

Bacteria Recreational Water Quality Criteria (RWQC) Update – Regulatory Issues and Resolutions

Terry Bousquet, NCASI WCRC

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Update Topics

- USEPA 2012 RWQC – Indicator Bacteria Refresher
- OR-DEQ 2016 Update to Water Quality Bacteria Standards
- Mill Monitoring - Fecal Sources
- EPA Approved Analytical Methods
- Evaluating Methods and Sources
 - Case study examples
 - Wood yard runoff study results
- Regulatory Updates
- NCASI Resources

USEPA 2012 RWQC

Criteria Elements	Recommendation 1 (estimated illness rate 36/1000)		Recommendation 2 (estimated illness rate 32/1000)	
	GM ^a (cfu/100 mL)	STV ^b (cfu/100 mL)	GM ^a (cfu/100 mL)	STV ^b (cfu/100 mL)
Indicator Bacteria				
Enterococci (fresh and marine)	35	130	30	110
<i>E. coli</i> (fresh)	126	410	100	320

Duration and frequency: The waterbody GM should not be greater than the selected GM magnitude in any 30 day interval. There should not be greater than a 10% excursion frequency of the selected STV magnitude in the same 30 day interval

^a GM = geometric mean

^b STV = statistical threshold value

OR-DEQ - Update

- Draft “Water Quality Bacteria Standards 2016”
<http://www.deq.state.or.us/eqc/docs/o81716eqc/o81716ItemI.pdf>
- Shellfish & Coastal Recreational Waters – enterococci
 - 35 counts/100mL 90 day GM (30 days NPDES)
 - 130 counts/100mL no more than 10% of time in 90 days
- Freshwater Recreation – *E. coli*
 - 126 counts/100mL 90 day GM (30 days NPDES)
 - 406 counts/100mL single sample maximum
- Shellfish – Fecal coliform
 - 14 counts/100mL median concentration
 - 43 counts/100mL not more than 10% of samples

OR-DEQ – Fecal Source Stipulation

- DEQ is clarifying that these requirements apply to **bacteria in effluent discharges associated with fecal sources**, which is consistent with the existing language in the bacteria standard:
- “Numeric criteria: Organisms of the coliform group commonly associated with *fecal sources* (MPN or equivalent membrane filtration using a representative number of samples) may not exceed the criteria described in paragraphs (a) and (b) of this paragraph.” OAR 340-041-0009(1)(emphasis added).
- This change acknowledges that **certain non-fecal discharges, such as pulp and paper effluent, may contain bacteria that are detected as *E. coli* or enterococcus, but are not pathogenic and do not indicate the presence of fecal contamination.** (Gauthier and Archibald 2001; Degnan 2007; Croteau, et al. 2007). **Due to the potential interference of plant-based bacteria** in enterococcus tests, it may be difficult for pulp and paper mills to achieve compliance with enterococcus criteria even if the discharge poses little risk to public health due to the lack of pathogenic bacteria in the discharge. The proposed provision will allow **flexibility to entities that can demonstrate to DEQ that their discharge does not come from fecal sources.** DEQ would require such entities to **demonstrate through biochemical species identification techniques that the effluent contains non-fecal based bacteria species.** Once the demonstration is made, DEQ would include appropriate effluent limits in the permit to ensure that public health is protected.

Mill Monitoring - Fecal Sources

- Sanitary source piped off site or to packaging plant
 - Exemption from final effluent monitoring for bacteria
- Sanitary is treated on-site (e.g. package plant, process area) and then combined with mill treatment system
 - Where is the appropriate sampling point?
- Other sanitary contributions – contractor trailers, failing infrastructure or cross connections
 - When and how should a sanitary survey be conducted?
- Non-sanitary contributions exceed limits
 - Wood yard runoff (species associated with plant decay)
 - Mixed Recycle Furnish
 - Birds, other wildlife

Effluent Bacteria Monitoring

- Enterococci monitoring - dischargers to marine waters
 - Better survival in saline environment than *E. coli*
 - Mill discharge is not saline – is it a better indicator of sanitary sources than *E. coli* in mill effluent?
 - Plant associated species (*E. casseliflavus*) identified in mill wastewaters exceeding RWQC
 - Analytical method bias – false positive identification
- *E. coli* monitoring – dischargers to fresh water
 - Better than fecal coliform – excludes Klebsiella
 - Incubation temperature of analytical methods important for reducing background bacteria levels
 - High levels contributed by wood yard runoff

Fecal Indicator Bacteria Test Methods

Parameter	Method Name	Method Type (units)
Escherichia Coli (E. coli)	EPA Method 1603	Membrane filtration (CFU ^b /100 mL)
	Colilert [®]	Enzyme substrate (MPN ^c /100 mL)
	SM ^a 9221F	Multiple tube fermentation (MPN/100 mL)
	mColiBlue	Membrane filtration (CFU/100mL)
Enterococci	EPA Method 1600	Membrane filtration (CFU/100 mL)
	Enterolert [®]	Enzyme substrate (MPN/100 mL)
	SM 9230B	Multiple tube fermentation (MPN/100 mL)

Methods approved by USEPA

Temperature modified Colilert[®] alternative test procedure submitted to USEPA for approval under 40 CFR part 136

Indicator Bacteria Testing

< Permit Limit

In compliance

> Permit Limit

Evaluate Methods

- Compare methods
- Validate Results

Resolved
< Permit
Limit

Comparison studies

- Incubation temperature
- MF vs MPN

Unresolved > Permit Limit

Validation studies

- Target species verification
- Non-target interferences

Weight of Evidence - No Sanitary Source

Sanitary Surveys

- Dye/chemical tracers

No Sanitary Source?

