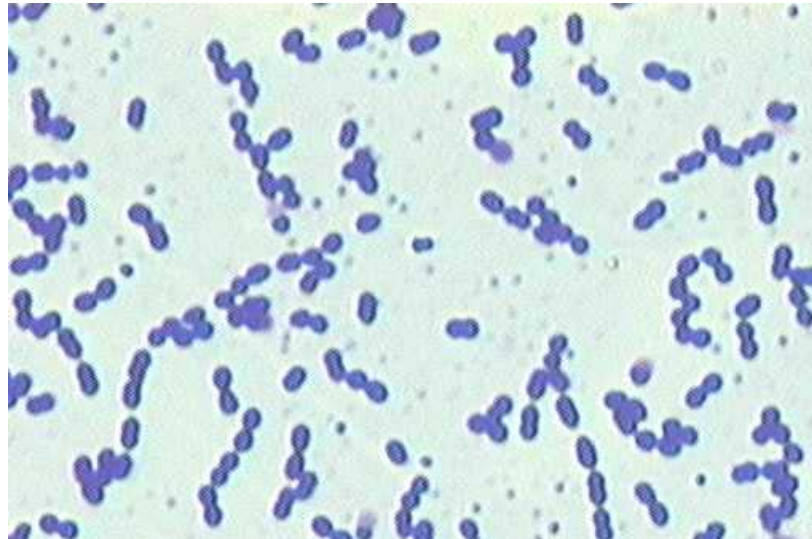


Description of the Genus *Enterococcus*

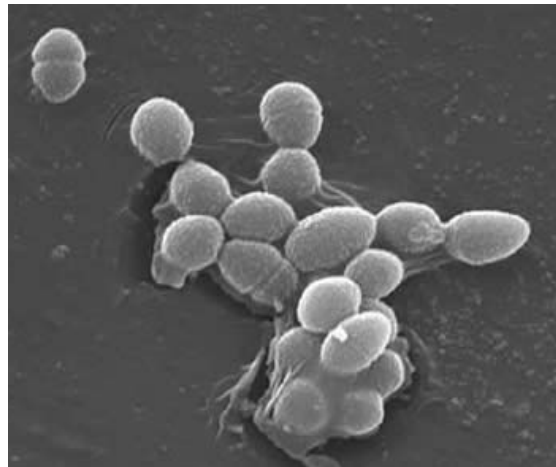
- Twenty-seven different species
- Gram positive cocci in pairs or short chains
- Grow aerobically or anaerobically
- Can grow in 6.5% NaCl
- Can grow at a pH range of 9.6 to 4.6
- Can grow at temperatures ranging from 10-45°C
- Present in human and animal guts in high concentrations
- Very robust, can survive well in the environment



Enterococcus



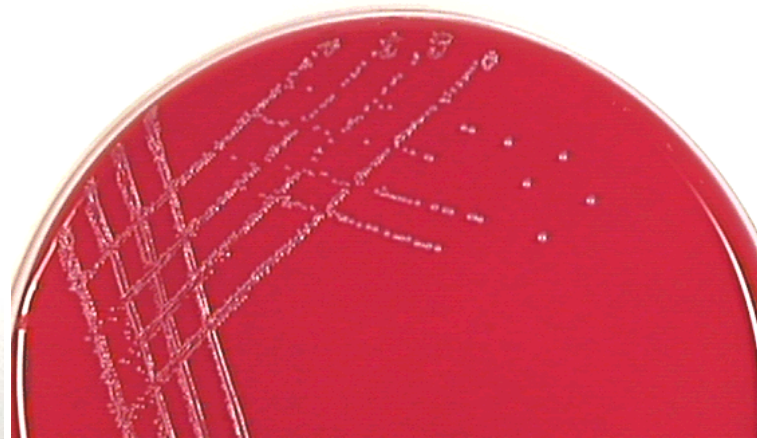
Gram Stain (1000X)



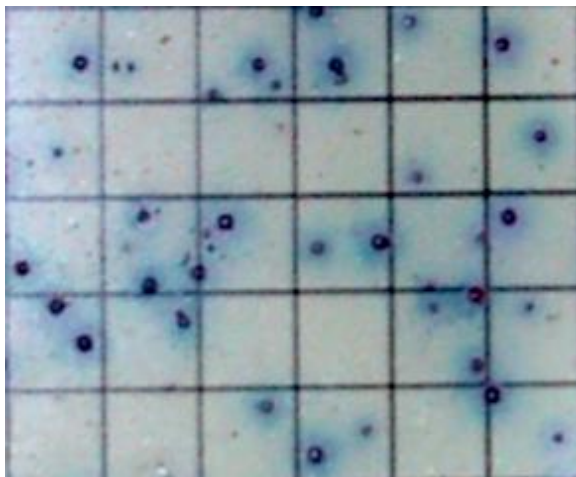
Electron micrograph (4000X)



