

Temporal and Spatial Variation in Genotypic Composition of *Cladophora*-borne *E. coli* Populations in Lake Michigan

Brian D. Badgley, John Ferguson, Amy Vanden Heuvel, Gregory T. Kleinheinz, Colleen M. McDermott, Todd R. Sandrin, Julie Kinzelman, Emily A. Junion, Muruleedhara N. Byappanahalli, Richard L. Whitman, and Michael J. Sadowsky



FIB survive in the environment

- Some indicator bacteria appear to be capable of extended persistence and growth in the environment
- This phenomenon probably violates two important assumptions of indicator bacteria:
 - Environmental decay rates mirror those of pathogens
 - Incapable of reproduction in the environment
- Resuspension of indicators from these reservoirs confounds interpretation of water column concentrations



Previous work in *Cladophora*

- High numbers of *E. coli* and enterococci attached to *Cladophora* filaments
 - Up to 10^6 CFU/g algal tissue
 - Ability of *E. coli* to grow on algal exudates
- *E. Coli* strains in rock-attached *Cladophora* distinct from known sources and vary across sites and years
- Evidence that *Cladophora* biomass correlates with locally elevated concentrations of *E. coli* in the water

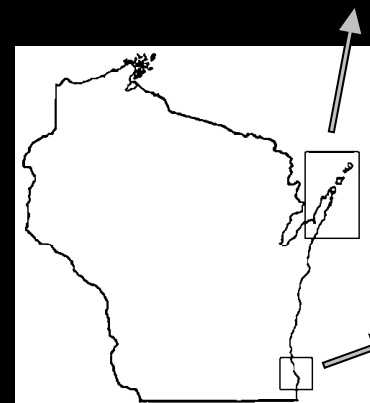
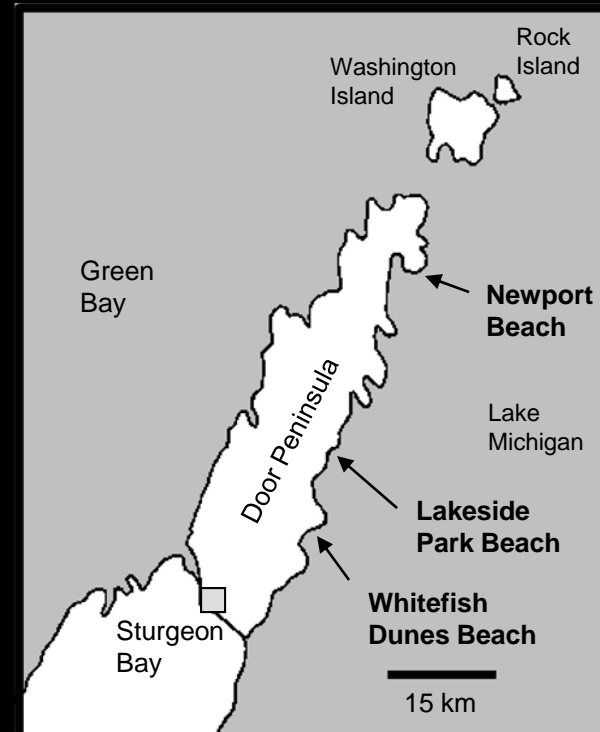


Goals of this study

- Learn more about the ecology of *E. coli* populations attached to free-floating *Cladophora* mats
- Use genetic fingerprinting techniques to examine short and long term changes in the population structure
- Intensive sampling produced a large dataset (4,285 isolates fingerprinted) that is well suited to look at a range of ecologically relevant scales

