A Preliminary Study of the Sources of *E. coli* Contamination at Marquette Park Beach by Random Amplified Polymorphic DNA Typing

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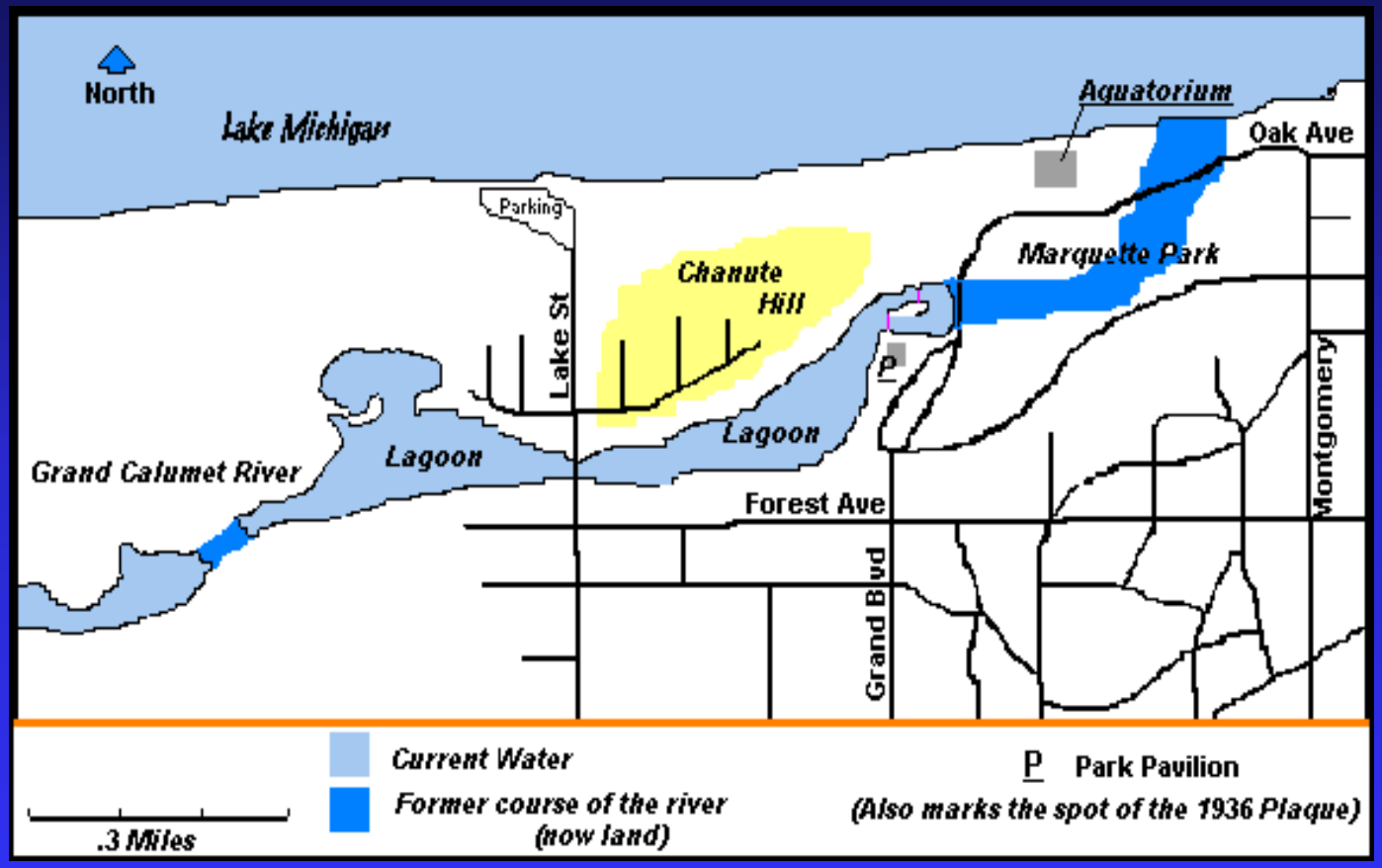
Background

- Beaches of south Lake Michigan are often closed due to high *E. coli* counts.
- It is suspected that combined sewer outflow (CSO) is a major contributing factor to the elevated *E. coli* counts after rainfall.
- City of Gary is interested in finding out whether the beach water is polluted by Gary sewage.

Marquette Park Beach



Marquette Park Beach

Objective

- The primary objective was to determine the impact of Gary sewage on the *E. coli* contamination of Marquette Park Beach water using RAPD fingerprinting.

- The secondary objective was to identify other possible sources of *E. coli* using the existing RAPD fingerprints database.

Materials & Methods

Sample Collection

- Lake water samples were collected from 3 sites on the beach on 2 dry and 2 wet days. Fifty *E. coli* isolates were obtained from each day.