Enterococcus



on Blood Agar



on m-EI Agar



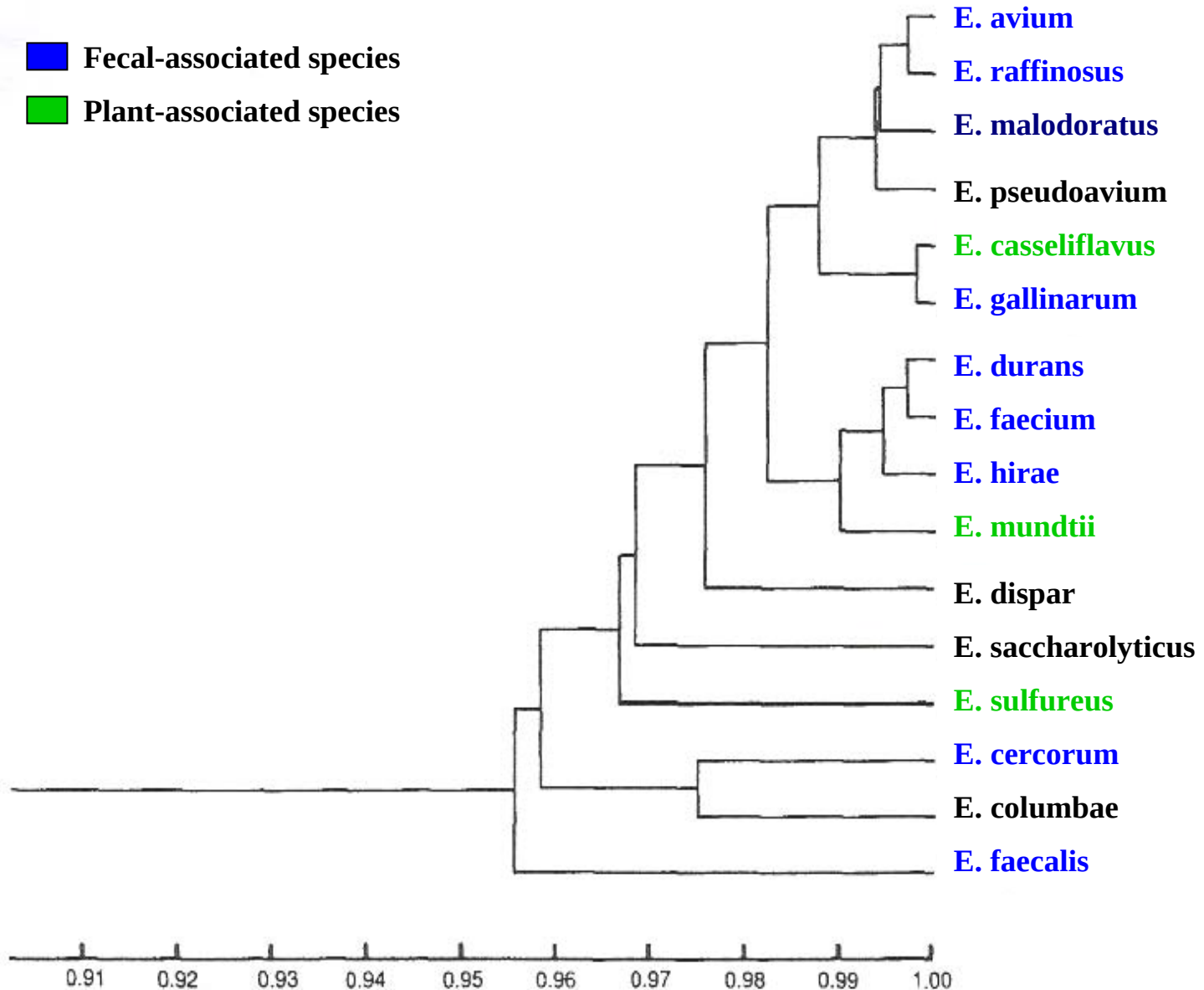
Bile Esculin 6.5 % Salt



Enterococcus species

 Fecal-associated species

 Plant-associated species



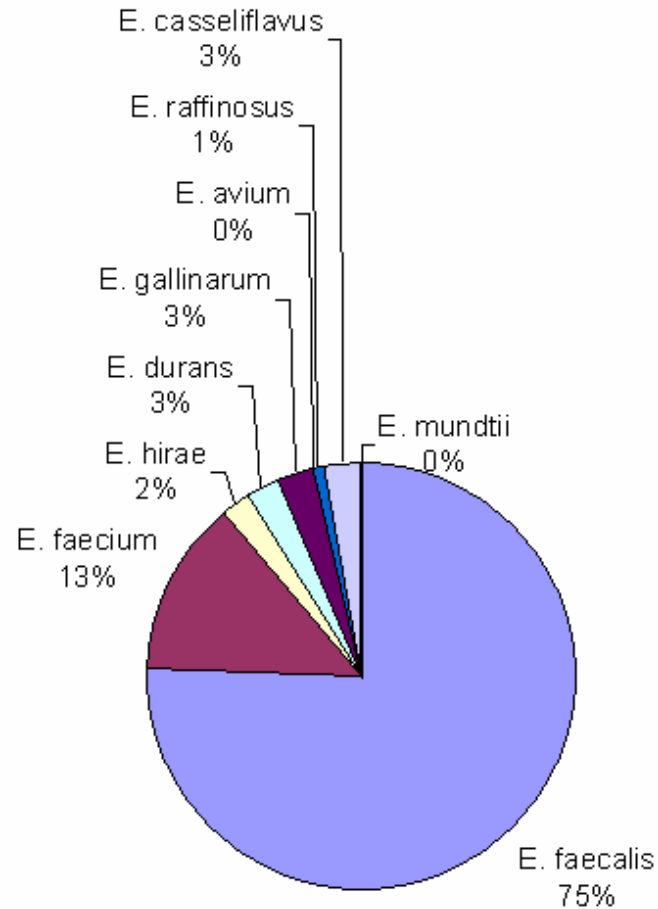
Enterococcus as an Indicator and for Source Tracking in California

- **Is enterococcus a good indicator for California?**
- **What species are we detecting?**
- **Can identification of different species be utilized to determine source of pollution?**