Source Tracking

qPCR genetic markers

- Human
- Wildlife
- Indicator species ID

Human Markers
Found

No Human
Markers Found

Species Identification

- Biochemical tests
- qPCR species ID markers

Natural Species/Markers
Identified

Mitigate or Disinfect

Sanitary
Source Found



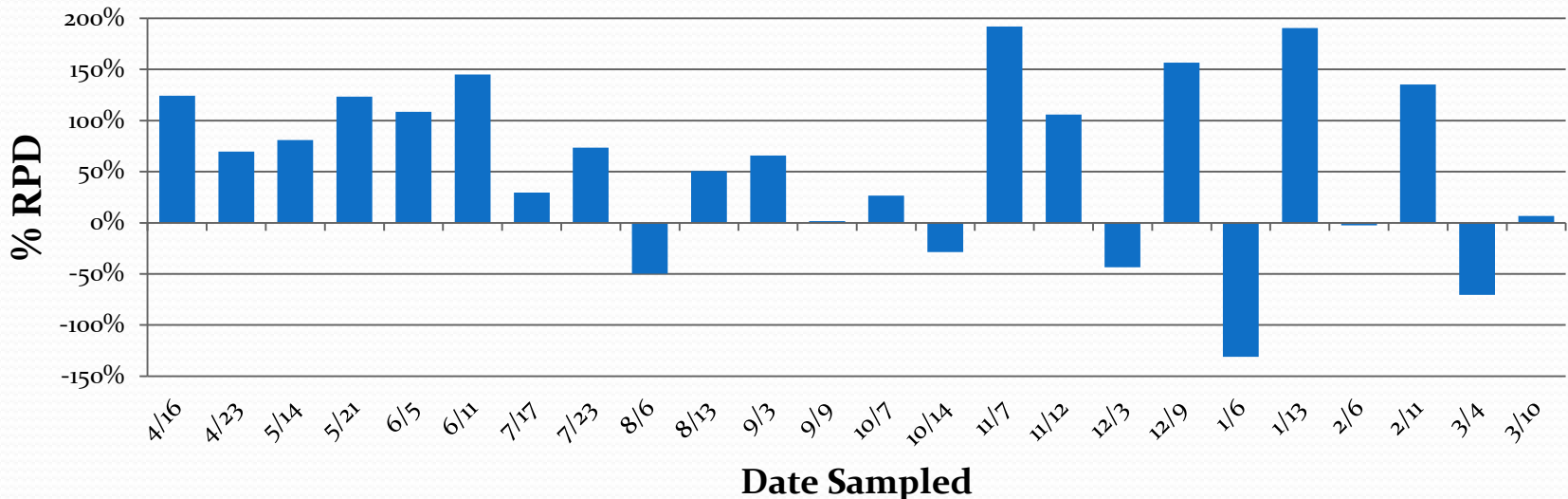
Enterococci Method Bias

Case Study 1 –Method comparison & species identification

- 8160 MPN/100mL by Enterolert & 84 CFU/100mL by EPA Method 1600 (n=1)
- Vitek biochemical species testing from Enterolert positive wells
 - Sphingomonas paucimobilis* & *Chryseobacterium indologenes*
- EPA 1600 MGP biochemical species tests - enterococci <10 - 20 CFU/100 mL (n=7)
 - 43% *E. cassiliflavus* or *E. gallinarum* & 57% *E. faecium* or *E. faecalis*

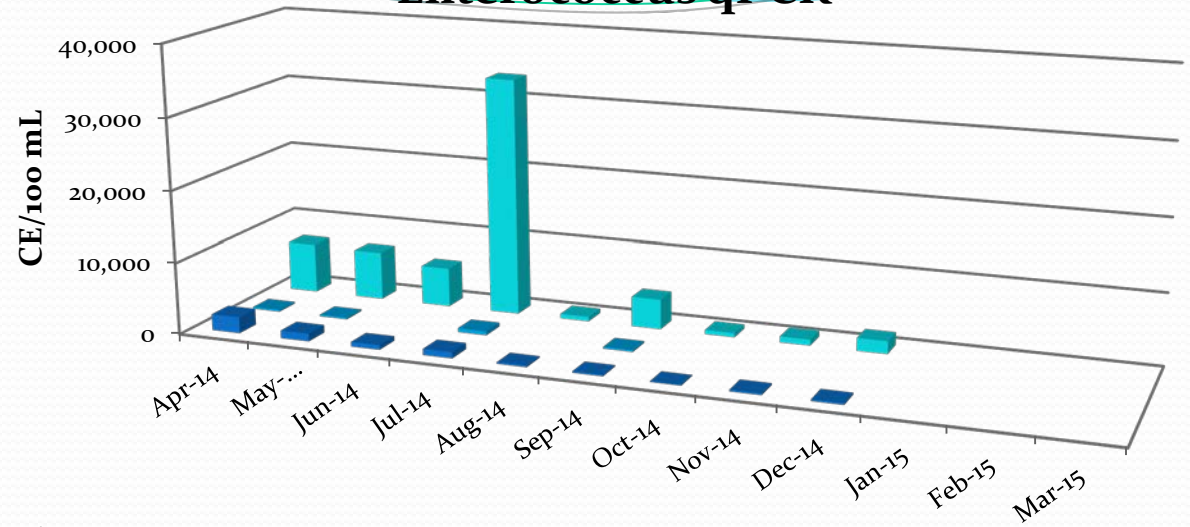
Case Study 2 – Method comparison - higher enterococci levels by Enterolert compared to EPA 1600 membrane filtration in 19 of 24 effluent sampling episodes