  Dry day – No rainfall occurred > 72 h before sampling (Low risk of fecal contamination)

  Wet day – A CSO occurred < 48h before sampling (High risk of fecal contamination)

- Raw sewage samples were collected from Gary sewage treatment facility. Fifty *E. coli* isolates were obtained from these samples.

(Cont)

RAPD fingerprinting

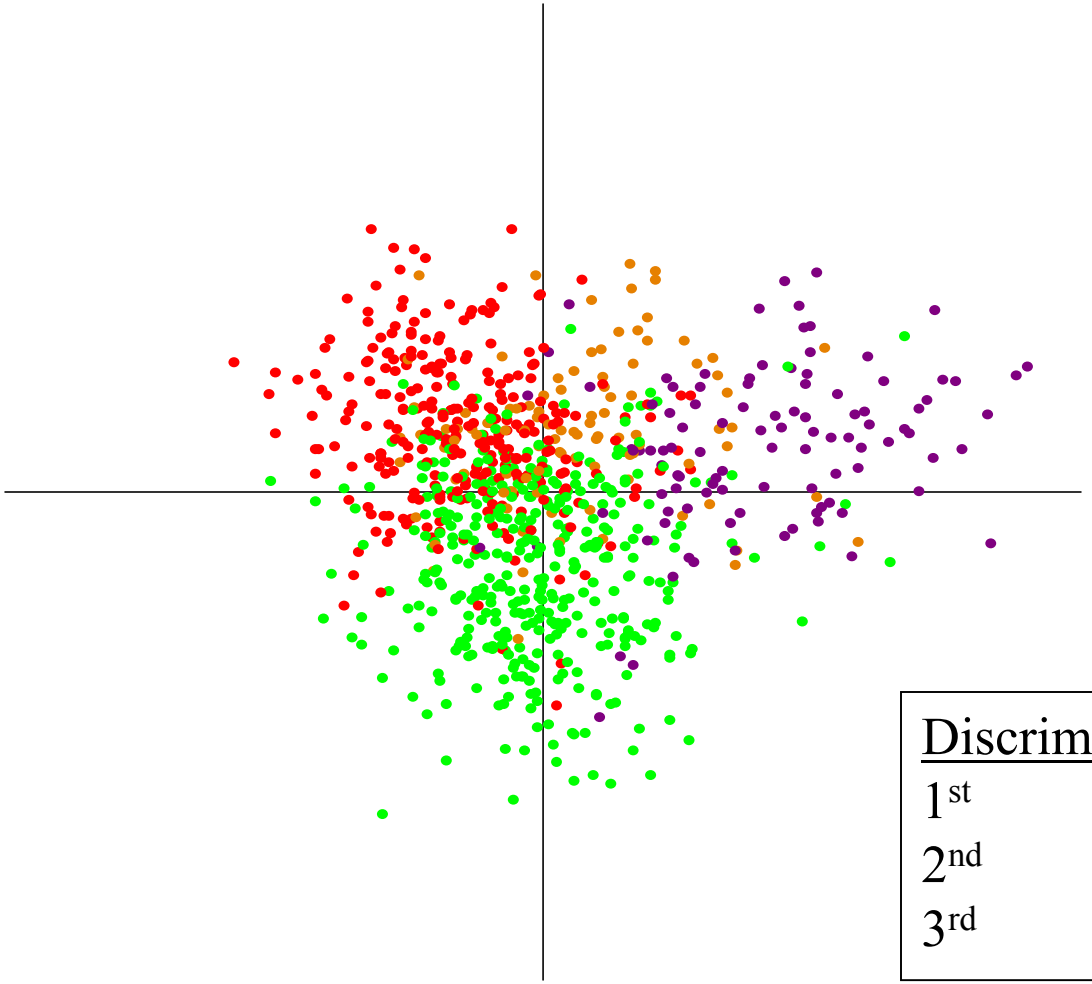
- DNA extraction
- DNA amplification w/ RCR
  (using 3 separate primers)
- Gel electrophoresis
- RAPD pattern documentation
- RAPD pattern analysis – BioNumerics™

Table 1. Identification of the possible sources of *E. coli* isolated from Marquette Park Beach water samples using the RAPD database of PUC.

Possible Source	Dry Day #1	Dry Day #2	Wet Day #1	Wet Day #2
HM				
Fecal or Urine (245)*	6/50	7/50	5/54	4/30
Sewage-Gary (50)	2/50	1/50	1/54	0/30
Other Sewage (44)	2/50	5/50	0/54	0/30
NHM				
Seagull (86)	12/50	5/50	14/54	8/50
Goose (55)	1/50	5/50	1/54	3/30
Farm Animals (140)	13/50	5/50	7/54	4/30
Raccoon, Deer (100)	6/50	3/50	12/54	0/30
No Close Match	8/50	19/50	14/54	11/30

* The numbers of RAPD fingerprints in the database.