Enterococcus sp. in humans

(Ruoff 1990, Stern 1994, Pinto 1999)

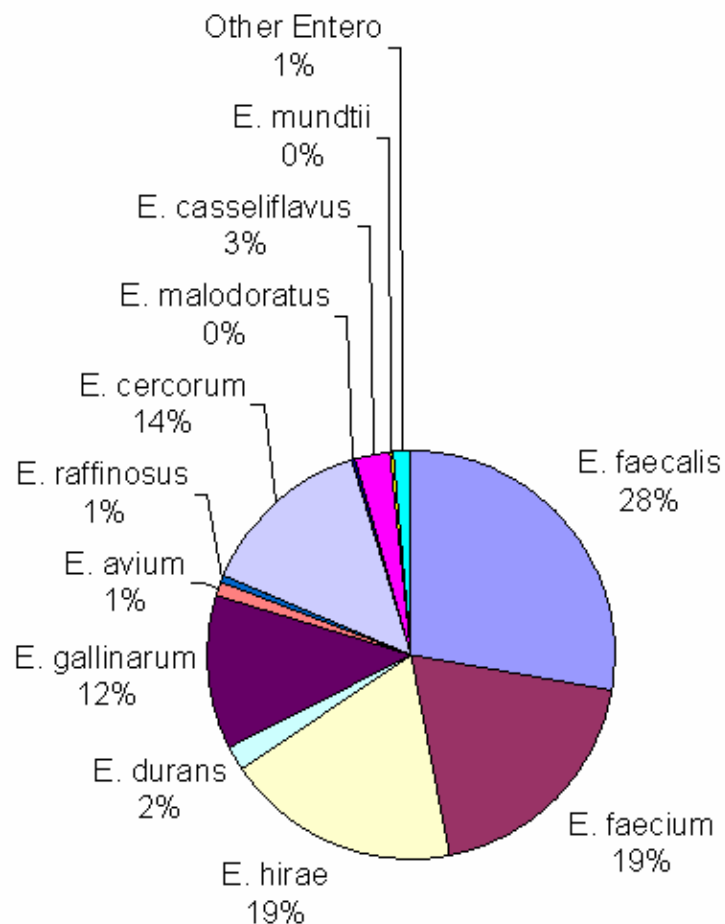


96.7% Fecal-Associated / 2.8% Plant-Associated



Enterococcus sp. in Animals

(Devriese 1987-'94, Stern 1994, Mac 2003, Poeta 2005)



