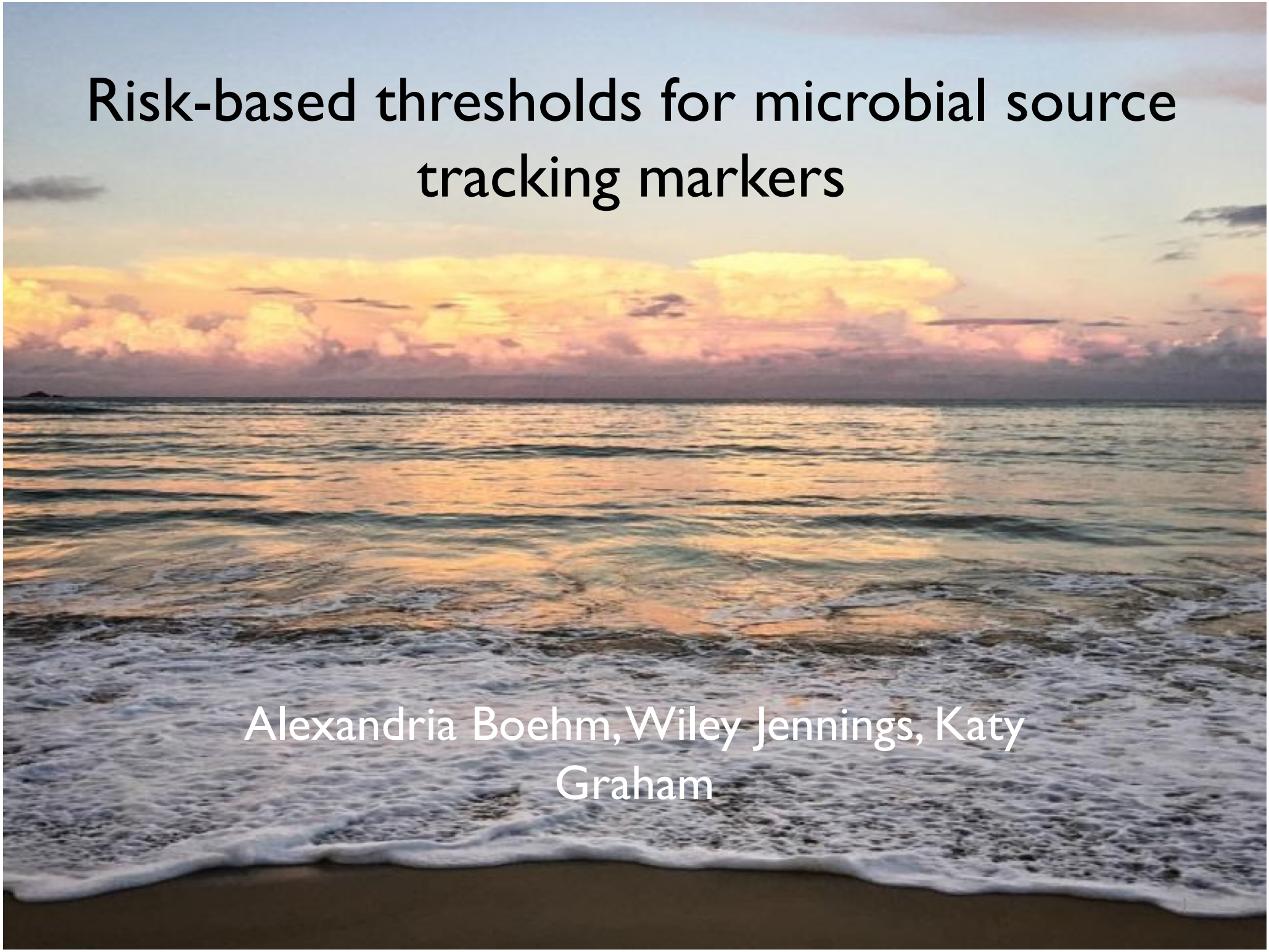


Risk-based thresholds for microbial source tracking markers

Alexandria Boehm, Wiley Jennings, Katy Graham

There are a number of sensitive and specific fecal source-associated MST markers

- Human - HF183 Taqman, HumM2
- Ruminant - BacR, Rum2Bac
- Gull - LeeSeagull
- Swine - Pig2Bac




We have great tools for identifying host associated fecal bacteria

photo credit: wikipedia

Taking MST markers to the field…

Example result:

HF183 Taqman = BLOQ

[LOQ = 500 copies / 100 mL]

LeeSeagull = 3000 copies / 100 mL

enterococci = 100 CFU/100 mL

Cowell Beach, Santa Cruz, CA

How would you interpret these results?