Enterolert vs EPA 1600



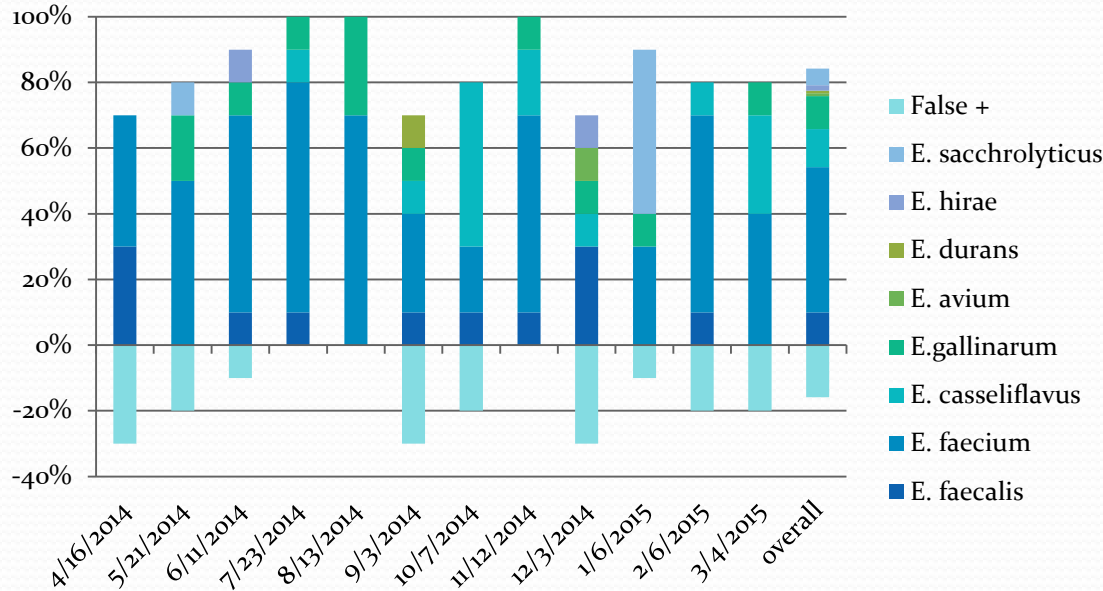
Case Study 2 – Species Testing

Enterococcus qPCR



Biochemical species tests

EPA Method 1600 Typical Colony Identification



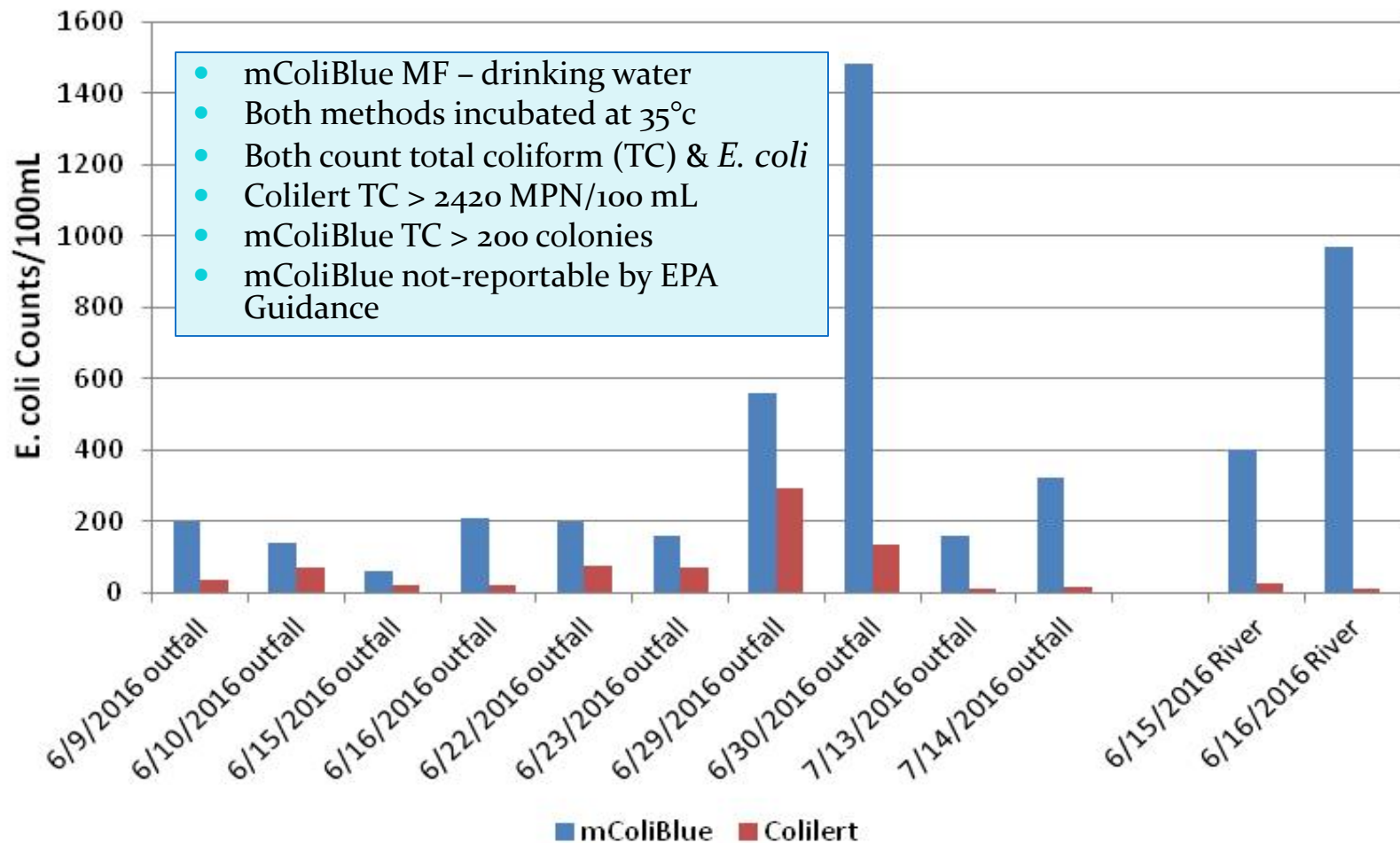
- 12 monthly effluent sampling events
- Broad species diversity
- Species associated with plants and wildlife
- Overall 18% false positive rate

Case Study 2 - Enterococci

- Study Components
 - Compared indicator test methods
 - Species testing showed *E. cassiliflavus* and other ubiquitous species
 - qPCR human markers not detected
 - Other human pathogen strains not detected
- Regulatory Outcome
 - State dropped enterococci monitoring requirement
 - Required testing every two years for human markers