95.5% Fecal-Associated / 4.5% Plant-Associated



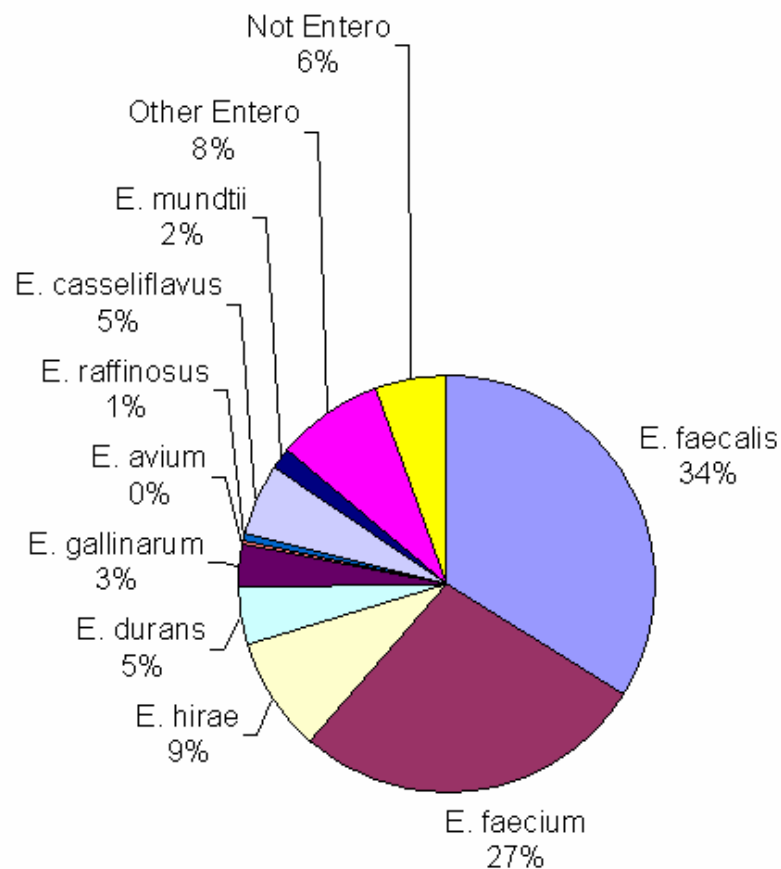


Enterococcus in Plants

- *E. casseliflavus*
- *E. mundtii*
- *E. sulfureus*

Enterococcus sp. in Environmental Waters

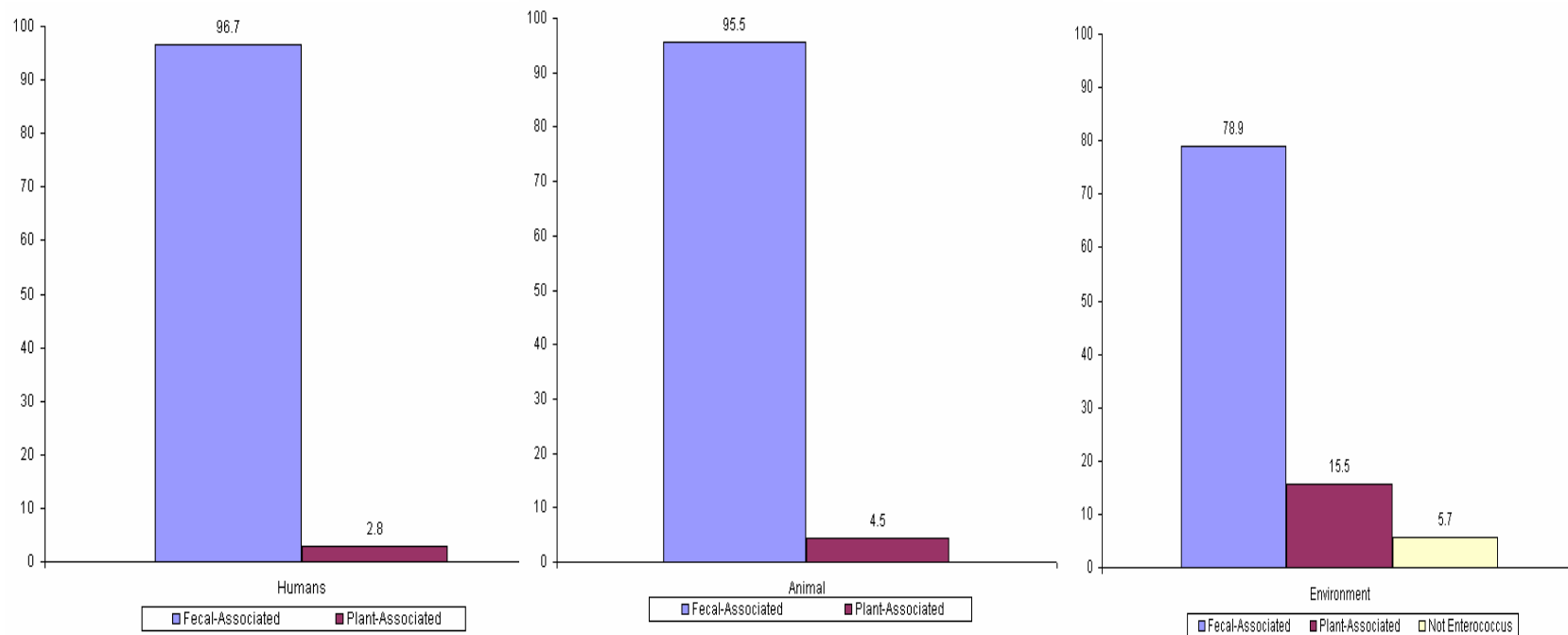
(Stern 1994, Pinto 1999, Svec 1999)



