

QPCR: A Rapid Method to Monitor Water Quality at a National Park

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Introduction

- Sleeping Bear Dunes National Lakeshore (SLBE): culture-based *E. coli* for monitoring beaches.
- *E. coli* levels are generally low at the park's popular recreational beaches.
- In the Great Lakes, beach managers desire analytical methods that reduce the time-lapse between sample collection and results:
 - ❖ Molecular methods (qPCR)
 - ❖ Predictive modeling
- SLBE is the first National Park (in Midwest) to evaluate qPCR as a rapid, alternate method for beach monitoring.

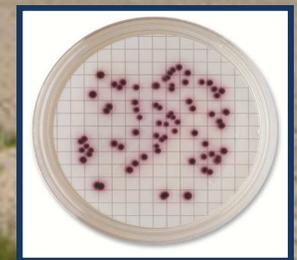
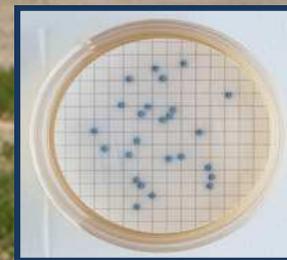
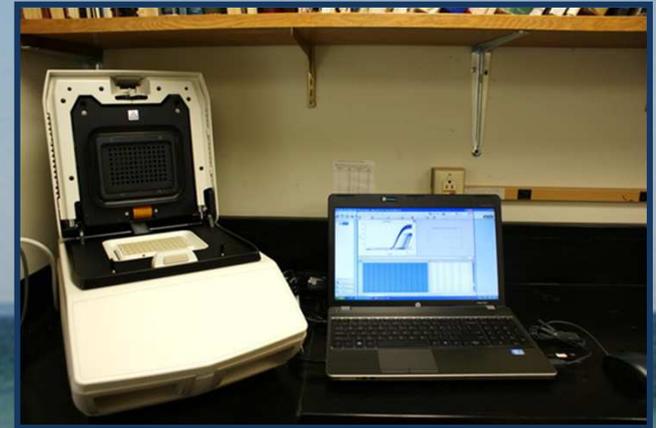


Mean *E. coli* (CFU/100 mL)
during 2001-2013:

- ❖ Esch Road: 59
- ❖ Otter Creek: 103
- ❖ Platte Point Bay: 31
- ❖ Platter River: 25

Study Objectives

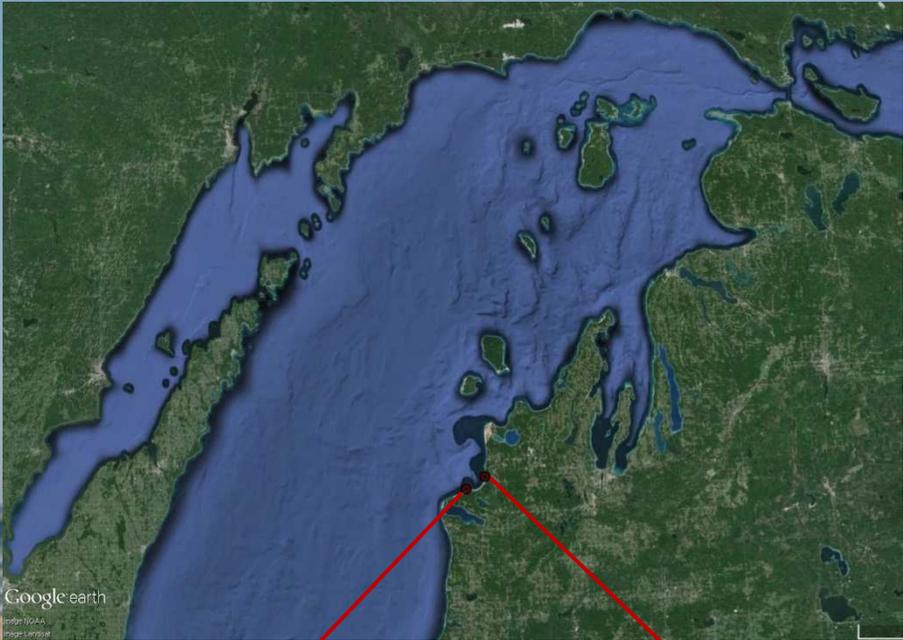
- USGS, in collaboration with SLBE, evaluated qPCR as a potential alternate method for monitoring water quality at select bathing/recreational sites in the summer of 2014.
 - qPCR performance was compared with culture-based method, using enterococci as the indicator bacteria.



