Note: Data shown here are preliminary, and not to be cited.

Microbial Source Tracking in the Love Creek Watershed of Delaware's Inland Bays

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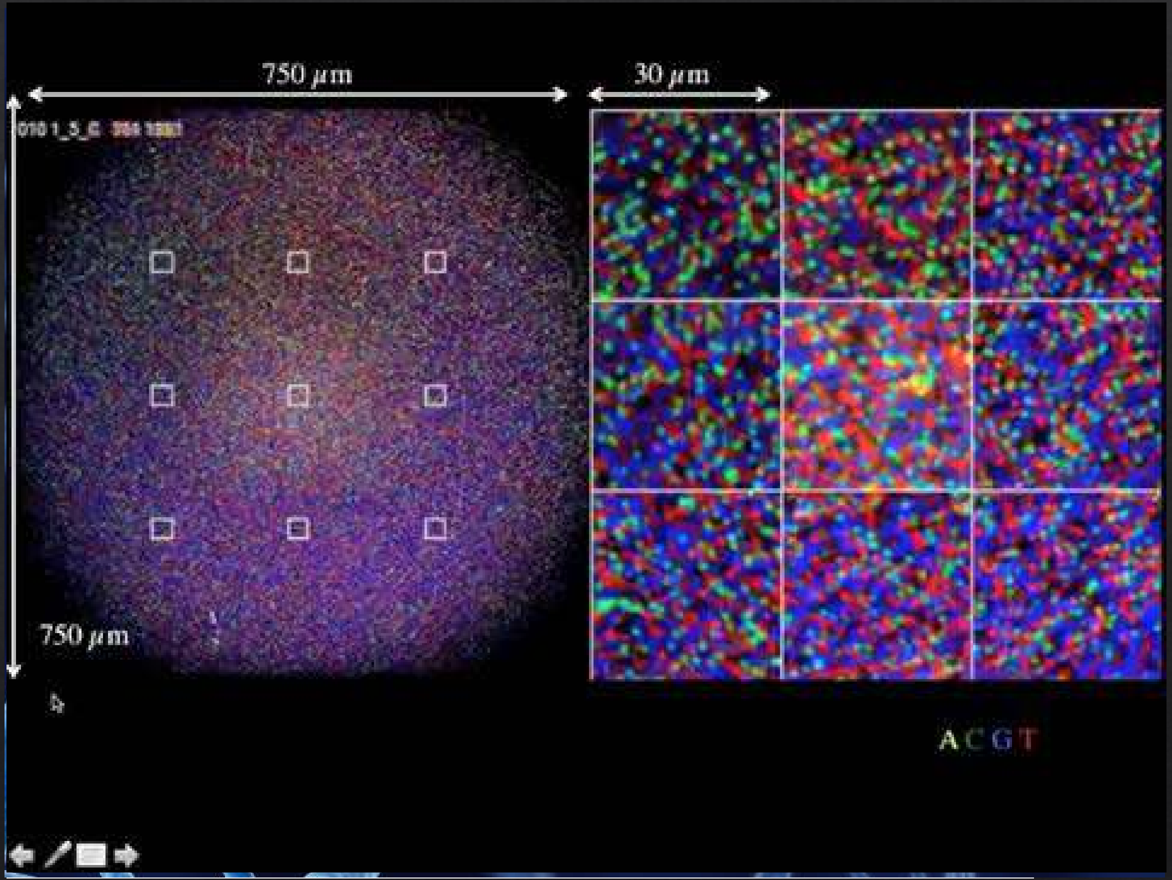
Objectives

- Develop a preliminary study to examine the microbial community within the Love Creek Watershed
    - Can we determine the source of high bacteria loads within the watershed?