We need guidance for allowable threshold concentrations of MST markers

Proposal: Risk-based thresholds

Is there enough human feces to represent a health risk?
Is there enough gull feces to represent a health risk?

health risk = risk >30 /1000 chance of getting diarrhea

From my last presentation to you (November 2017)

MST marker (source)	Risk-based threshold (copy/100 mL)
HF183 (raw sewage)	4200
HumM2 (raw sewage)	2800
HF183 (treated effluent)	20000
CAT (gull feces)	7000
HF183 (raw sewage) & CAT (gull feces)	$\log_{10} \mathrm{HF} = 2.95 + \frac{-2.85}{(\log_{10} C_{\mathrm{CAT}} - 4.55)^2 + 0.26}$

Derived using QMRA (quantitative microbial risk assessment)
Assumed the fecal material was not aged in the environment

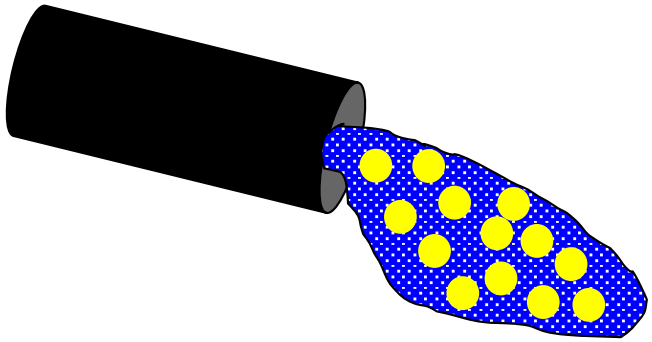
Research question

What is the risk-based threshold of human marker if the source (raw sewage) is aged? Or if the age is unknown?

Approach: Use quantitative microbial risk assessment (QMRA)

QMRA scenario

raw sewage with human markers and pathogens



leaks into surface water

surface water with dilute sewage and human markers

some time τ later

swimmer exposed to human markers and pathogens

pathogen

water with human marker (white dot)

QMRA scenario

1. Raw sewage discharged into surface water
   – raw sewage contains human markers and pathogens
2. Some time τ passes
3. Swimmer is exposed to specified concentration of human markers
4. Concentration of human markers is used to predict the amount of sewage in water
5. Pathogen concentration inferred from human marker concentration and τ
6. Infection and illness risk predicted
   – dose-response equations
   – probability of illness given infection

Example QMRA using point values

- Raw sewage has 10^7 copies / 100 ml human marker and 10^5 norovirus / 100 ml
- Human marker concentration is 10^3 copies / 100 ml at the beach when swimmer swims
- τ = 0 days
- Assuming human marker comes from raw sewage, concentration of norovirus is 10 norovirus /100 mL at the beach.
- Swimmer consumes 30 ml water
- Swimmer consumes ~ 3 norovirus
- Probability of infection is 0.4
- Probability of illness is 0.2

$$C_{i\_surface} = C_{meas} C_{i\_sewage} \exp((k_{hf} - k_i)\tau) / C_{hf\_sewage}$$

$C_{i\_surface}$ = pathogen i concentration in surface water

C_{i_sewage} = pathogen i concentration in sewage

C_{hf_sewage} = human marker concentration in sewage

k_{hf} = first order decay constant of human marker

k_i = first order decay constant of pathogen i

τ = time

C_{meas} = concentration of human marker measured

$$C_{i\_surface} = C_{meas} C_{i\_sewage} \exp((k_{hf} - k_i)\tau) / C_{hf\_sewage}$$

$C_{i\_surface}$ = pathogen i concentration in surface water

C_{i_sewage} = pathogen i concentration in sewage

C_{hf_sewage} = human marker concentration in sewage

k_{hf} = first order decay constant of human marker

k_i = first order decay constant of pathogen i

use values from literature

τ = time

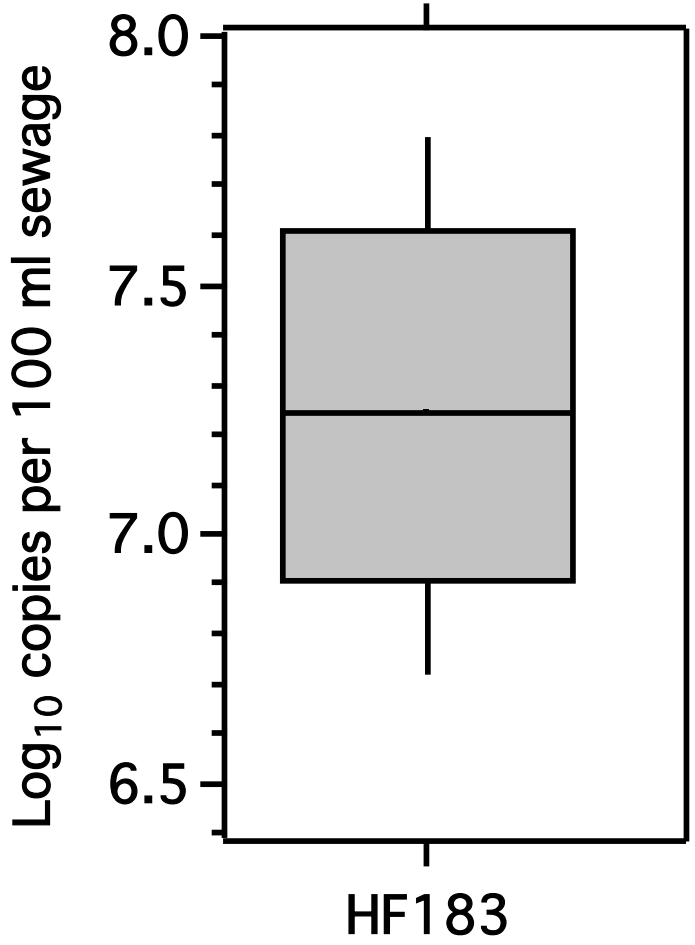
C_{meas} = concentration of human marker measured