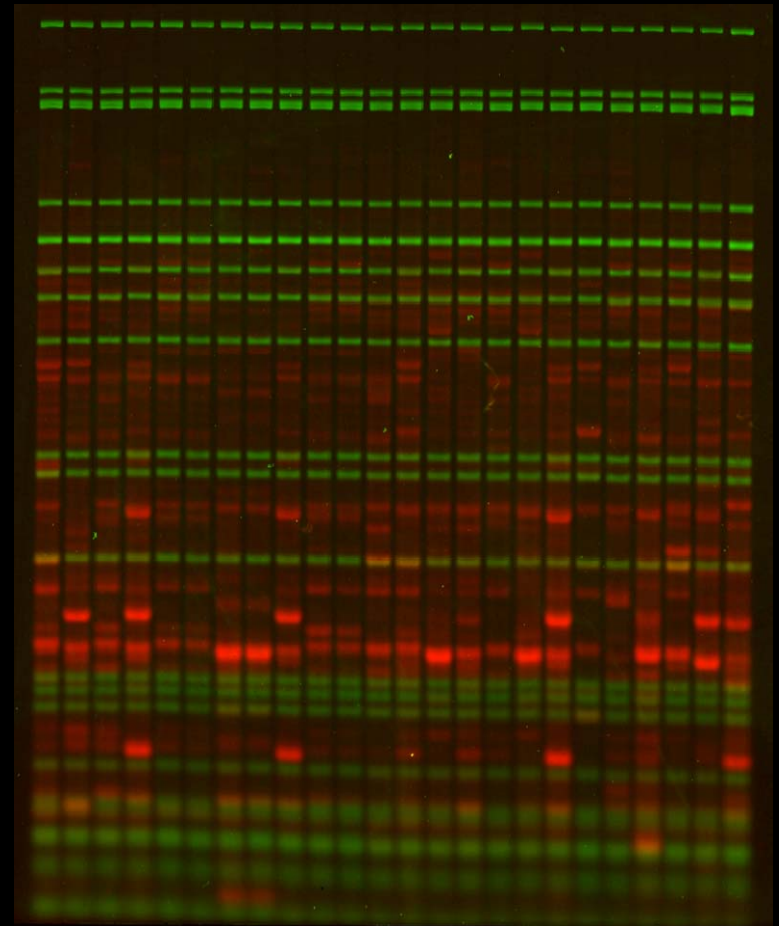
Sampling *E. coli* from *Cladophora* mats

- Four beach sites monitored daily for presence of *Cladophora* mat during summer 2007-2009
- When a mat was present, water and *Cladophora* samples were taken daily for 3-4 days
- Culturable *E. coli* enumerated in each sample, with a subset of isolates from algae saved for fingerprinting



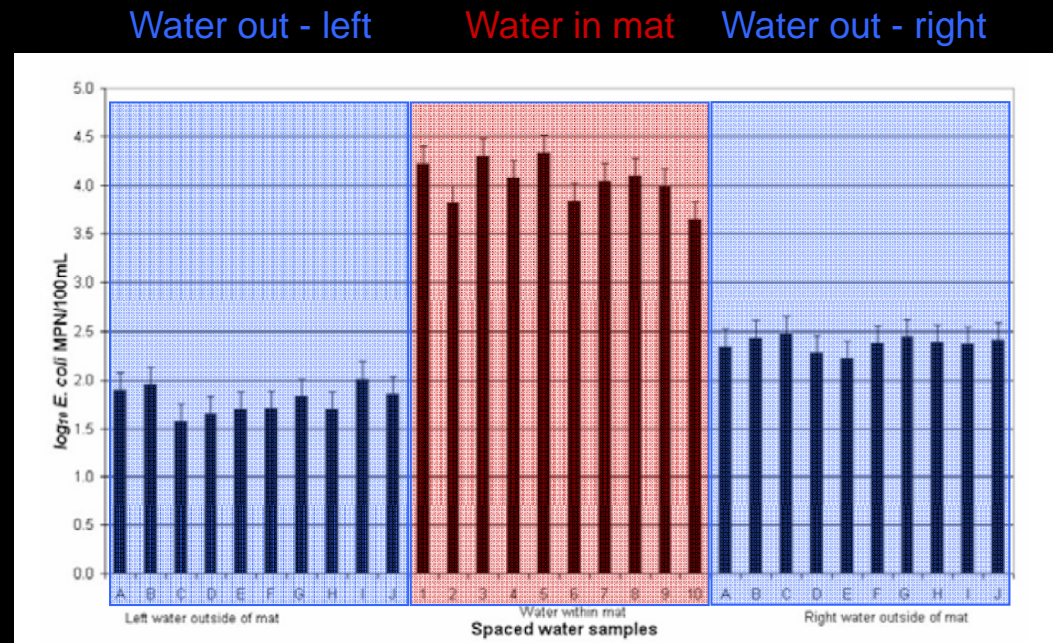
Determining genetic structure of *E. coli* isolates