DNA Sequencing

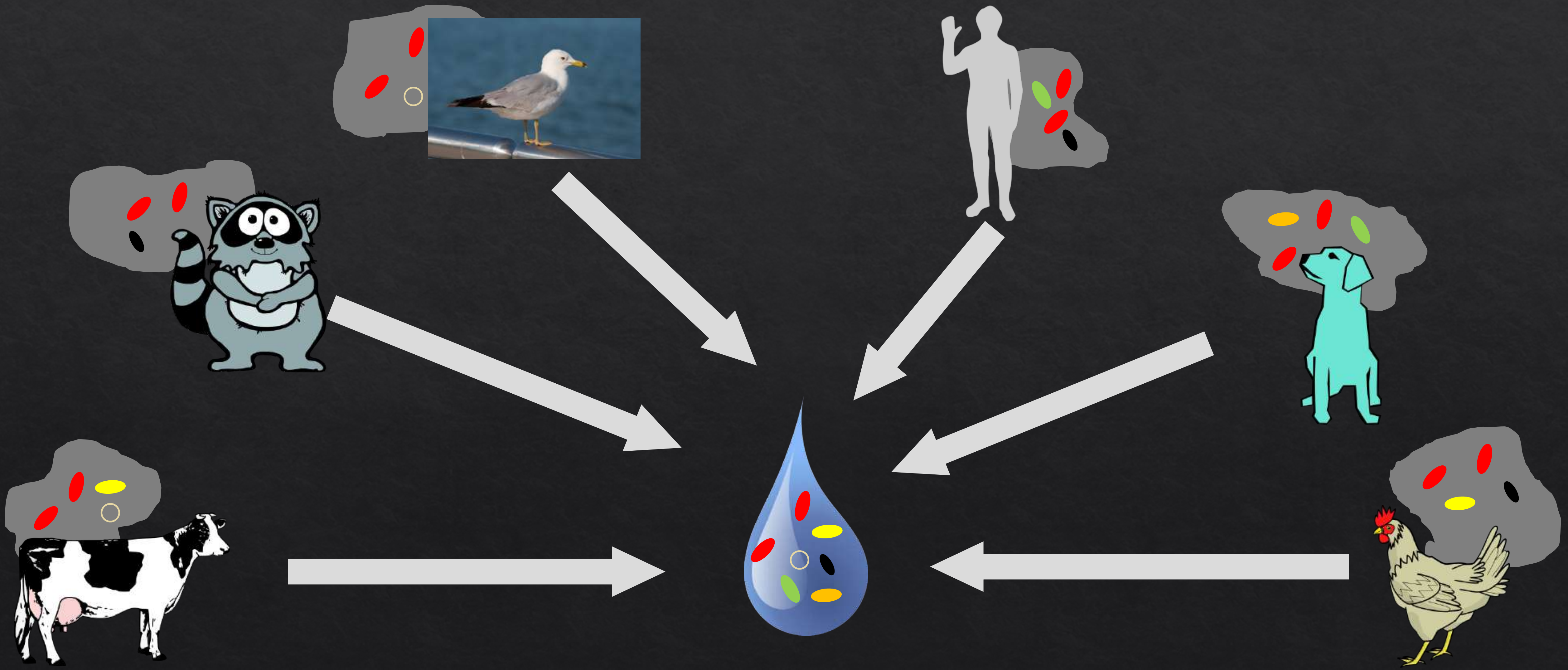
- Sanger Sequencing (1st Generation)
  - The Gold Standard for sequencing
  - Larger sequences (>700 bp)
  - Increased cost, sequencing and analysis times
- Next Generation Sequencing (NGS)
  - Shorter sequences (50 – 300 bp)
  - Less cost and DNA required
  - Increased computational power



From: http://www.intechopen.com/books/next-generation-sequencing-advances-applications-and-challenges/next-generation-sequencing-in-aquatic-models