vary ourselves to preset values

QMRA implementation

- Risk estimates for
  - C_{meas} (human marker) at 1, 10, 100, 1000, 10000 copies/100 ml surface water
  - τ = 0, 0.5, 1, 1.5, 2, 2.5, 3 days
- 10000 iterations per C_{meas}-τ combo using Monte Carlo simulations
- Model requirements:
  - volume of water ingested
  - human marker & pathogen concentrations in raw sewage
  - k values for human marker and pathogens
  - dose-response models and $P_{ill|infected}$
  - model parameters drawn from distributions
- 10000 model outputs for each C_{meas}-τ combo:
  - P_{ill_j} from each reference pathogen j
  - $P_{ill} = 1-\Pi(1-P_{ill\_j})$

C_{hf_sewage}
HF183 in raw sewage



54 samples of raw sewage from 37 states

Shanks et al. 2010. Environ. Sci. Technol. 2010, 44 (16) 6281– 6288

C_{i_sewage} and dose-response

Organism	C_{sewage} range (log_{10} per L)	P_{inf}	$P_{ill|inf}$ (distribution)
Salmonella spp.	[0.5, 5]	$1-(1+ \mu/2884)^{-0.3126}$	0.17-0.4 (uniform)
Campylobacter	[2.9, 4.6]	$1- 1-{}_1F_1(0.024,0.024+0.011,-\mu)$	$1-(1+n\mu)^{-r}$
E. coli O157:H7	[-1, 3.3]	$1-(1+ \mu/48.8)^{-0.248}$	0.2-0.6 (uniform)
Cryptosporidium	[-0.52, 4.7]	$1 - \exp(-0.09\ \mu)$	0.3-0.7(uniform)
Giardia	[0.51, 4.2]	$1 - \exp(-0.0199\ \mu)$	0.2-0.7 (uniform)
Norovirus	[4.0, 1.1] *	$1- {}_1F_1\ (0.04, 0.04+ 0.055, -\mu)$	0.6

* log-normal distribution: log_{10} mean and log_{10} stdev

Volume ingested during swimming

log_{10} normal with mean of 1.146 and standard deviation of 0.545 units of ml (Dufour et al. 2017)

Still need k values! No literature compilation of k values so we had to do a systematic review

Systematic review of literature on human marker and pathogen decay rate constants

"(X) AND (water OR seawater OR stormwater) AND (die-off OR persistence OR survival OR inactivat* OR decay)"