- PCR amplification of repeating element in the genome (BOXA1R)
- Software calculates similarities amongst all fingerprints
- Investigate differences among groups of isolates to look for patterns



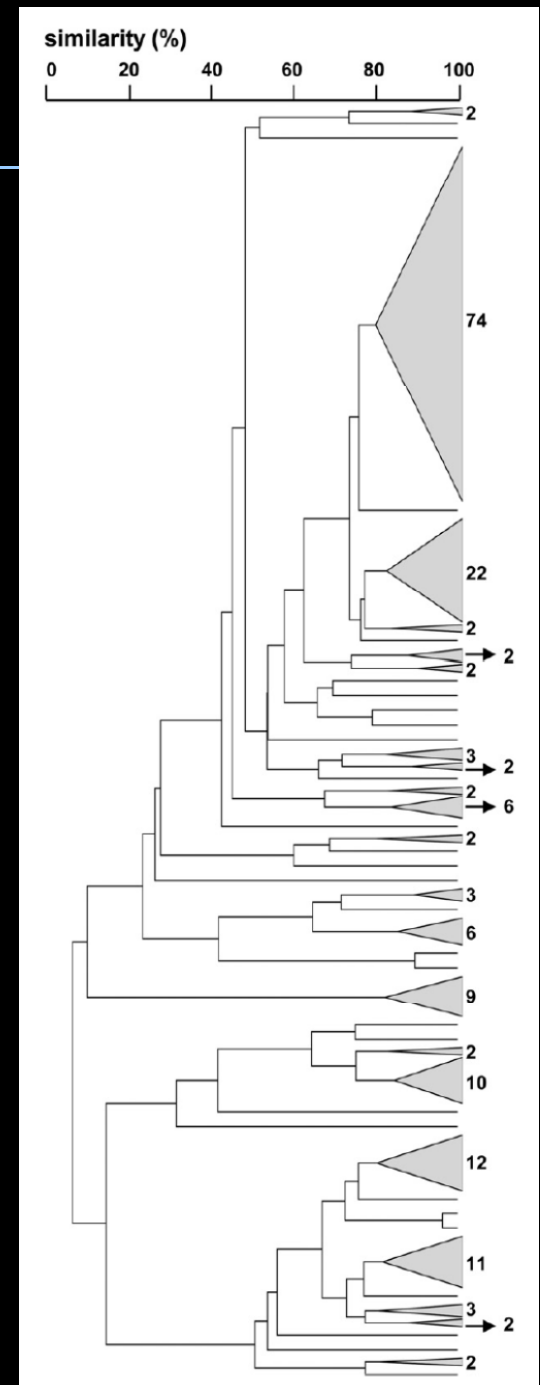
High *E. coli* concentrations associated with *Cladophora*

- Mean concentrations of *E. coli* attached to *Cladophora* ranged from $10^1 - 10^4$ CFU/g
- Mean concentrations in the water ranged from $10^0 - 10^3$ CFU/100 mL
- *E. coli* concentrations in water inside *Cladophora* mats consistently averaged 1-2 orders of magnitude higher than water outside the mats



Complex *E. coli* population structure

- Among Door County isolates, similarity values ranged from 6 to 100%
- 33% of isolates were contained in 55 different clonal groups
 - $\geq 92\%$ similarity
- Four clonal groups contained at least 50 isolates each, from multiple sites and years
 - Suggests possibility of naturalized strains



