Current status on quantifying sources of microbial contamination

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Define sources, please!

- **Land uses**

e.g., <u>discharge types</u> like residential runoff, commercial/industrial, agricultural, combined sewer overflows, urban, forested land runoff, POTW.

- **Sanitary surveys**

can be instrumental in determining why FIB levels may be in exceedance. However, assessment of predominant fecal sources (such as leaking sewer lines or illicit discharges) by sanitary surveys is never quantitative. Once identified as a major source, action can be taken.

Define sources, please!

- **Fecal hosts**

(livestock, birds, wildlife, human sources)

We could count all the relevant animals, determine an average fecal load per individual and the representative number of FIB per g feces, and estimate the “host contribution” to the FIB concentrations found in surface waters.

Generally, this is not considered feasible or acceptable.

Conclusion: Developed microbial source tracking methods to identify and quantify fecal animal sources in water

What fecal sources are contaminating the water ?

Which is the dominant fecal source?

Alternative: **What are the main discharge types?**

Which is the dominant discharge type?

Source: Trish Holden

Define quantification, please!

- Approach 1: Quantify pathogens in sources

  (= discharge types)

  - Choose reference pathogens (EPA)

- Approach 2: Quantify relative contributions from animal and human sources in a water sample

  - Which method(s)? How quantitative?

Approach 2: How Quantitative is Microbial Source Tracking?

Example: Host-associated *Bacteroidales* genetic markers

1. Inherent uncertainty in host-associated genetic assays (<100% sensitivity and specificity)
   - qPCR assays do not equate to quantitative MST

⇒ Developed statistical model to predict “true” concentrations of *Bacteroidales* genetic markers

A probabilistic approach

Measured genetic marker concentration by human-associated assay of *Bacteroidales*

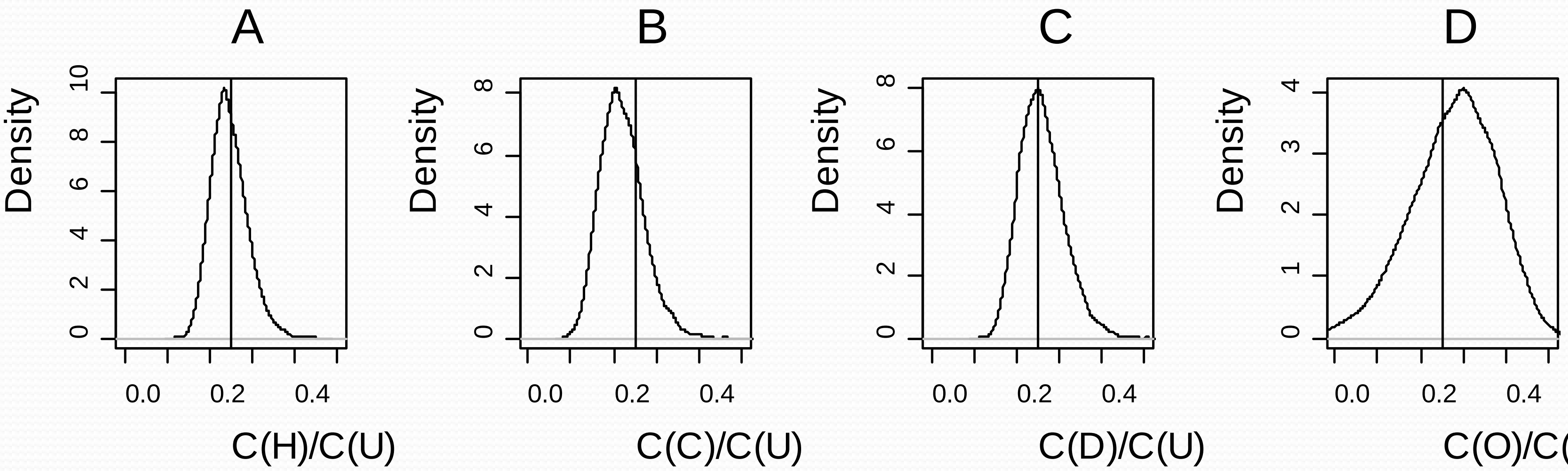
= Σ_i (DNA originating from source *i*, amplified by human-associated assay) + measurement error

where

"*i*" = human, cow, dog, other

And similarly for other specific assays

Model output distributions of fecal source composition for a mixed fecal sample containing equal amounts of total *Bacteroidales* from human, dog, cow and other sources