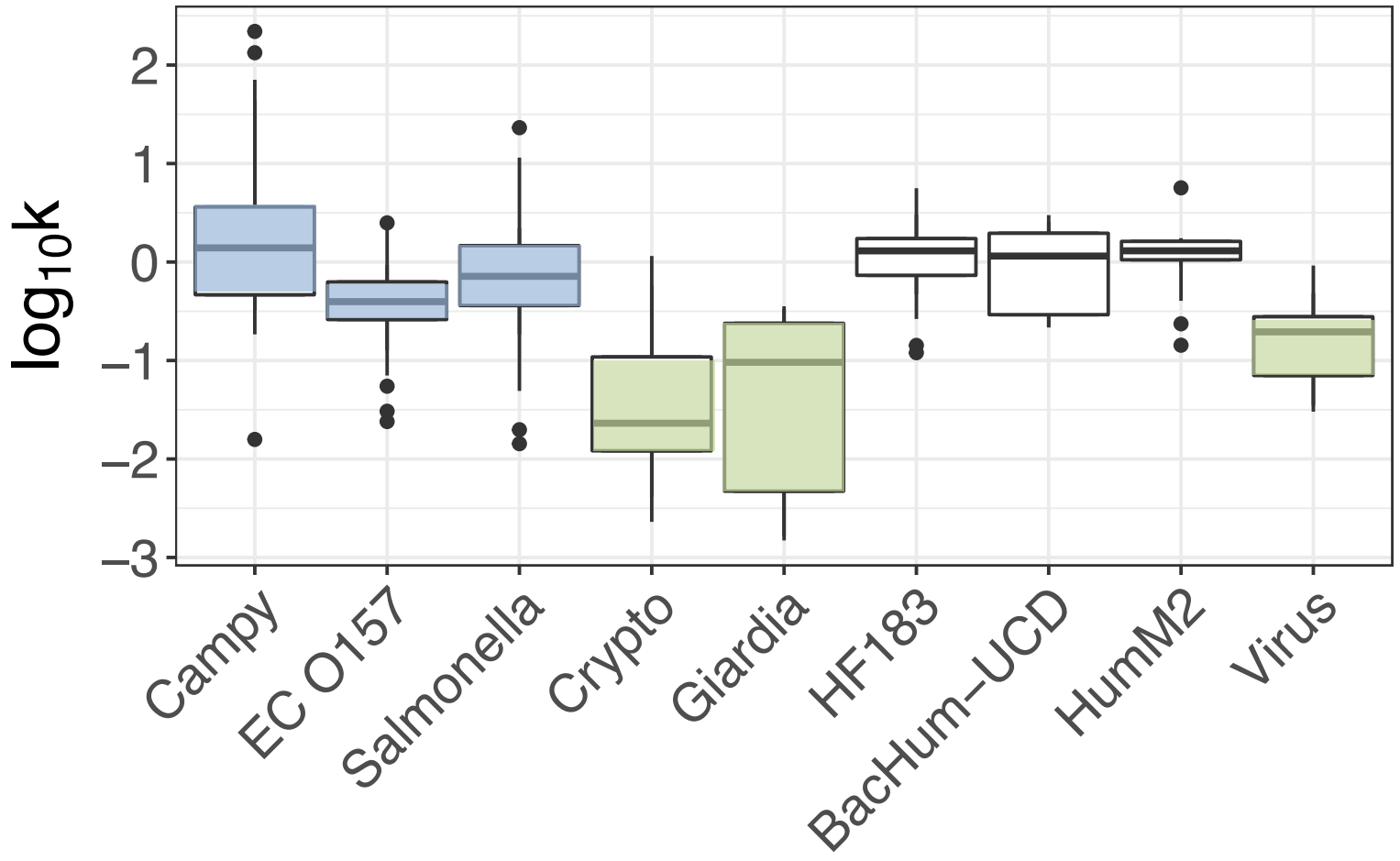
Target	Date of search	Target-specific search term	Number unique papers identified through databases	Number papers identified from references of review or other papers	Total number papers screened	Number for full text review	Number included
Human norovirus	8/1/17	(norovir* OR norwalk vir* OR calicivir*)	857	8	865	50	2
Campylobacter	8/10/17	(campylobacter)	608	0	608	50	9
Salmonella	8/24/17	(salmonella)	3064	3	3067	75	25
E. coli O157:H7	8/24/17	("E. coli O157:H7" OR "Escherichia coli O157:H7")	762	11	773	44	15
Giardia	8/24/17	(Giardi*)	624	3	627	25	2
Cryptosporidium	8/24/17	(Cryptosporidi*)	1121	6	1127	53	7
Human associated fecal indicator	8/25/17	("human Bacteroides" OR "microbial source tracking markers" OR HF183 OR (bth AND bacteroides) OR bachum OR bachum-UCD OR humbac OR BsteriF1 OR (gyrB AND fragilis) OR "human Bacteroidales" OR bacH OR humm2 OR "human marker" OR "human-associated marker")	55	10	65	34	17
Murine norovirus	8/1/17	(norovir* OR norwalk vir* OR calicivir*)	857	8	865	50	2
Feline calicivirus	8/1/17	(norovir* OR norwalk vir* OR calicivir*)	857	8	865	50	1

k values (d^{-1})

Target	N	log_{10}-mean	log_{10}-stdev	Geo-mean k
HF183	52	0.063	0.34	1.16
HumM2	15	0.050	0.37	1.12
BacHum-UCD	13	-0.038	0.43	0.92
Salmonella	84	-0.17	0.51	0.68
Campylobacter	41	0.28	0.84	1.91
E. coli O157:H7	84	-0.43	0.37	0.37
Giardia	14	-1.36	0.96	0.04
Cryptosporidium	22	-1.39	0.80	0.04
Virus	8*	-0.81	0.50	0.15

N = 2 for human norovirus, N = 4 for feline calicivirus, N = 2 for murine norovirus