Interannual genetic variation among all *E. coli* isolates

All Door County

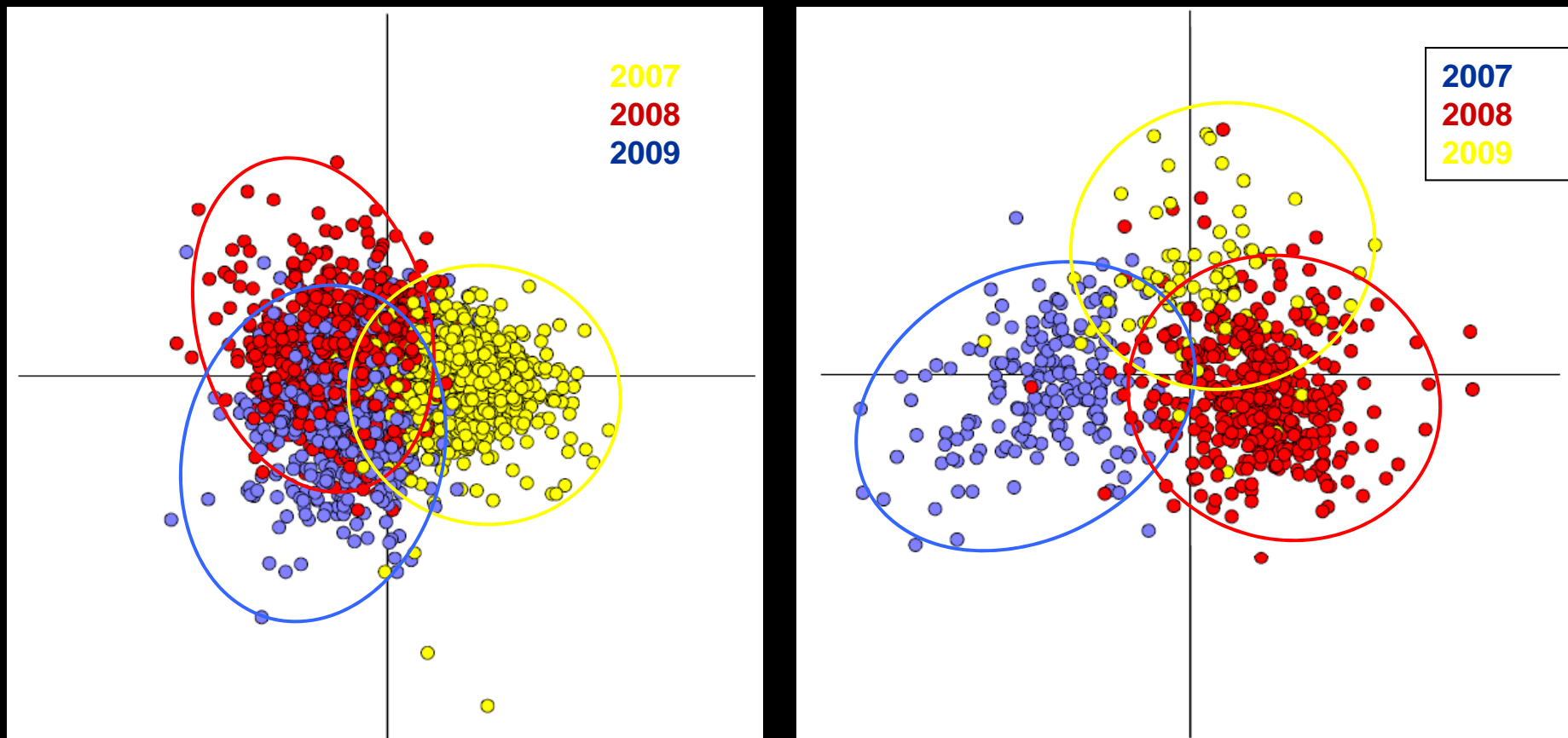
All Racine

2007
2008
2009

2007
2008
2009

second discriminant

first discriminant



Short-term variation in population structure