78.9% Fecal-Associated / 15.5% Plant-Associated



Comparison of Fecal-Associated & Plant-Associated Enterococcus

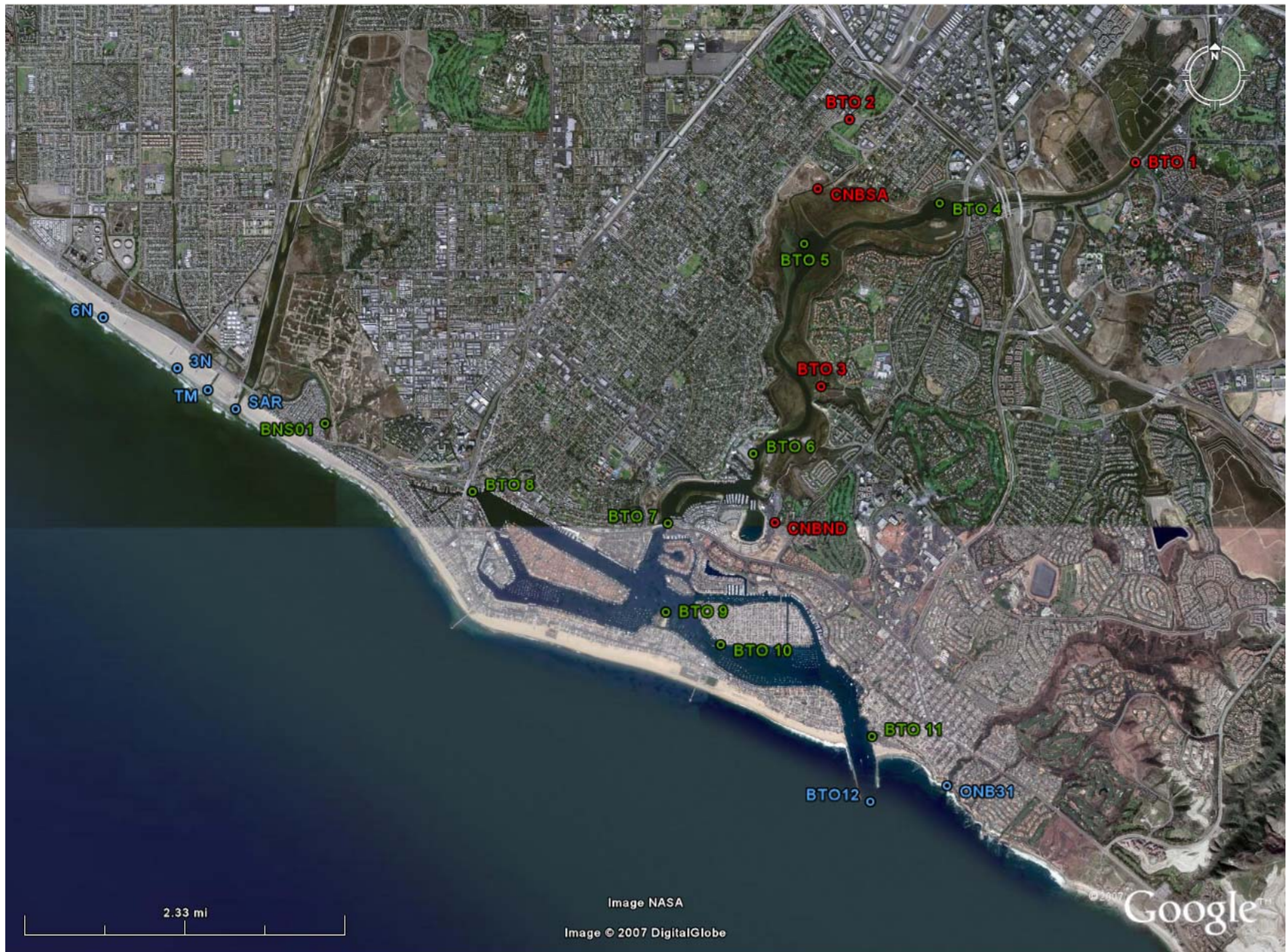


Orange County Enterococcus Speciation Study

- Urban Runoff: 5 sites, 202 isolates
- Bays/ Wetlands/Harbors: 10 sites, 176 isolates
- Ocean: 6 sites, 262 isolates
- Sewage: 2 sites, 98 isolates
- Total: 23 sites, 738 total isolates collected between February '06 – July '07



Site Locations

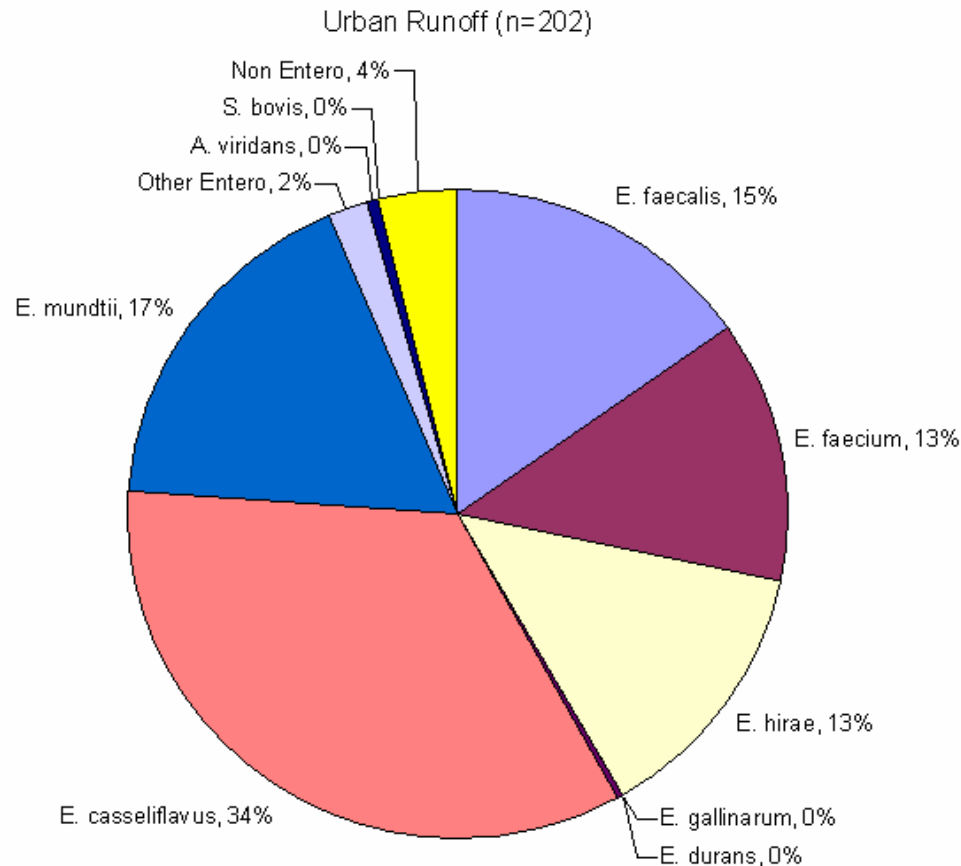




Study Protocol

- Up to 5 colonies picked for each sample
- Isolates purified
- Species determined utilizing automated and manual biochemical and growth tests
- Sequencing utilized for confirmation

