

Identification of *E. coli* Sources in the Conesus Lake Watershed Using PCR

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Introduction

- *E. coli* contamination is a well documented problem around Conesus Lake with several instances of elevated *E. coli* levels in the watershed.
- This is generally thought to be due to agricultural practices in the watershed.
- Focus on two watersheds:
 - Graywood (Experimental watershed)
 - Long Point (Control watershed)
- Using a different approach (PCR) to identify sources of *E. coli* contamination.
- Poor bacterial water quality is event driven.

Question 1

- Which sources (cows, geese, humans) are contributing to bacterial contamination in the Graywood watershed?
 - Quantification (membrane filtration) alone provides little understanding of the particular sources contributing to poor bacterial water quality.
 - PCR (Polymerase Chain Reaction) provides a tool to identify sources of *E. coli* contamination based on genetic fingerprinting.

Question 2

- Will Best Management Practices effectively reduce *E. coli* coming from farms?
 - Isolate *E. coli* from known sources and stream samples.
 - Obtain PCR amplified fragments (genetic fingerprints).
 - Compare fingerprints from source groups with fingerprints from stream samples.
 - Estimate the relative percentages of *E. coli* coming from each source group.
 - Show decreasing trend in *E. coli* contamination from cattle.

Question 3

- Do sources of bacterial contamination differ during event and non-event periods?
 - Which source (cows, geese or humans) contributes the most during event periods?