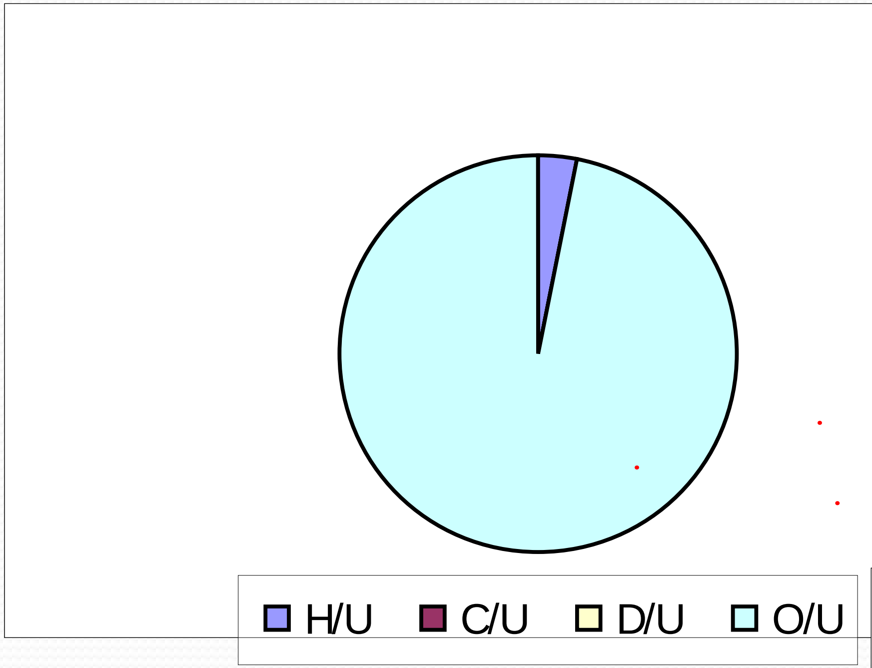


Measured contribution to fecal pollution in the sample from human, dog and cow was (**0.2445, 0.0122, 0.0172**)

Predicted contribution = (**0.2650, 0.2311, 0.2611**)

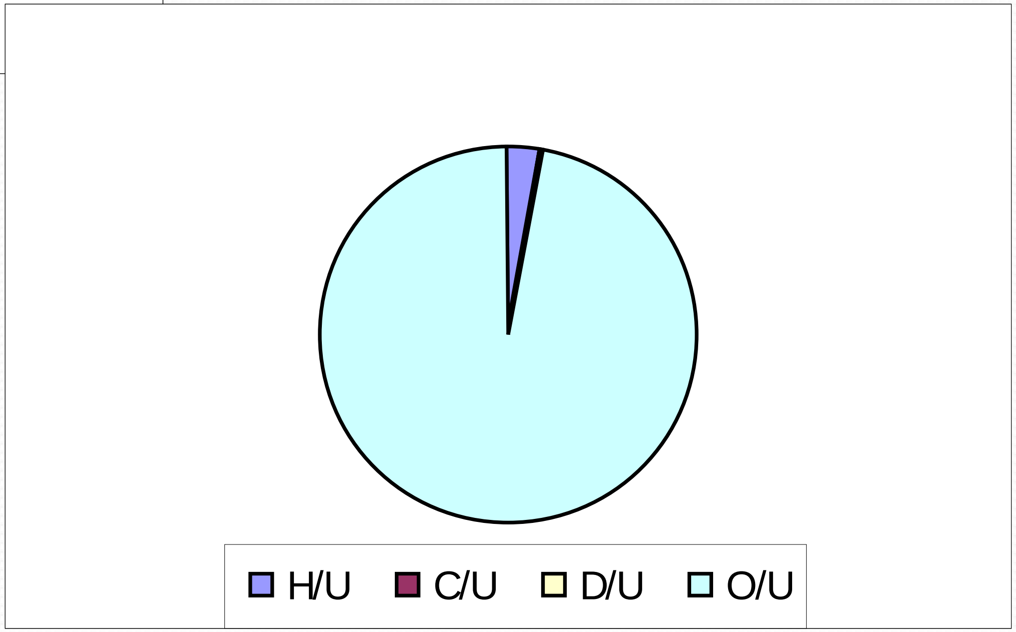
True contribution = (**0.25, 0.25, 0.25**)