Enterococcus in Orange County Urban Runoff

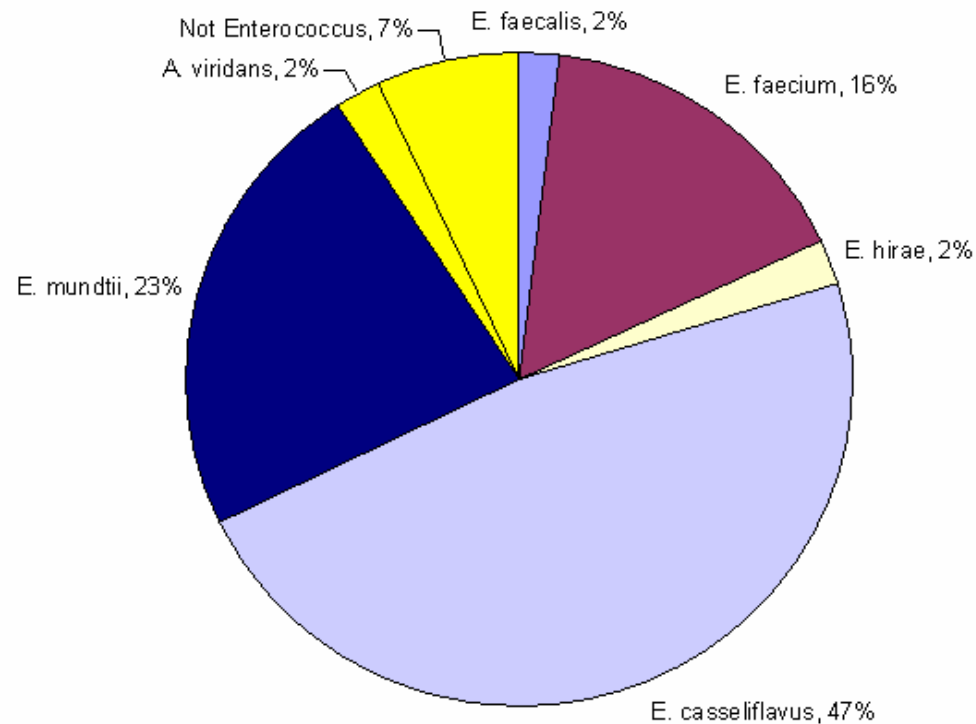


42% Fecal-Associated / 54% Plant-Associated



Enterococcus at San Diego Creek Site

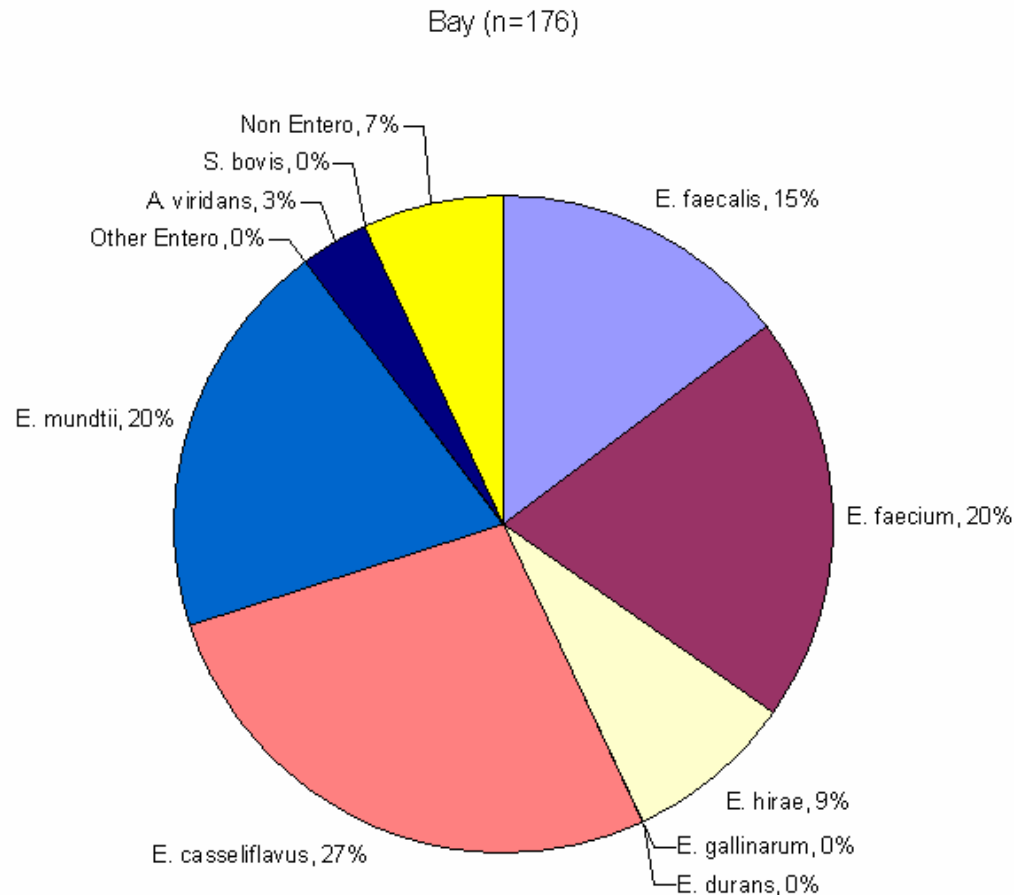
San Diego Creek (n=43)