Whitefish Dunes 2007

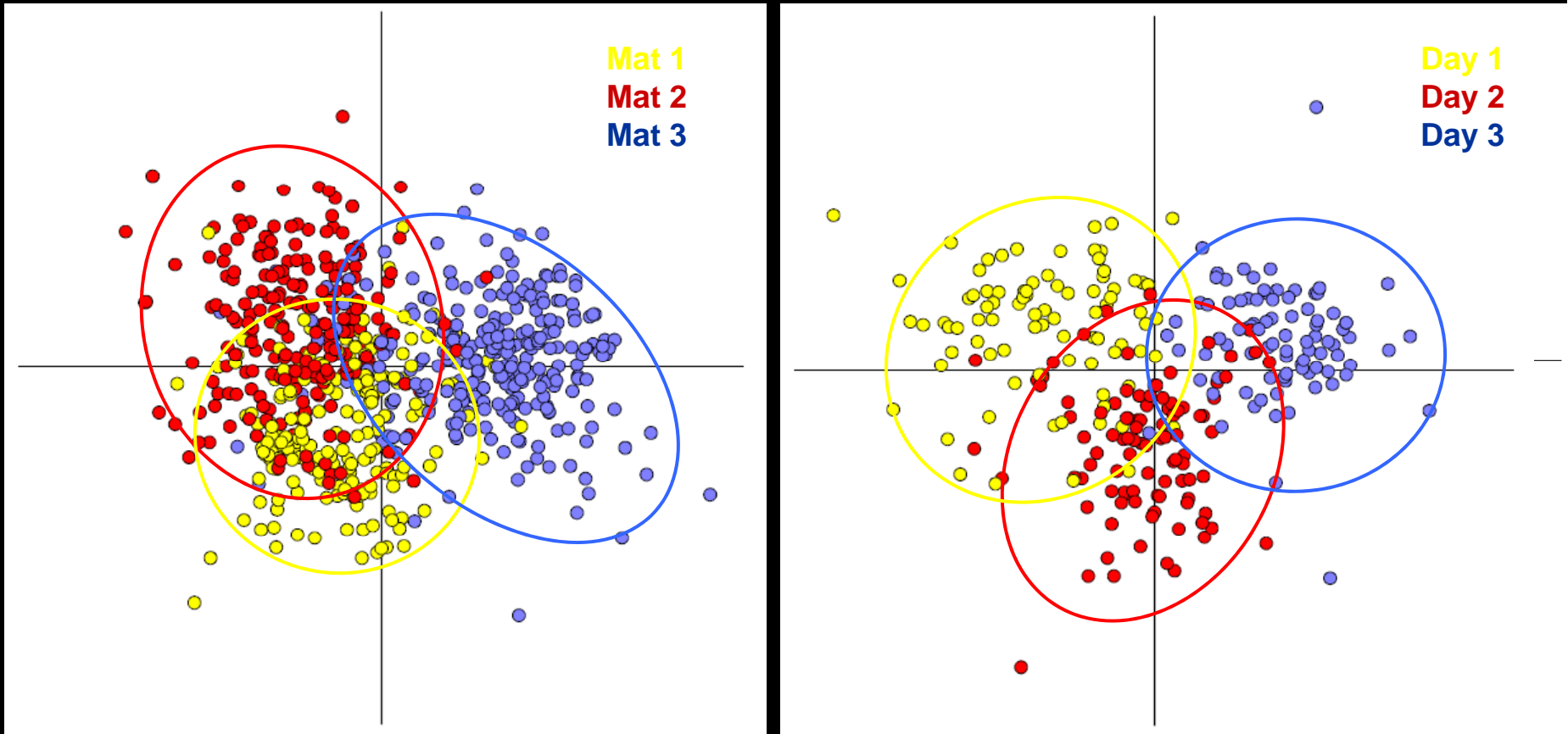
All 2009 Mats

2009 Mat 3

Mat 1
Mat 2
Mat 3

Day 1
Day 2
Day 3

second discriminant



first discriminant

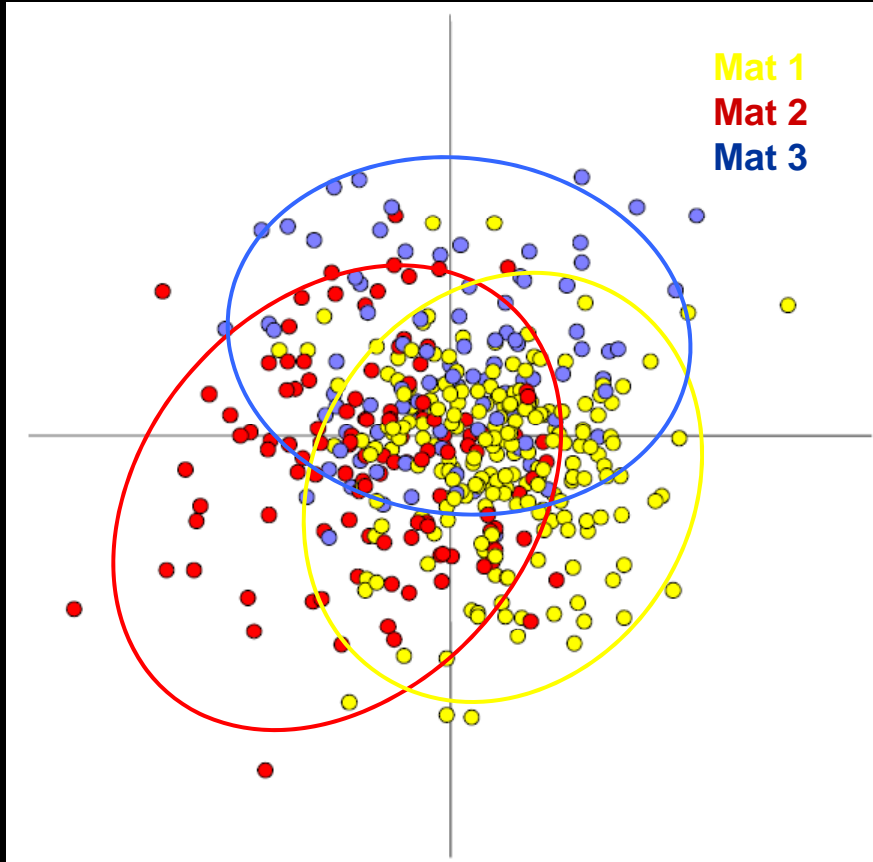
Short-term variation in population structure