E. Coli - mColiBlue MF vs Colilert (mill support)

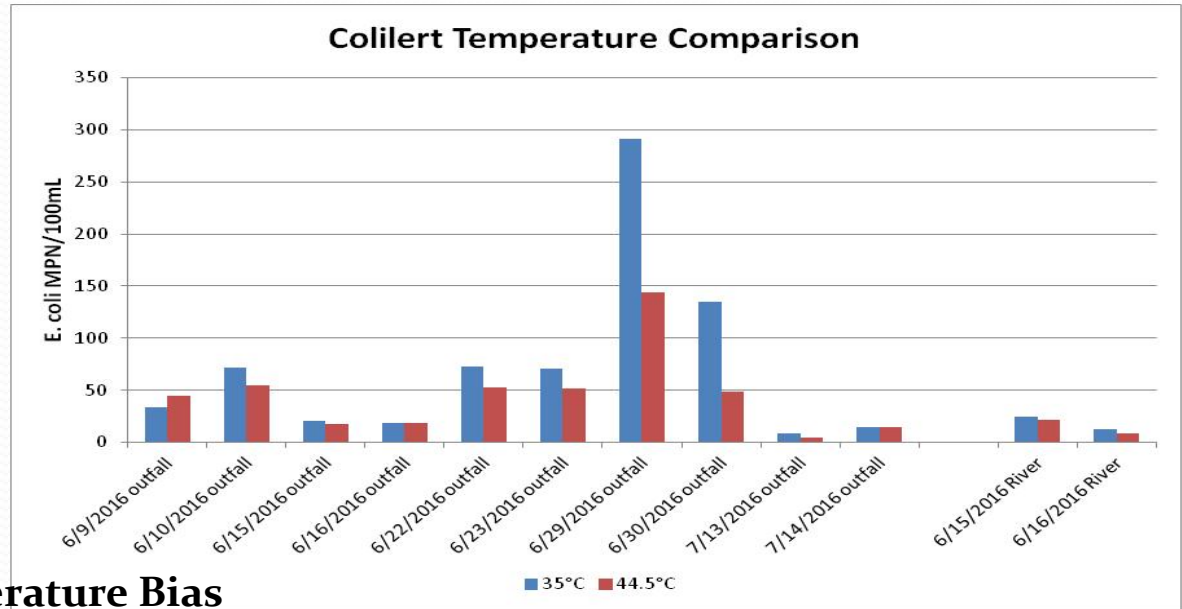
E. coli Method Comparison



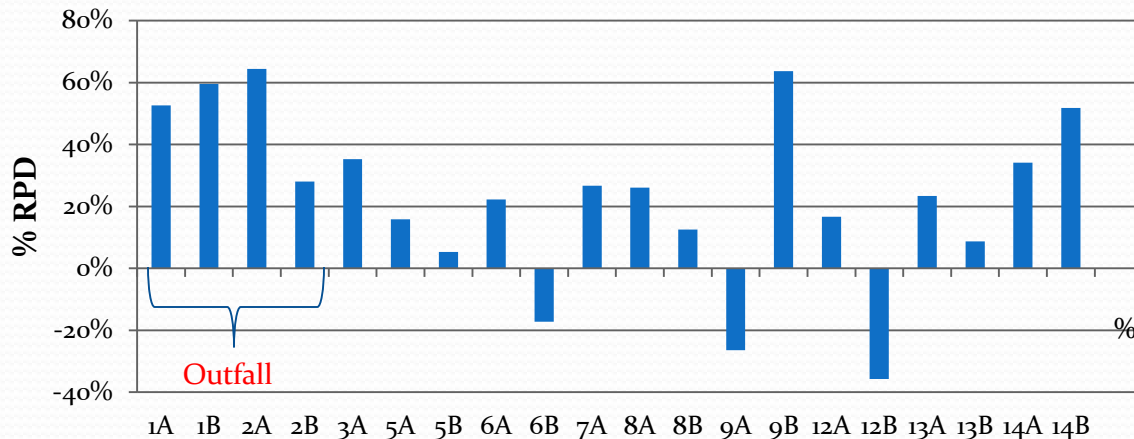
Incubation Temperature Studies

TC & *E. coli* @ 35°C incubation, FC & *E. coli* @ 44.5°C incubation

Mill support – 11/12 effluent & river water samples had higher *E. coli* @ 35°C than 44.5°C