20% Fecal-Associated / 60% Plant-Associated



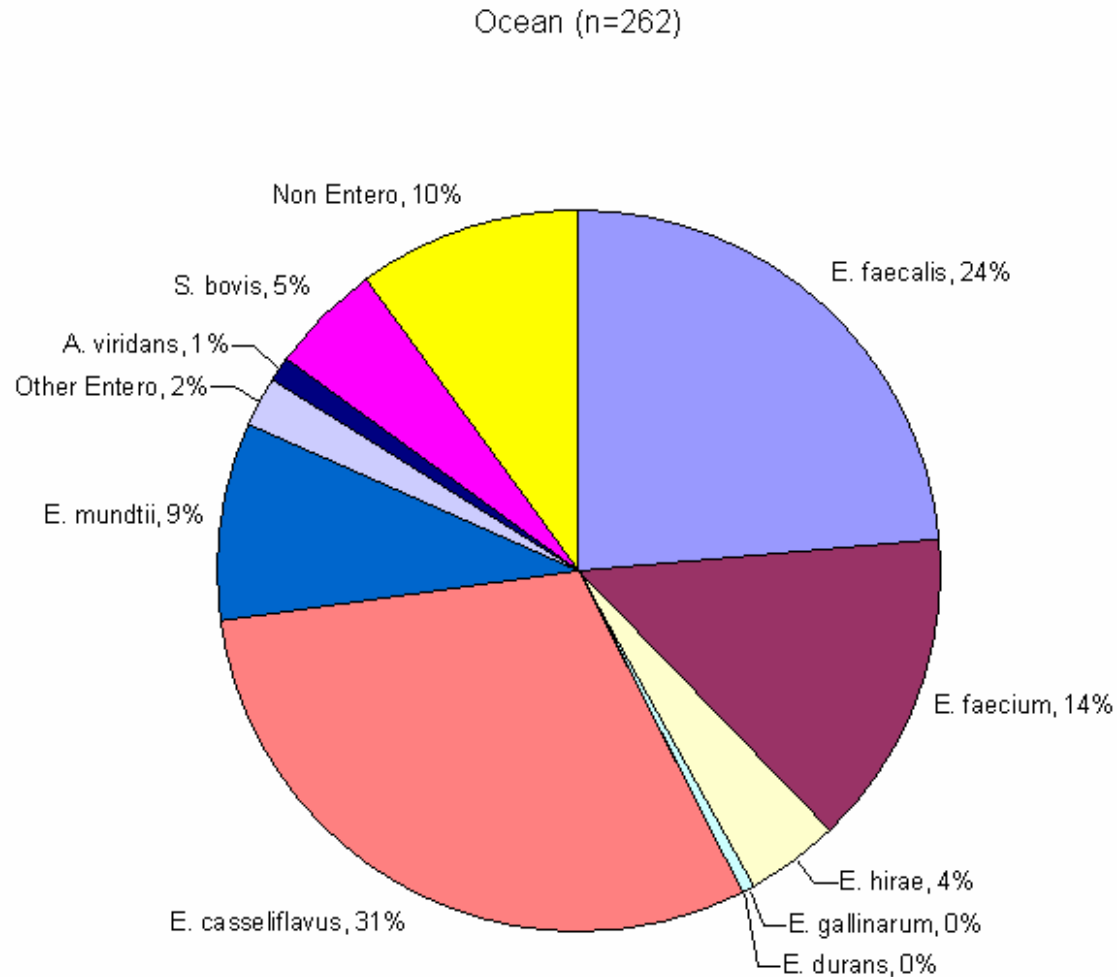
Enterococcus in Orange County Bays (harbors, bays, and wetlands)



43% Fecal-Associated / 47% Plant-Associated



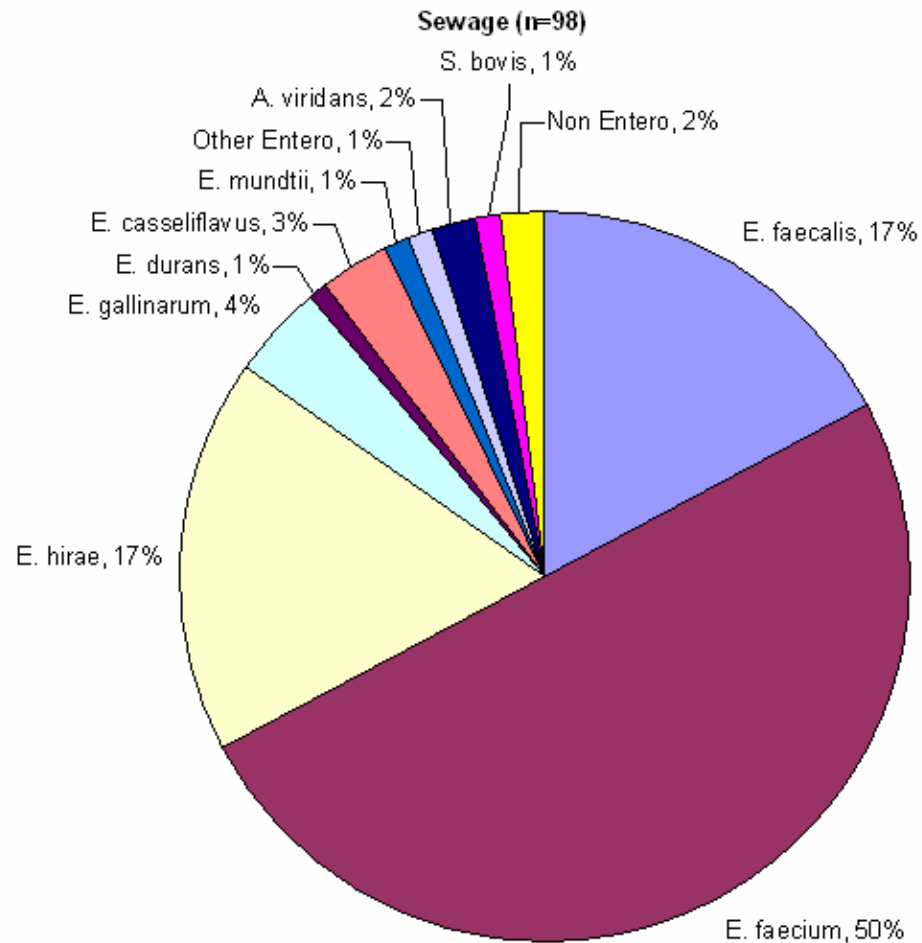
Enterococcus in Orange County Ocean Sites