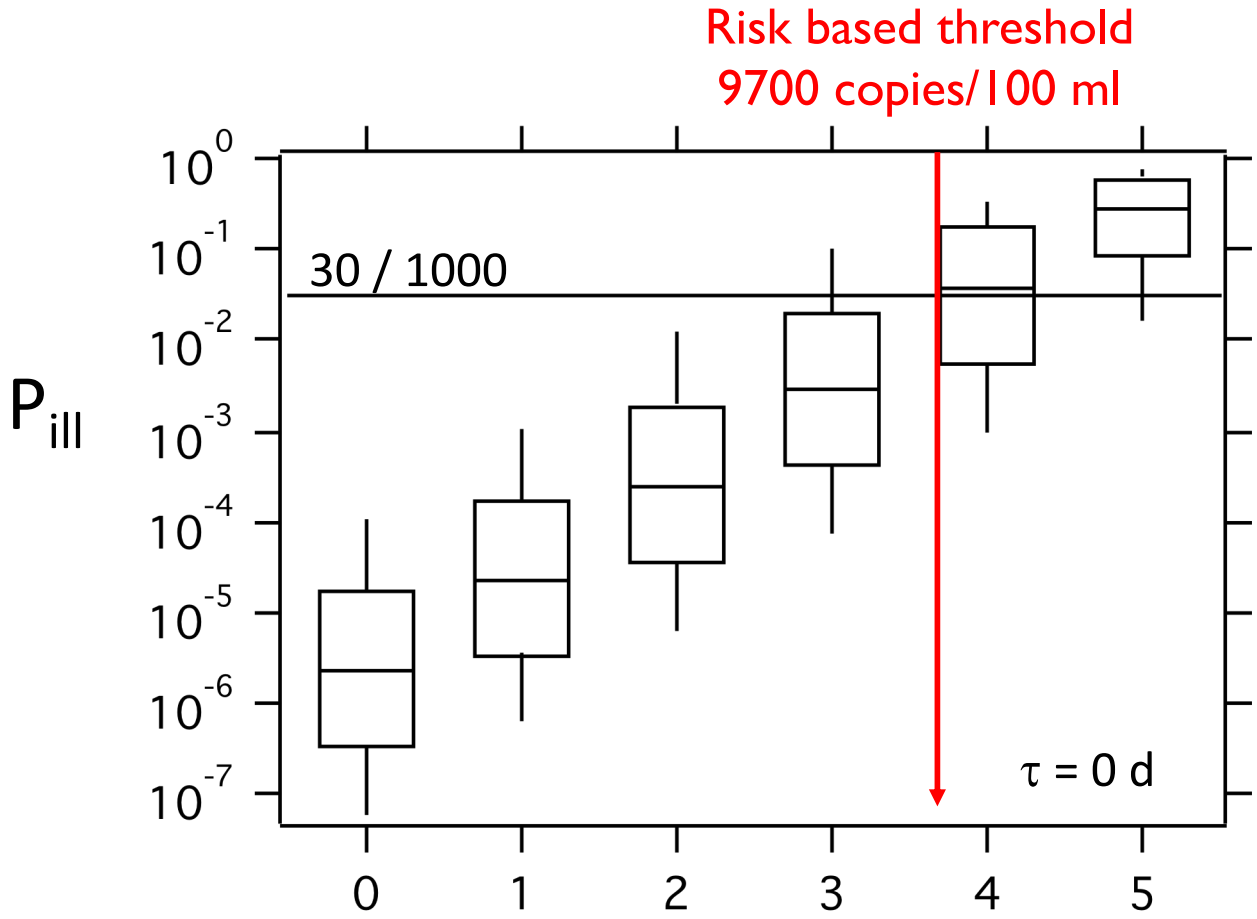
k (d^{-1}) in graphical form



Now we can run our model!

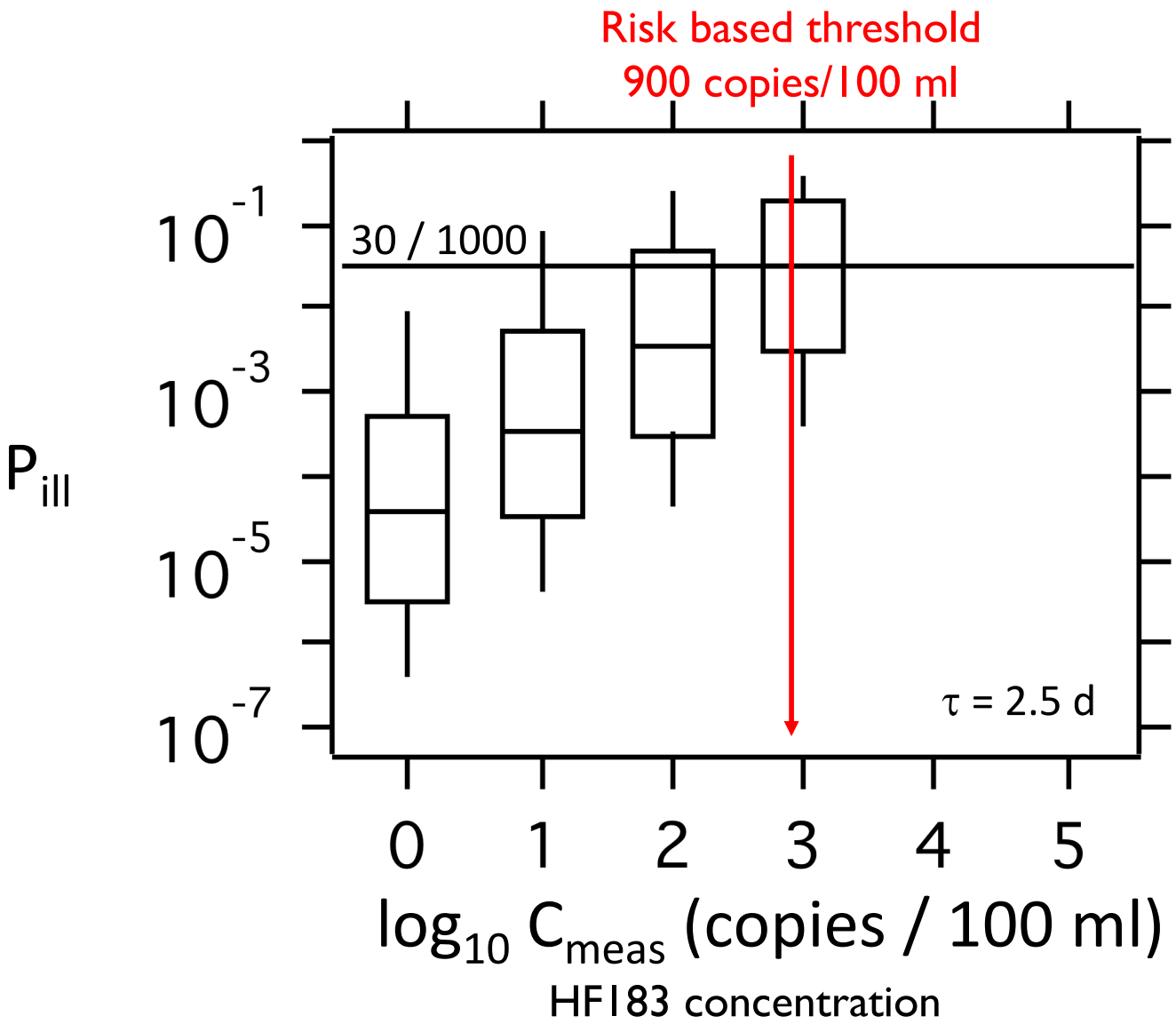
1. Modeler specifies C_{meas} (HF183 conc.) and τ
2. Run model in Matlab
3. Output: 10000 P_{ill} estimates that consider the range of model parameters