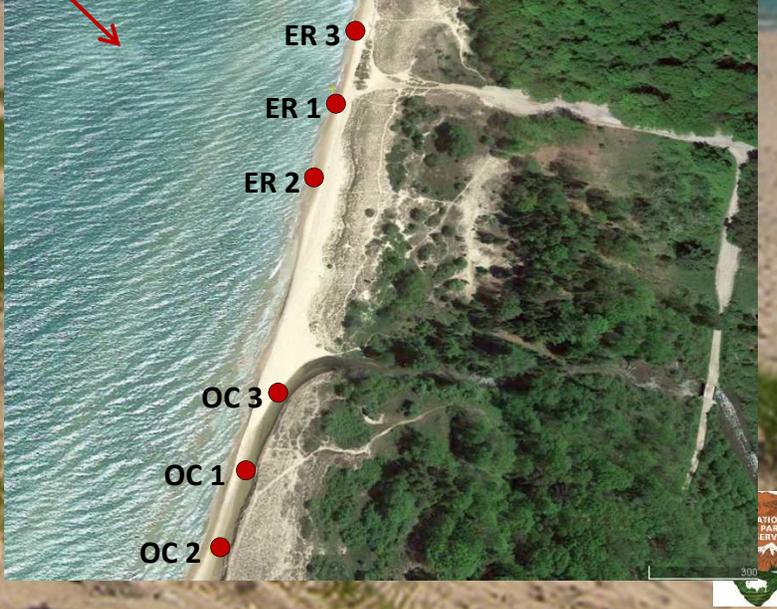
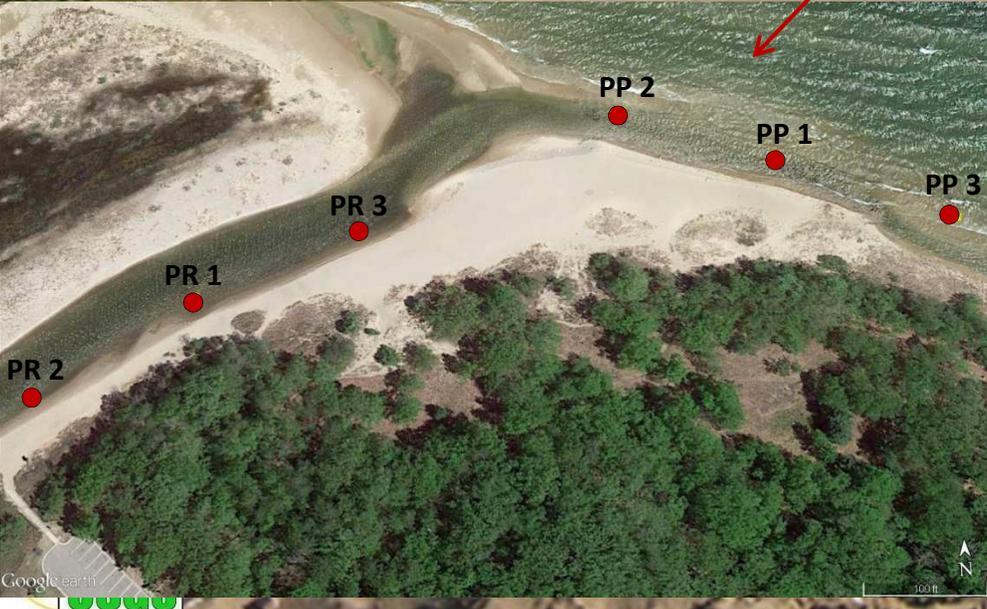
Study Area: Two Beaches and Their Adjacent Outfalls



**Platte Point Bay (PP)
and Platte River (PR)**



**Esch Road (ER) and
Otter Creek (OC)**



Methods

➤ Sampling:

- Seven weeks: 8/4/14 to 9/18/14
- Intensive sampling (ER and OC): On select days (n=12), 5 replicate samples collected

➤ Microbiological Analysis:

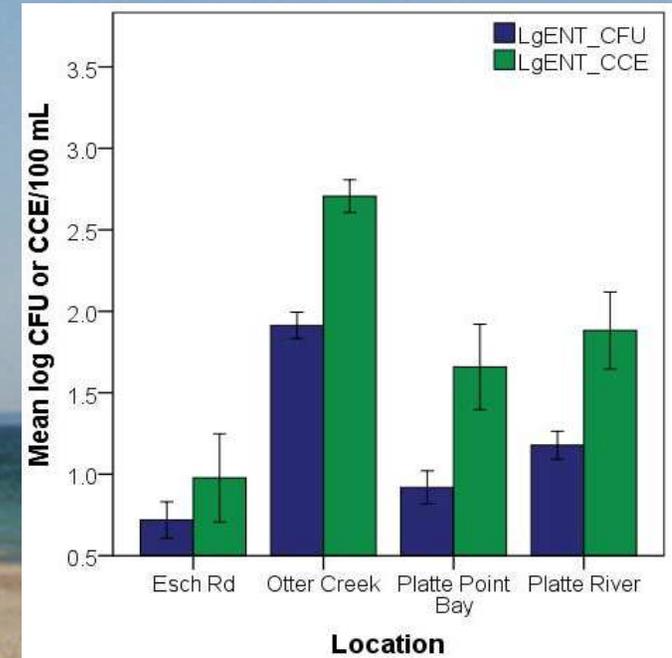
- Culture-based: *E. coli* (EC) and enterococci (ENT) by membrane filtration, MF (counts expressed as CFU)
- Molecular (qPCR): ENT (Modified EPA Method 1611); calibrator cell equivalents, CCE



Culturable and qPCR ENT Results Were Variable

Location	Mean (\pm 1 SD)		
	Log EC _{CFU}	Log ENT _{CFU}	Log ENT _{CCE}
ER	0.37 (0.430)	0.75 (0.470)	0.98 (1.12)
OC	1.59 (0.415)	1.91 (0.343)	2.71 (0.422)
PP	0.66 (0.482)	0.92 (0.430)	1.66 (1.12)
PR	0.93 (0.523)	1.18 (0.381)	1.88 (1.04)

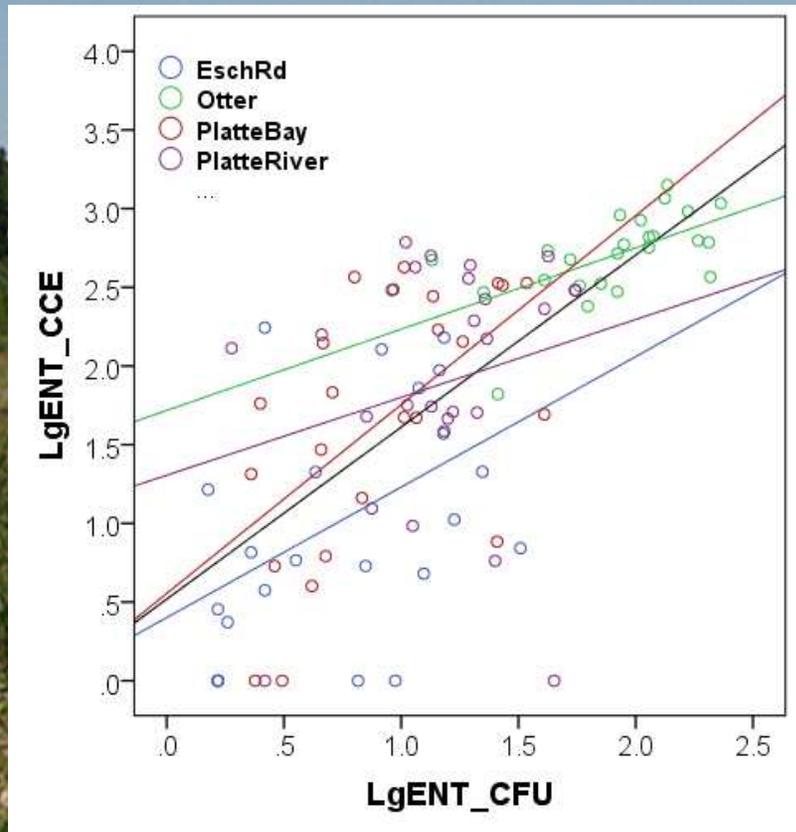
- CCE was significantly higher than CFU at all locations, except Esch Rd
- Otter Creek CFU and CCE was significantly higher than other locations



River vs Beach:

- Otter Creek CCE and CFU significantly higher than Esch Rd
 - Otter Creek and Esch Rd CFU were correlated
- Platte River CFU significantly higher than Platte Point Bay
 - Platte River and Platte Point Bay CFU were correlated
- No CCE correlations