Example: Measured and predicted *Bacteroidales* concentrations at site X in June

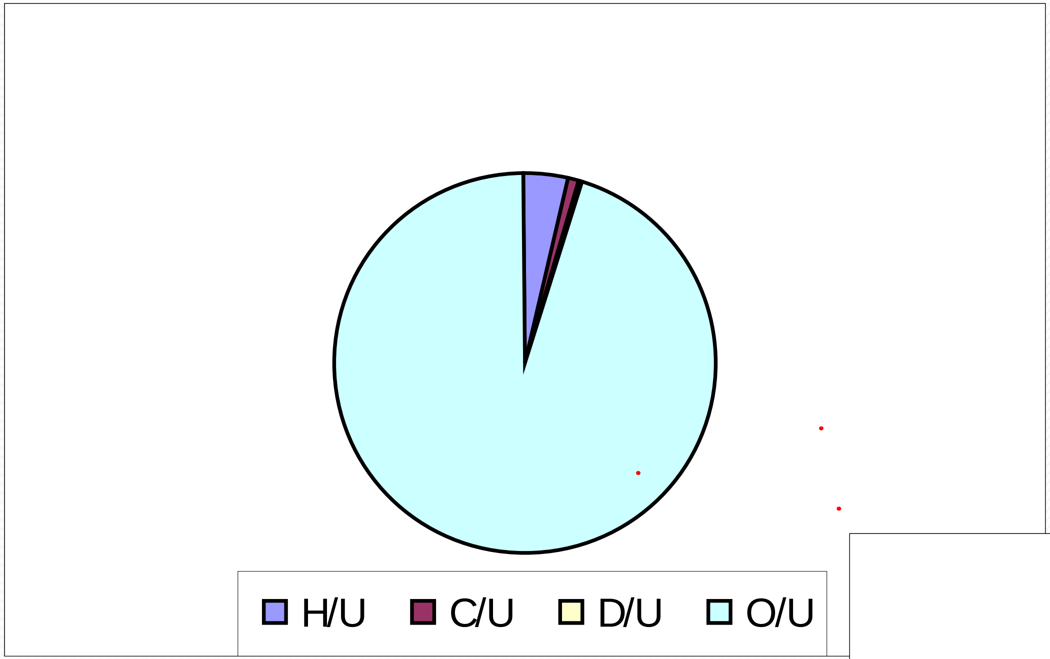


←Measured

Predicted →

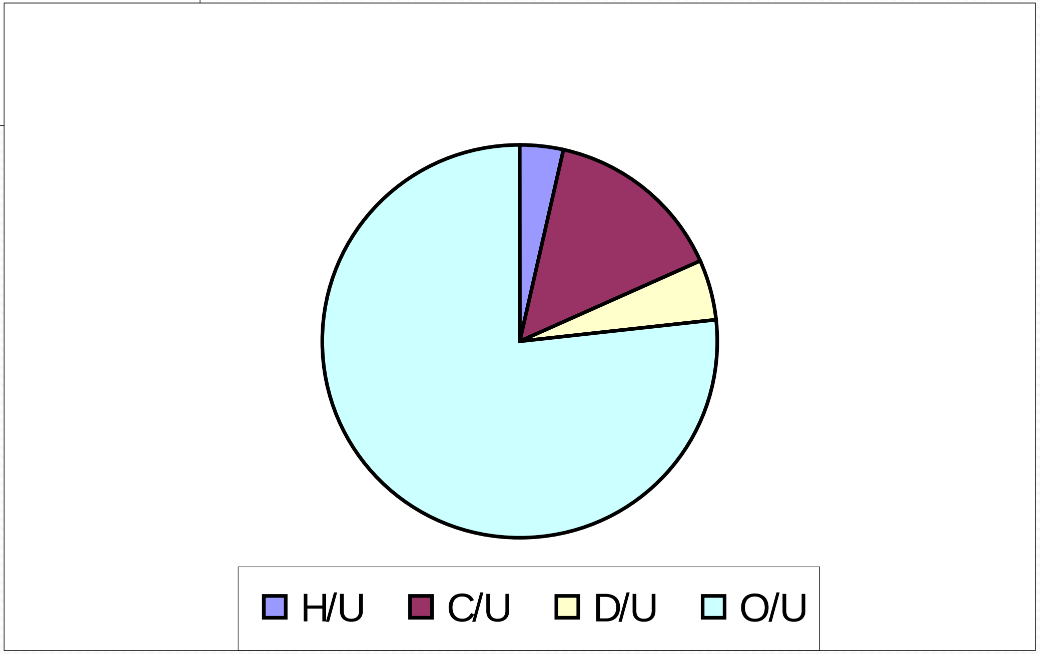


Example: Measured and predicted *Bacteroidales* concentrations at site Y in January



←Measured

Predicted →



You are thinking….