Model output for τ=0



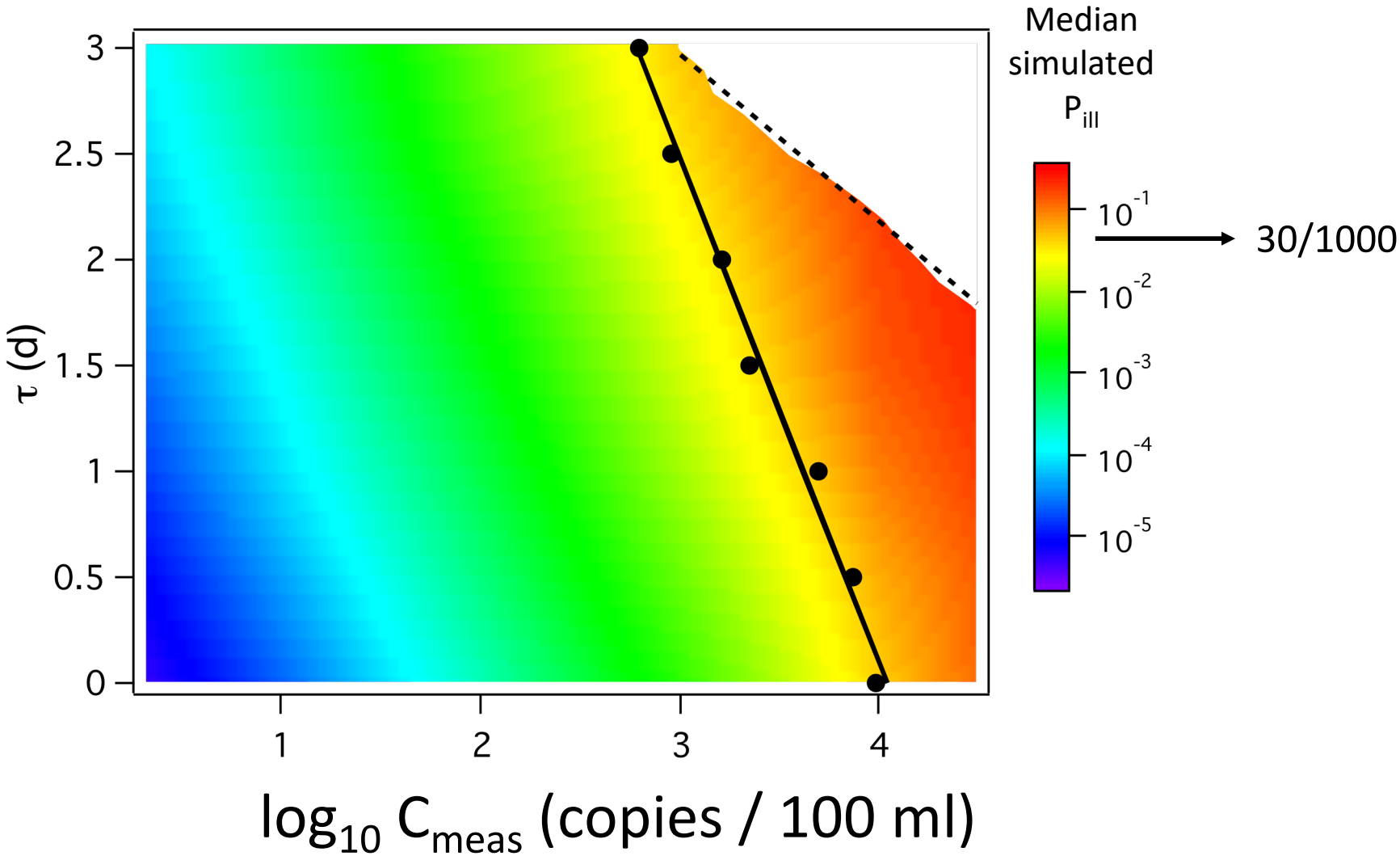
HF183 concentration

Model output for τ=2.5 d



We will focus on the median of P_{ill} distributions in coming slides

Risk based threshold decreases with τ