Colilert® Incubation Temperature Bias

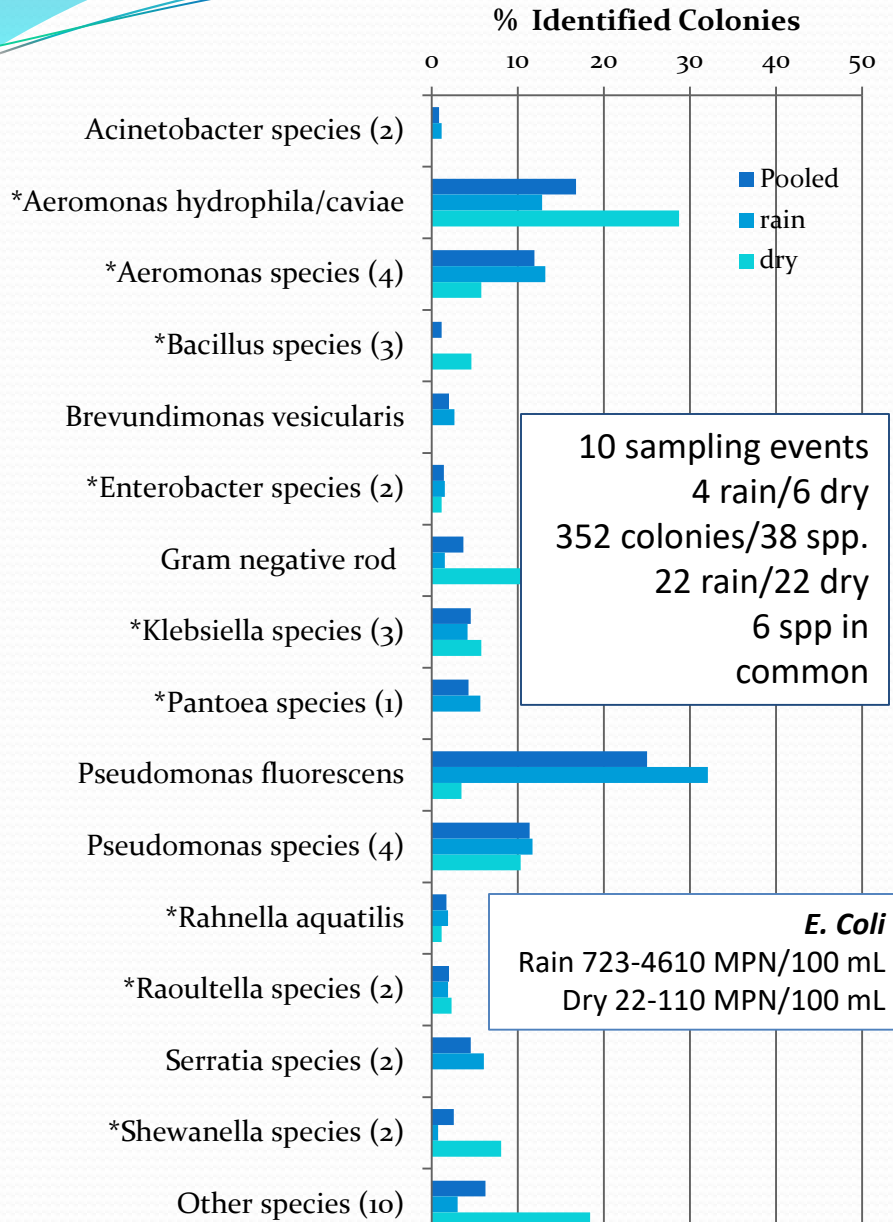


Case Study 3 – 17/20 sample locations had higher *E. coli* @ 35°C than 44.5°C

$$\%RPD = \frac{35^{\circ}\text{C count} - 44.5^{\circ}\text{C count}}{\text{Average count}} \times 100$$

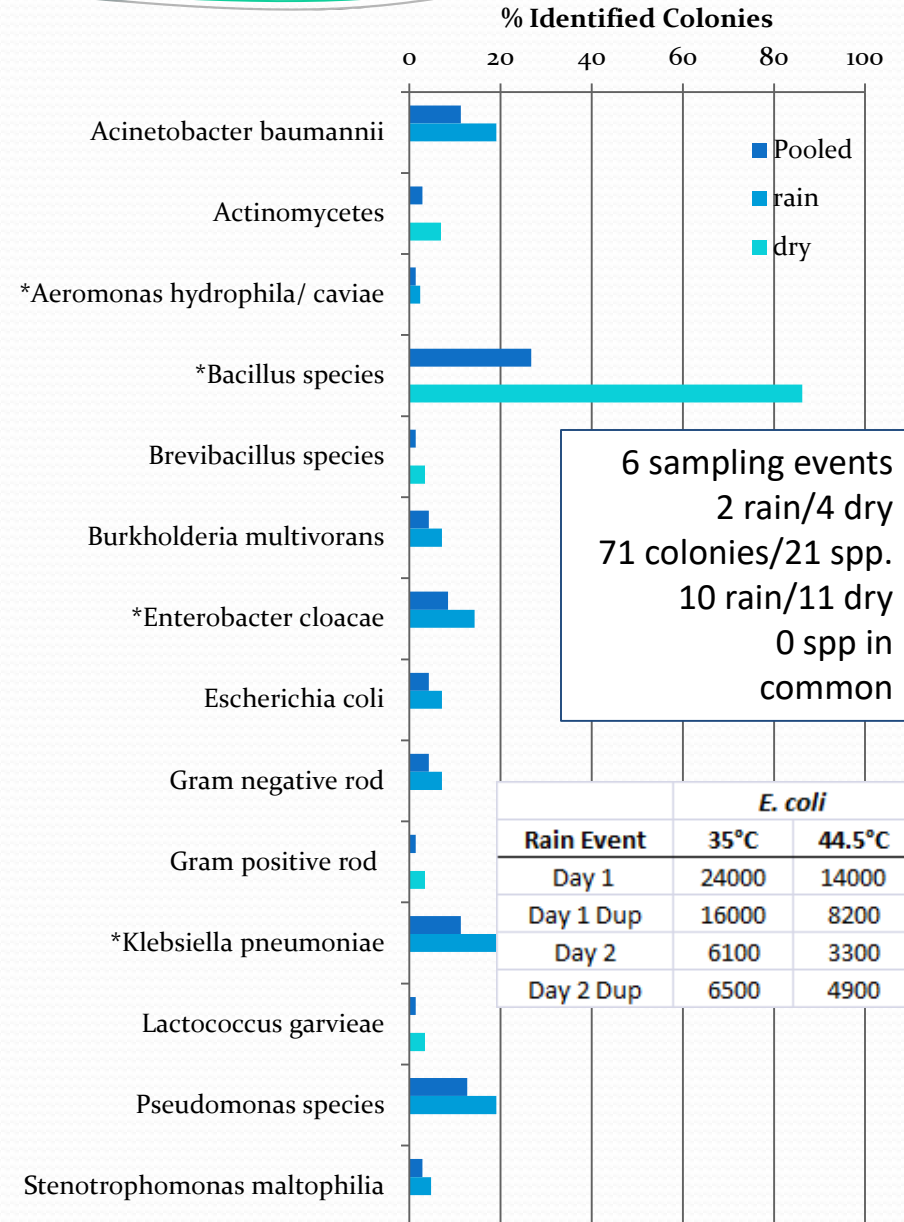
Outfall Species Diversity 35°C

Outfall Species Diversity 44.5°C



10 sampling events
4 rain/6 dry
352 colonies/38 spp.
22 rain/22 dry
6 spp in common

E. Coli
Rain 723-4610 MPN/100 mL
Dry 22-110 MPN/100 mL



6 sampling events
2 rain/4 dry
71 colonies/21 spp.
10 rain/11 dry
0 spp in common