Fig.1. MANOVA analysis of RAPD patterns of *E. coli* isolated from HM (●-feces, urine, sewage), NHM (●-feces of cow, horse, pig, goose, seagull, chicken, raccoon, and deer), lake water (●- dry day , ●- wet day).



Discriminant	%	*LP*	(%)
1st	46	0.01975	0.001
2nd	31	0.3967	0.001
3rd	23	0.6642	0.001

Fig. 2 Possible sources of *E. coli* in Marquette Park Beach water samples collected on 2 dry and 2 wet days.

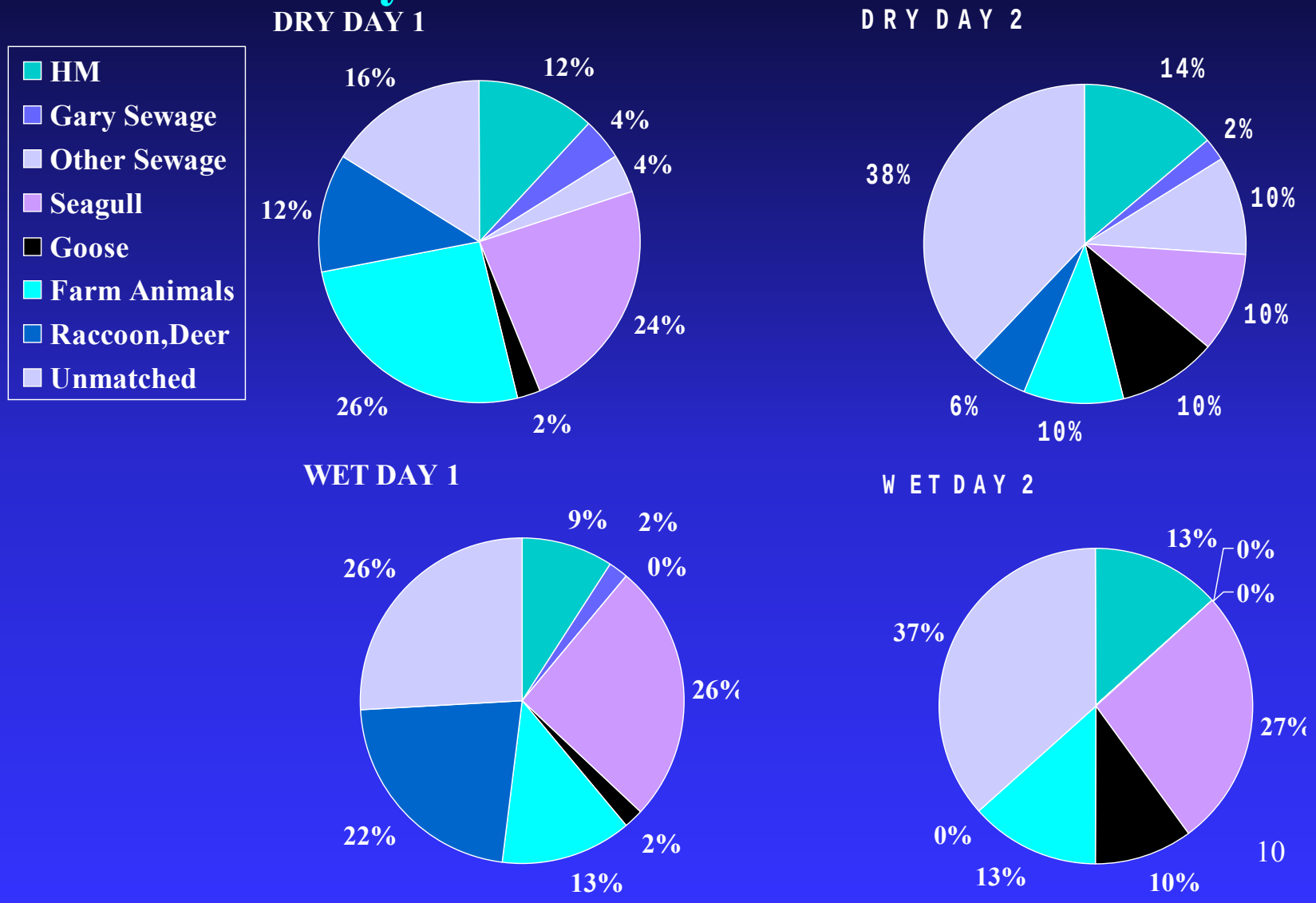


Fig. 3. Possible sources of *E. coli* in all 4 Marquette Park Beach water samples.