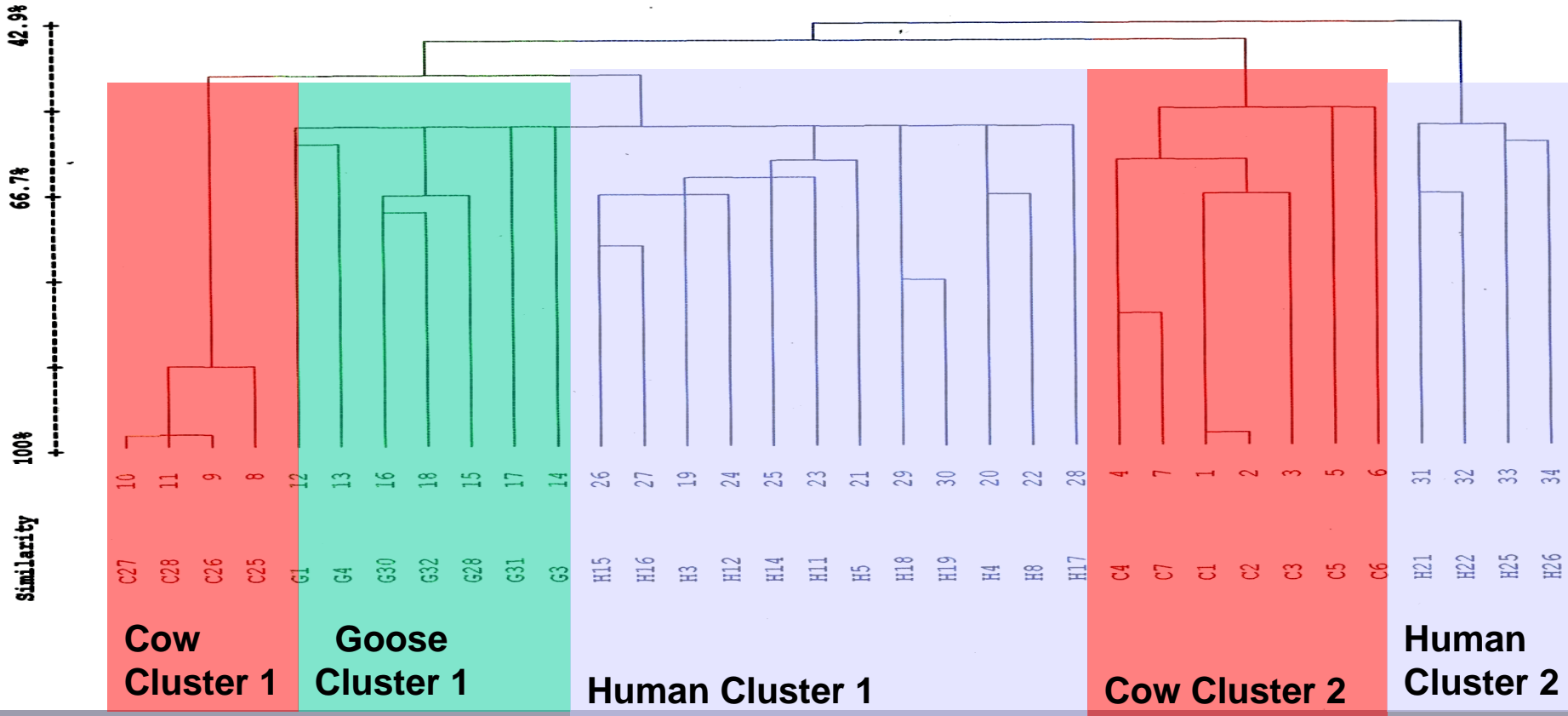
Conesus Lake

- Three major *E. coli* sources:
 - Wildlife
 - Geese at Graywood outlet to lake, on Maxwell's farm, Long Point Park (200g droppings/bird/day * 50 geese = 10,000g).
 - Estimated population of deer in Conesus watershed: 2,092
 - Humans
 - Septic leaks-twice at Graywood
 - Domestic/Agriculture
 - Cattle are only domestic animal with significant numbers on farms associated with experimental watersheds.

Methods

- *E. coli* was isolated using membrane filtration onto mColiBlue24 broth.
- Methods for isolation of genomic DNA, PCR and electrophoresis followed Rademaker and de Bruijn (1997) without exception.

Dendrogram Shows Source Groups Cluster Together



- Source groups cluster together.
 - Each source isolate is more similar to isolates in its group than to those in other groups.
 - Able to assign unknown isolates to groups.
 - Source groups have multiple strains.

Results

- Range of % similarity of unknown isolates to source groups was 22-71%
- Average was 53%
- Literature uses $\geq 80\%$ to assign an unknown isolate to a source group.