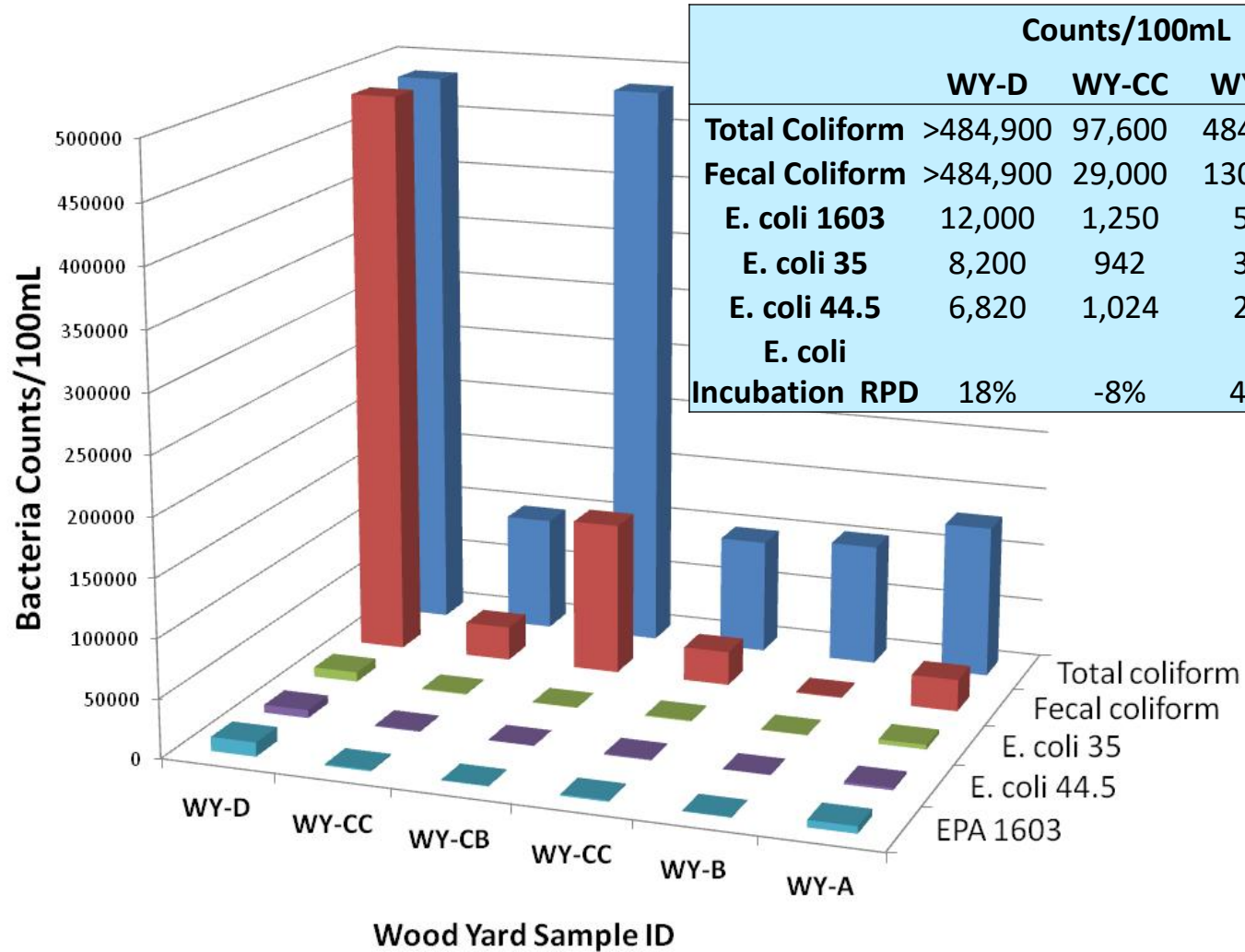
	<i>E. coli</i>	
Rain Event	35°C	44.5°C
Day 1	24000	14000
Day 1 Dup	16000	8200
Day 2	6100	3300
Day 2 Dup	6500	4900