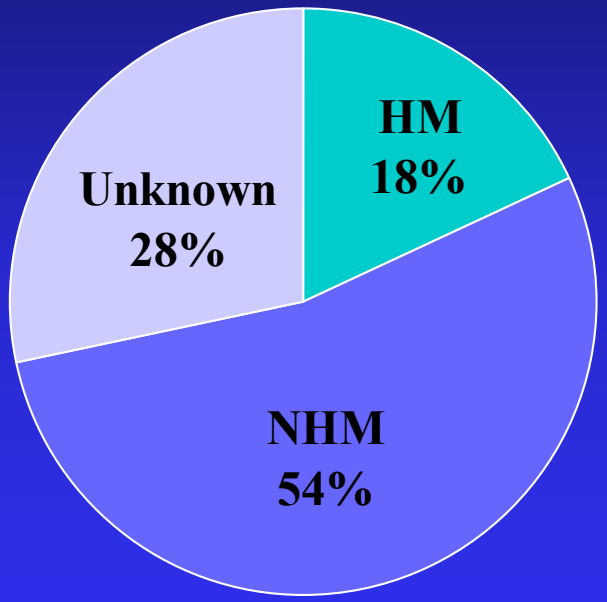
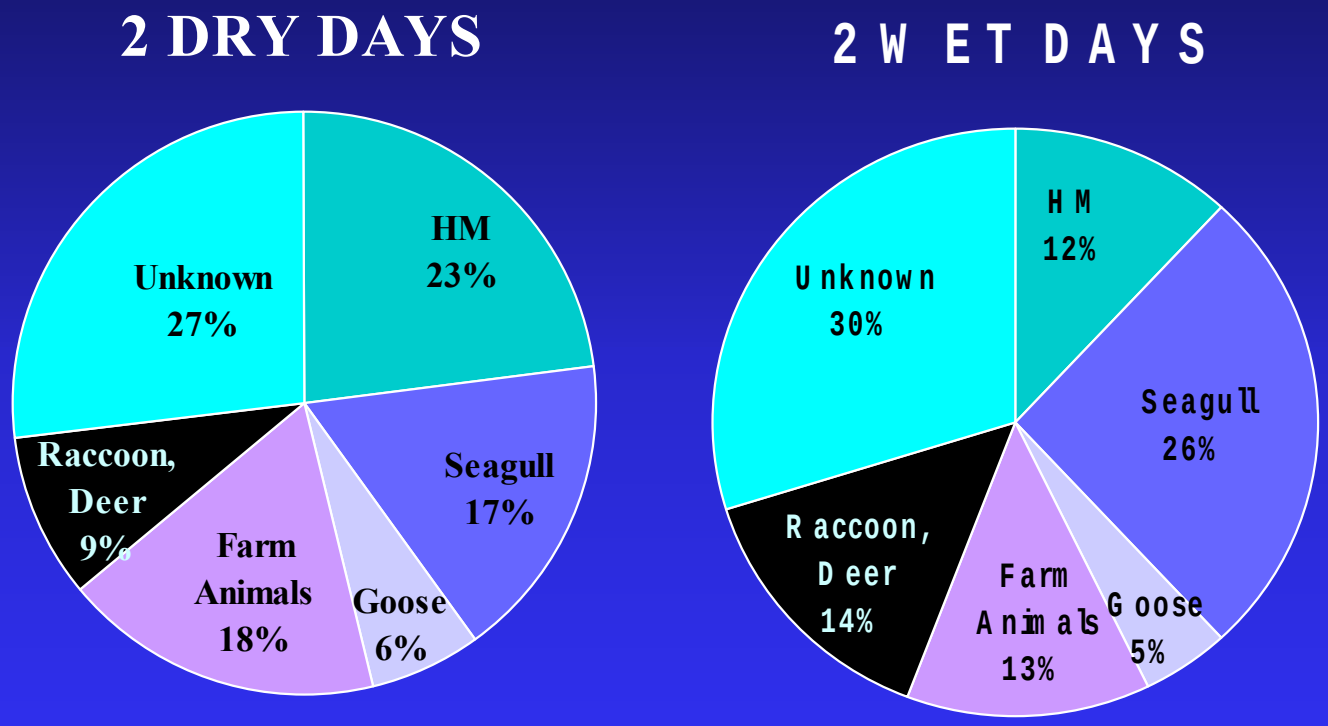


Fig. 4. Comparison of sources of *E. coli* in Marquette Park Beach water samples collected from 2 dry and 2 wet days.



Conclusions

- No evidence indicated that Gary sewage or other sewage was the major source of *E. coli* contamination in the Marquette Beach water samples on the 2 dry and 2 wet days that we analyzed.
- The lake water was contaminated by multiple sources.
- More *E. coli* were from non-human sources than from human sources.
- Among non-human sources, seagull appeared to be an important source of *E. coli* in beach water.

Acknowledgement

- This project is funded by the City of Gary.