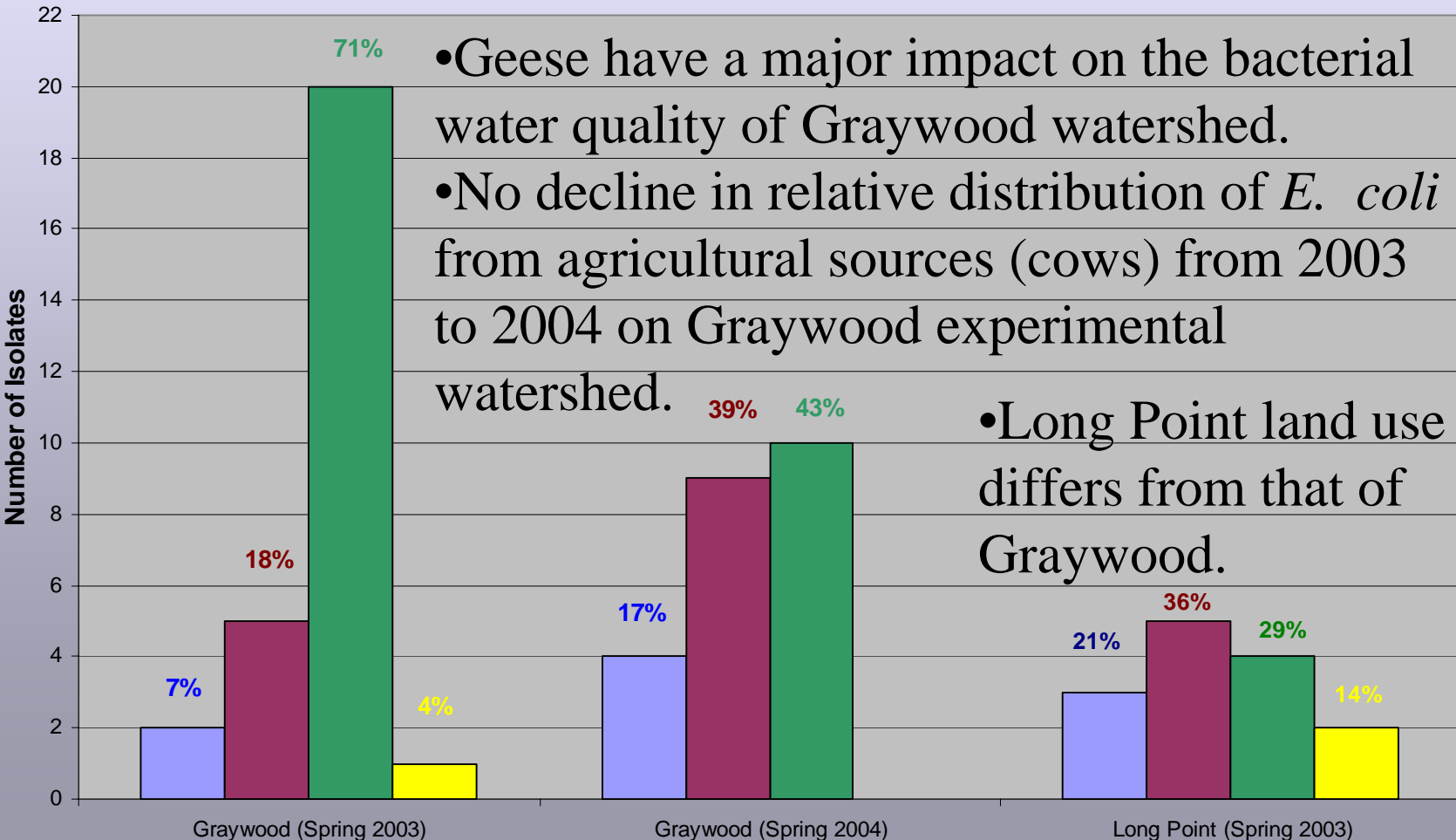
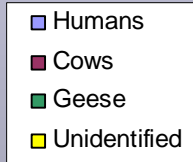
Results

- A total of 150 *E. coli* isolates were PCR amplified and an average of 30 isolates per source group were used for comparison with unknown samples.
- A total of 68 *E. coli* isolates were identified from stream water during spring of 2003 and 2004.
 - 17 isolates from Long Point Control Watershed (8 Event, 9 Non-Event)
 - 51 isolates from Graywood Gully Experimental Watershed (22 Event, 29 Non-Event)