* Indicates species that cause false positive *E. coli* results

Case Study 3 - Storm water *E. coli*

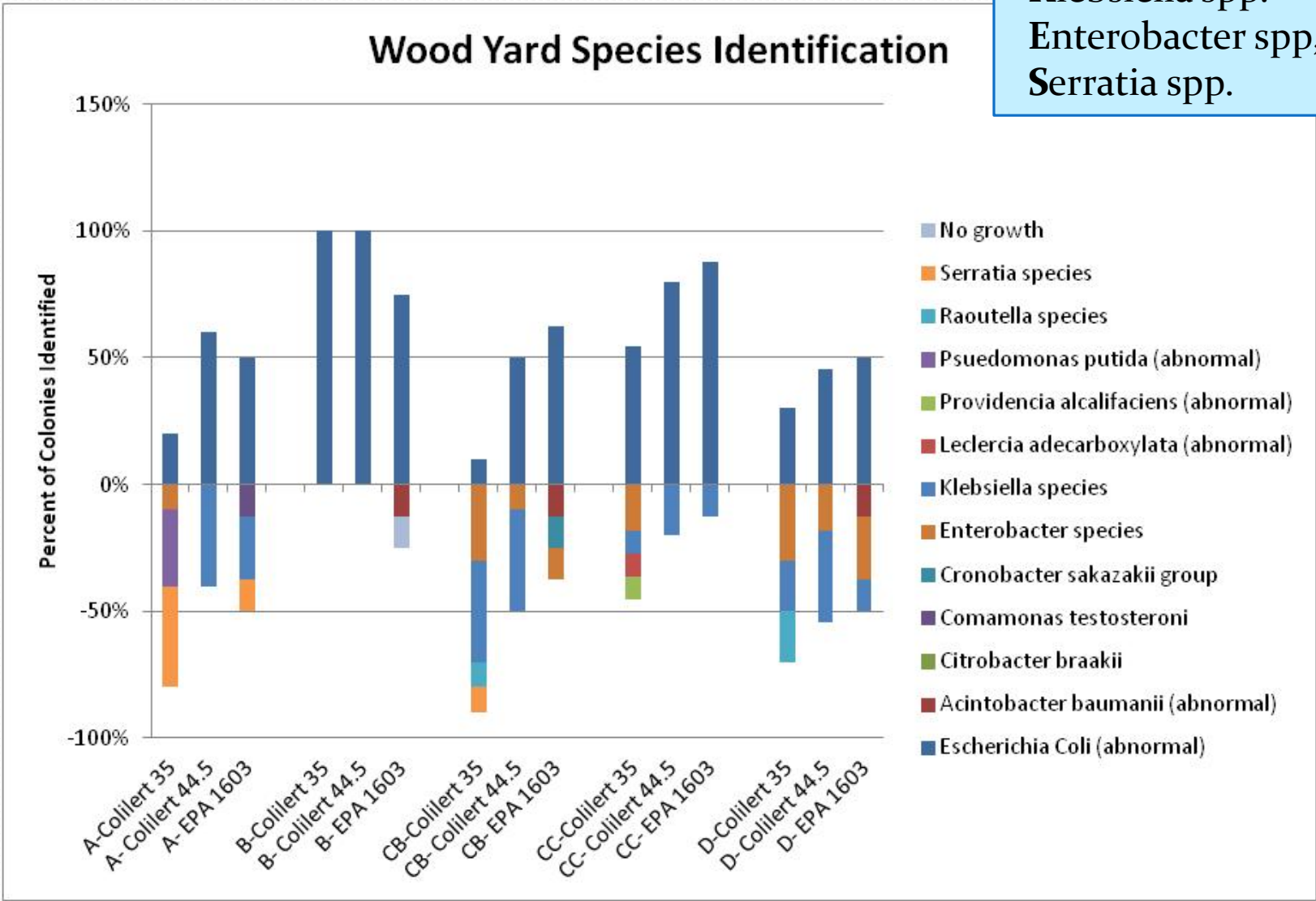
- Study Components
 - Colilert® incubation temperature bias study
 - Species testing identified interfering bacteria
 - qPCR human markers traced sanitary sources
 - High *E. coli* in wood yard runoff
 - human marker testing suggests no sanitary source
 - species testing shows interferences (NCASI wood yard study)
- Regulatory Implications
 - Facility is remediating sanitary issues
 - May not be able to meet limit due to *E. coli* in wood yard runoff – hope continued human marker testing will demonstrate *E. coli* has no sanitary links to wood yard

Indicator Levels in Wood Yard Runoff



Wood Yard Species Diversity

Common WY species.
 Klebsiella spp.
 Enterobacter spp,
 Serratia spp.



Key Case Study Findings

- Sources
 - Infrastructure failures common source of indicator bacteria
 - Mixed recycled furnish a highly variable source
 - Wood yard runoff a contributor of indicator bacteria
- Indicator Test Methods
 - Indicator tests don't differentiate sources
 - Incubation temperature biases Colilert® *E. coli* results
 - Interferences bias indicator test results
- Species testing
 - *Enterococcus casseliflavus* is a predominant species of enterococci in mill effluents which is often associated with plant sources
 - Indicator species testing useful for identifying interferences or confirming species identification to locate sources
- Human Bacteroides genetic markers useful for isolating or ruling out sanitary sources or connections

Tracking Regulatory Developments

- EPA approval of Colilert® at higher incubation temperature for thermotolerant (fecal) species expected in 2017 update to 40CFR, part 136
- Coliphage bacteria
 - To be proposed in 2017 USEPA RWQC update
 - Replacement or in-addition to indicators is unknown
 - Proposed water quality limit is unknown
 - Presence in mill effluents is unknown
 - NCASI initiating preliminary assessment in effluents
- Continuing to track State regulatory updates

NCASI RESOURCES

- TB No. 905 Bacteriological Assessment of Pulp and Paper Mill Effluents, Sept. 2005
- TB No. 971 Assessment of Test Methods for Enumeration of *Escherichia Coli* (*E. coli*) and Enterococci in Pulp and Paper Mill Wastewaters, Dec. 2009
- Summary of RWQC for Indicator Bacteria and Case Studies – Technical Bulletin No. (in-print)
- Wood Yard Runoff Study Report is in progress
- Terry Bousquet – 541-752-8801 ext 321
tbousquet@ncasi.org