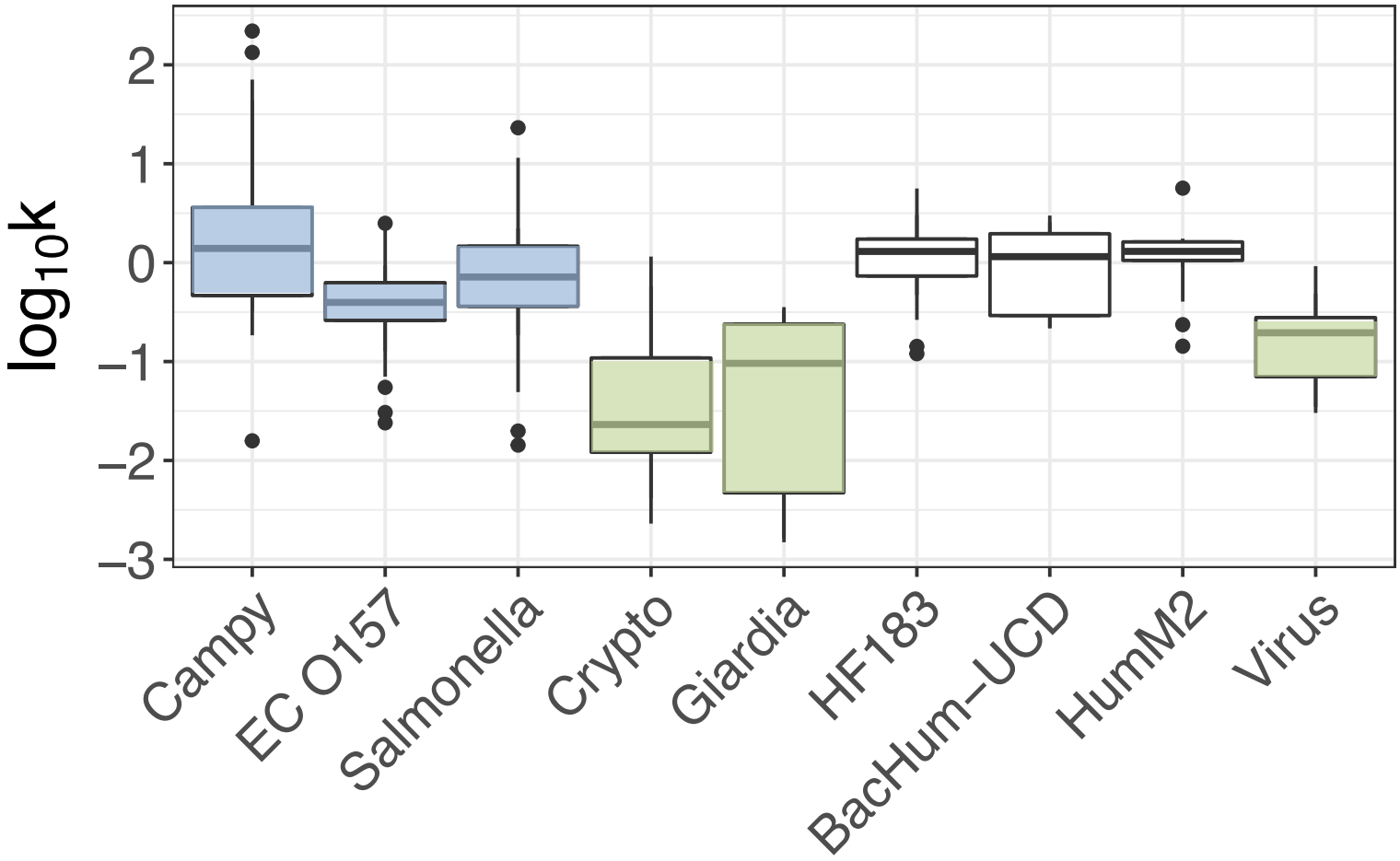


Threshold decreases ~ 0.4 log units per day of aging

After 3.3 d unlikely to have median risk > 30/1000

Why does the risk based threshold decrease with τ?