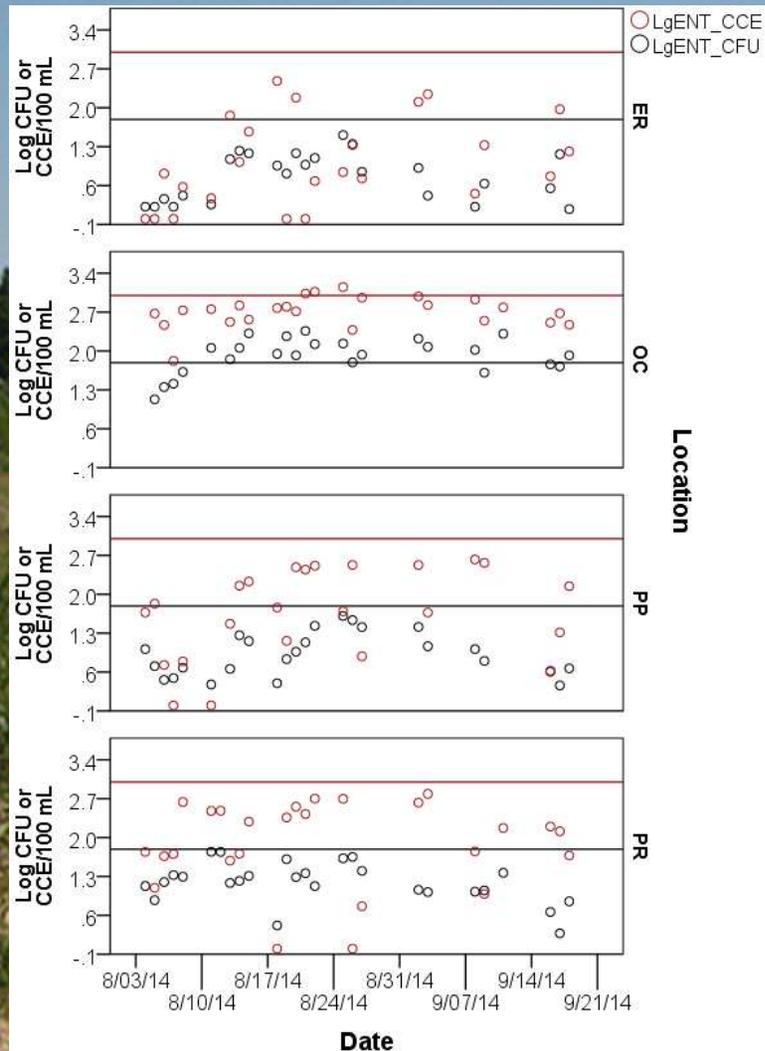
Enterococci CFU and CCE were Significantly Correlated*



Location	R ²	Pearson correlation
Overall	0.471	R=0.686** , P< 0.000, N=98
Esch Rd	0.195	R=0.441* , P= 0.031, N=24
Otter Creek	0.350	R=0.592** , P= 0.002, N=24
Platte Bay	0.326	R=0.571** , P= 0.004, N=24
Platte River	0.054	R=0.231, P= 0.255, N=26

***Indicated that enterococci qPCR was measuring the same target as the culture-based method, albeit rapidly and in real-time.**

Fewer Water Quality Exceedances Based on ENT_{CCE} U.S. EPA Criteria



Horizontal lines represent EPA standard

- All exceedances occurred at Otter Creek :
- Fewer exceedances with CCE relative to CFU
 - CFU=16% (16/98)
 - CCE=3% (3/99)



Intensive Sampling: 4 (OC)-22 (ER) Samples were Needed for a 70% Precision with ENT Measured by qPCR

Power analysis

Location	ENT _{CFU}			CCE _{CCE}			EC _{CFU}		
	CV=0.2	CV=0.3	CV=0.4	CV=0.2	CV=0.3	CV=0.4	CV=0.2	CV=0.3	CV=0.4
ER	5	2	1	49	22	12	9	4	2
OC	3	1	1	8	4	2	5	2	1

- CCE more variable than CFU
- EC_{CFU} more variable than ENT_{CFU}
- Esch Rd more variable than OC

Number of samples required to attain 70% precision



Location	ENT _{CFU}	ENT _{CCE}	EC _{CFU}
Esch Rd	2	22	4
Otter Creek	1	4	2



Inhibition and Non-Detects

CCE non-detects	Questionable data
30% (117/392)	3% (12/392)

- qPCR non-detects (30%): No Target Ct, probably due to low ENT_{CFU} densities and/or target DNA
- Questionable data (3%): Unresolved inhibition
 - Technological improvements can minimize this problem, but PCR inhibition cannot be totally eliminated

Conclusions

- ENT results from MF and qPCR methods were correlated
- Low ENT targets (viable cells, DNA) and inhibition could affect qPCR results
- There were fewer water quality exceedances based on ENT results from qPCR : MF (17), CCE (3)
- Take-home message: ENT qPCR may be a viable, alternate method for monitoring water quality at SLBE beaches.



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qPCR Training Fun



Thank You!



Photo credit: Ed Reschke

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