42% Fecal-Associated / 42% Plant-Associated



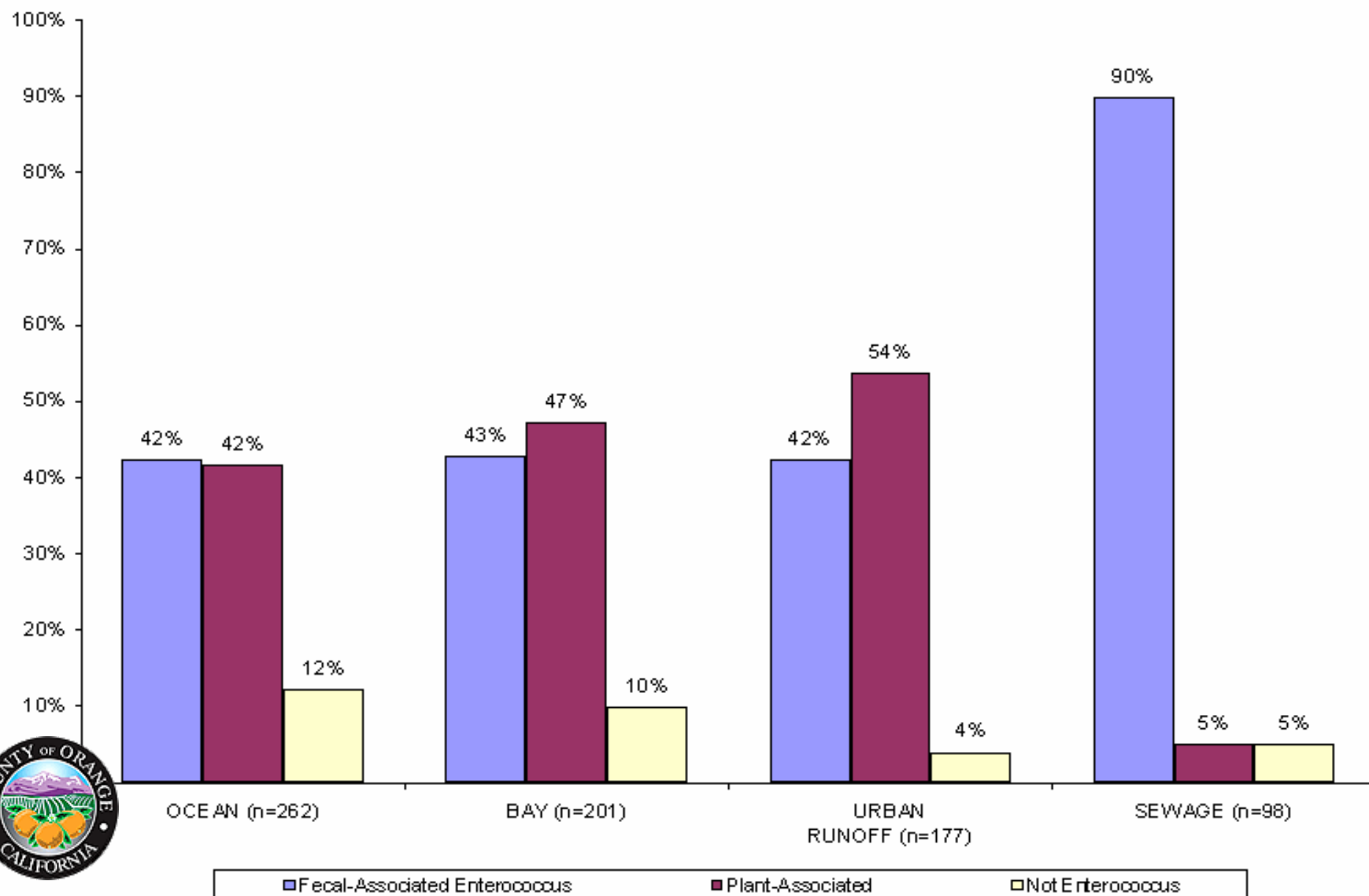
Enterococcus in Orange County Sewage



90% Fecal-Associated / 9% Plant-Associated



Orange County Fecal-associated and Plant – associated species



Results

- 42-54% of enterococcus isolated from urban runoff, bays and the ocean are *E. casseliflavus* and *E. mundtii*, plant-associated species. Remainder are fecal-associated species
- 90% of sewage isolates were *E. faecalis* and *E. faecium* as expected.
- False positives (non enterococcus) ranged from 4-5% for urban runoff to 10-15% for bays and oceans.
- Distribution of species was similar for urban runoff, bays and oceans.





Discussion: Enterococcus as an Indicator In California

- EPA epidemiology studies show good performance in sewage impacted waters.
- Recent San Diego epidemiology study shows no correlation with bather illness.
- Orange County speciation studies show lack of specificity due to the detection of plant associated species.
- Since animals also carry fecal-associated enterococci species there is a lack of specificity if any animal fecal material is present.
- Other studies have shown potential for re-growth of enterococcus in selected environmental sites.
- Present enterococcus test cannot reliably indicate human fecal vs. environmental pollution in Southern California.

Discussion: Enterococcus Speciation for Source Tracking

- Speciation can differentiate plant associated from fecal-associated species and demonstrated a large percentage in urban runoff, bays and ocean sites.
- Speciation can also determine percentage of false positive results.
- Since animals and humans both carry *E. faecium* and *E. faecalis*, speciation can not differentiate animal from human sources.
- Overall species distribution results are consistent with urban runoff being the source of most pollution in Orange County.
- Plant sources and wild and domestic animal sources could be the major source of enterococcus found in urban runoff.