- Does it work?
- Did they actually apply it?

An example of work in progress……

MST sampling locations in a Nor Cal harbor

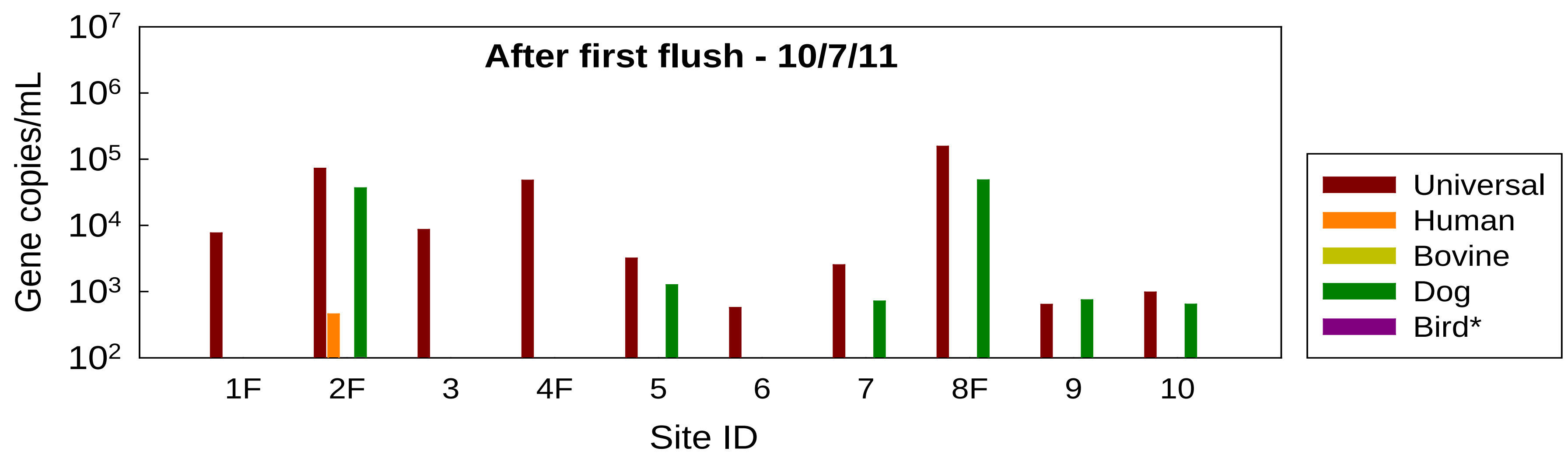
Locations (Yellow):

1. Capistrano Outfall Pipe
2. Bathhouse Outfall Pipe
3. Capistrano Beach
4. Denniston Creek
5. Pillar Point Marsh Beach
6. Mavericks Beach
7. Beach House Beach
8. Deer Creek Outlet
9. Inner Harbor Beach
10. Yacht Club Beach

⬆ Inflow sites

○ Most contaminated beach

Presence of host-associated genetic markers in water at different sites after first flush in 2011



- **First flush (2011) MST results**
  - Human marker hardly detected.
  - Bovine marker detected at site 8 before and during rain.
  - Dog marker elevated at inflow site 2, 4, and 8 during rain. Dog-associated *Bacteroidales* detected at high concentrations on several beaches after rain.

Conclusion

Dr. Dan Wang

It follows that source identifiers like *Bacteroidales* can help characterize fecal sources, e.g., the proportion of human fecal contribution.

Wang, D., S.S. Silkie, K.L. Nelson and S. Wuertz. (2010) Estimating true human and animal host source contribution in quantitative microbial source tracking using the Monte Carlo method. Water Research 44:4760-4775.

➔ Look out for new and simplified ratio-based method relating host-associated *Bacteroidales* to *Enterococcus* *- Ali Boehm lab*

What are limitations and additional research needed for quantification?

- Quantification by qPCR needs to be standardized and QA/QC procedures put in place (we have come a long way already)
- No assay is 100% specific and sensitive for a host
- Learn to live with these limitations by developing appropriate probability based “models” to account for errors and uncertainty in host-specificity

Additional research needed

- Understand that life sciences are undergoing a scientific revolution – massive sequencing of fecal and environmental samples and systems biology analysis can produce superior biomarkers
  - ➔ ‘environmental health’ of beaches/coastal areas
  - ➔ human health risks
- Realize that more specific assays would greatly improve any fecal source identification -- decide whether necessary
- Consider investigative tools which can distinguish many different fecal sources without being quantitative (similar to Phylochip)

Students and Collaborators

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