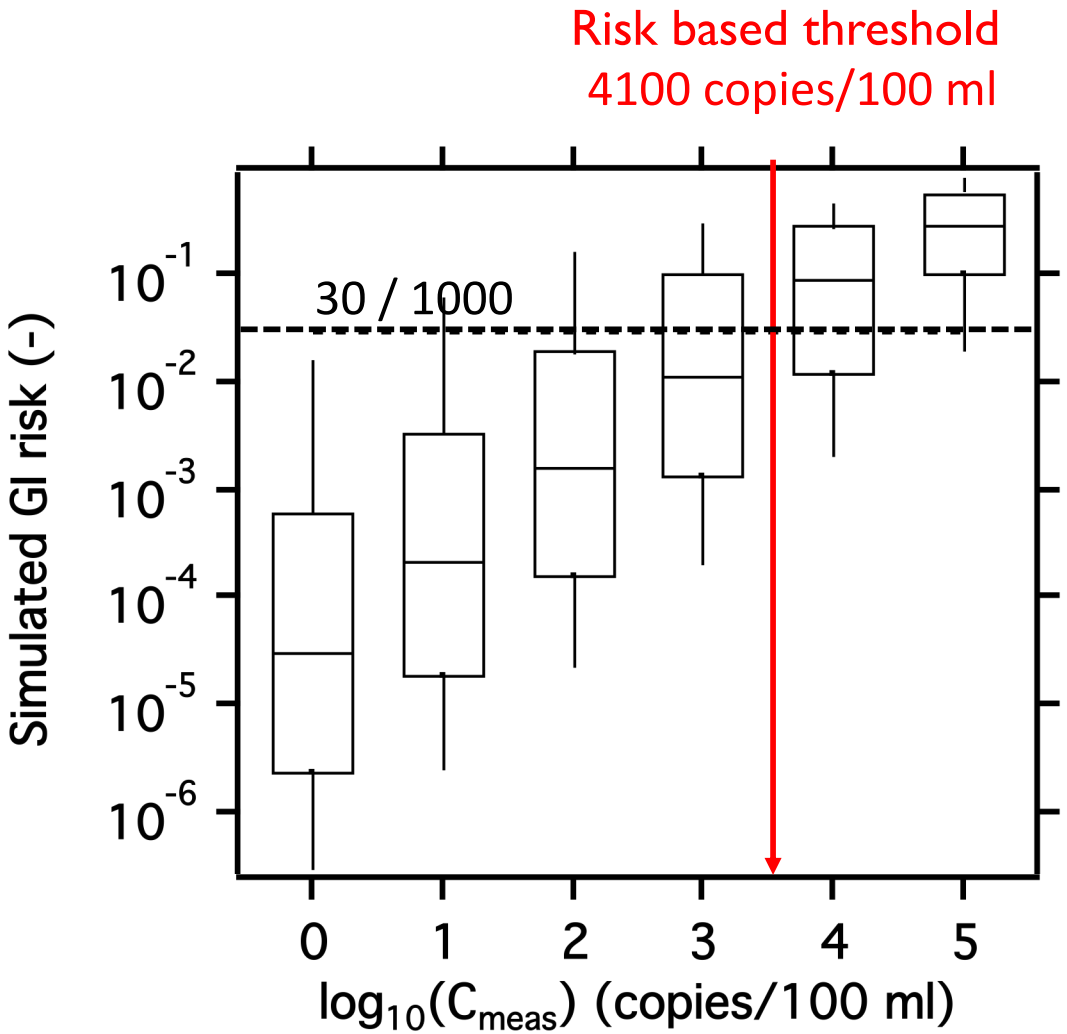
pathogen k is smaller than human marker k



What risk based threshold should we use? We don't know τ!

- Re-ran QMRA
- Specify C_{meas}
- Draw τ from a uniform distribution from 0 to a maximum realistic value (varies with C_{meas}) because we don't know τ
- All other methods the same

Results for τ unknown



Two things to remember

1. As sewage pollution ages, the risk associated with a particular HF183 concentration increases
2. Risk based threshold for HF183 is 4100 copies / 100 ml

- Risk based threshold = concentration of HF183 at which the median simulated risk is 30/1000
- Considers uncertainty in pollution age
- Risks in excess of 30/1000 are possible at lower HF183 concentrations
- A different risk-basis can be used for a threshold

Summary of work to date

MST marker (source)	Risk-based threshold (copy/100 mL)	Reference
HF183 (raw sewage)	4200	Boehm et al. 2015
HumM2 (raw sewage)	2800	Boehm et al. 2015
HF183 (treated effluent)	20000	Brown et al. 2017a
CAT (gull feces)	7000	Brown et al. 2017b
HF183 (raw sewage) & CAT (gull feces)	$\log_{10} \mathrm{HF} = 2.95 + \frac{-2.85}{(\log_{10} C_{\mathrm{CAT}} - 4.55)^2 + 0.26}$	Brown et al. 2017a
HF183 (raw sewage, uncertain age)	4100	Boehm et al. 2018

Your input needed

1) Do you have suggestions for future work?
2) Would you use these risk-based thresholds for MST markers for interpreting results at your beaches?