Newport 2009

All 2009 Mats

Mat 1
Mat 2
Mat 3

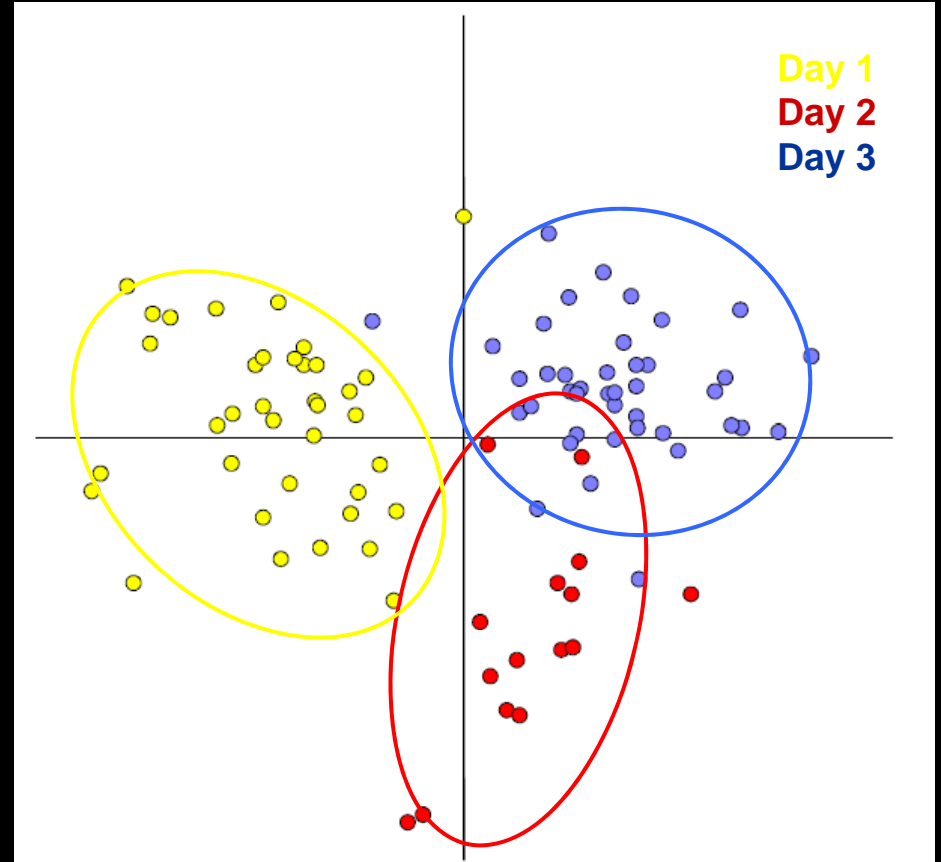
second discriminant



2009 Mat 1 23

Day 1
Day 2
Day 3

first discriminant



Conclusions

- *Cladophora* mats may harbor naturalized *E. coli* that can affect water quality in Lake Michigan
- Population structure varied annually, but also displayed high short-term variability
- These factors, combined with the high level of genetic diversity, limit the use of *E. coli* as an indicator of fecal pollution or sources.

Questions?