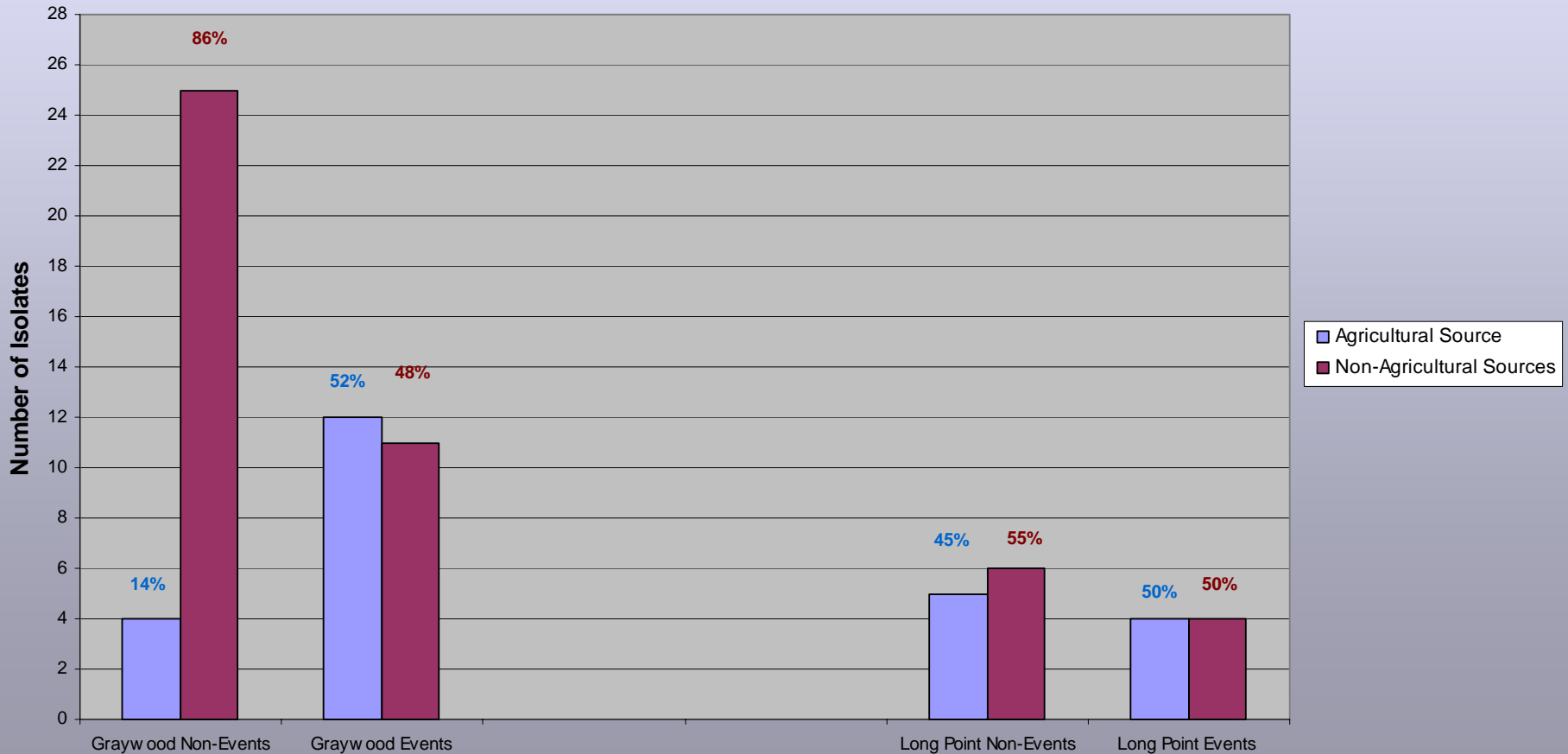
Graywood Gully (Experimental Stream): Number of *E. coli* Isolates in Each Source Group

• Geese have a major impact on the bacterial water quality of Graywood watershed.
 • No decline in relative distribution of *E. coli* from agricultural sources (cows) from 2003 to 2004 on Graywood experimental watershed.

• Long Point land use differs from that of Graywood.



Agricultural Contribution of *E. coli* During Event and Non-Event Periods



- Though there have been overall decreases in numbers of *E.coli*, agricultural sources (cows) contribute greatly to Graywood bacterial pollution during event conditions.
- Long Point control stream demonstrates an even contribution of agricultural and non-agricultural sources during both event and non-event conditions.

Summary

- PCR shows promise as a tool for identifying sources of *E. coli* contamination.
- Although cows were believed to be the main source of pollution, geese are another major source of bacterial contamination during the spring in Graywood Gully.
- Preliminary results show that events play an important role in bacterial pollution from agricultural sources.
- No evidence so far that BMP's are effectively changing the distribution of *E. coli* contamination from agricultural sources to non-agricultural sources.

Future of the Research

- Analyze deer isolates-Expand and enhance library.
- Look at temporal changes in distribution of *E. coli* sources in Graywood.
- Analyze other experimental streams.

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