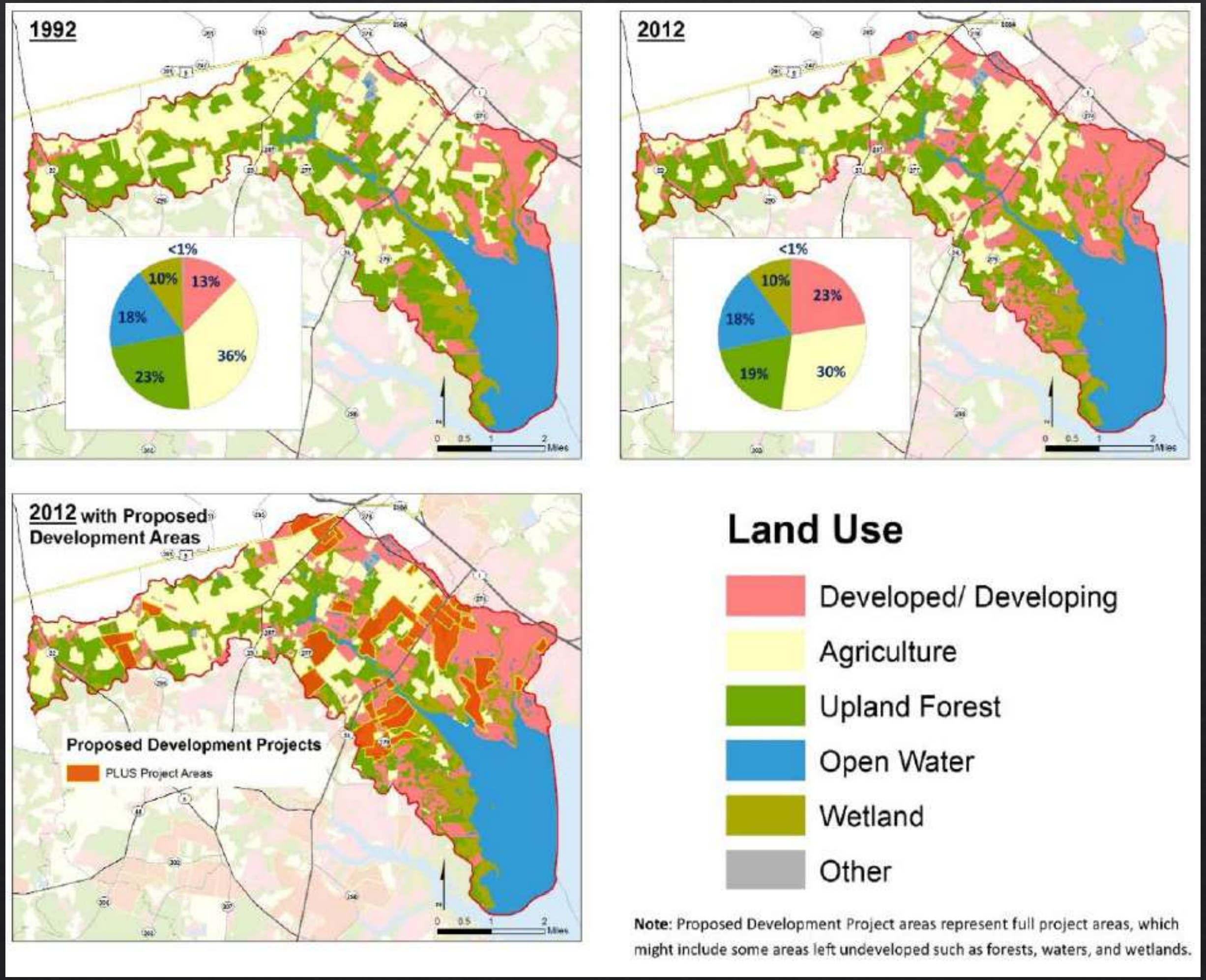
From: https://www.abmgood.com/marketing/knowledge_base/next_generation_sequencing_introduction.php

Microbial Source Tracking



Love Creek Watershed

- Major tributary of Rehoboth Bay
- Watershed approximately 24 square miles
- Last several decades increased urbanization with decreased agriculture and forested areas

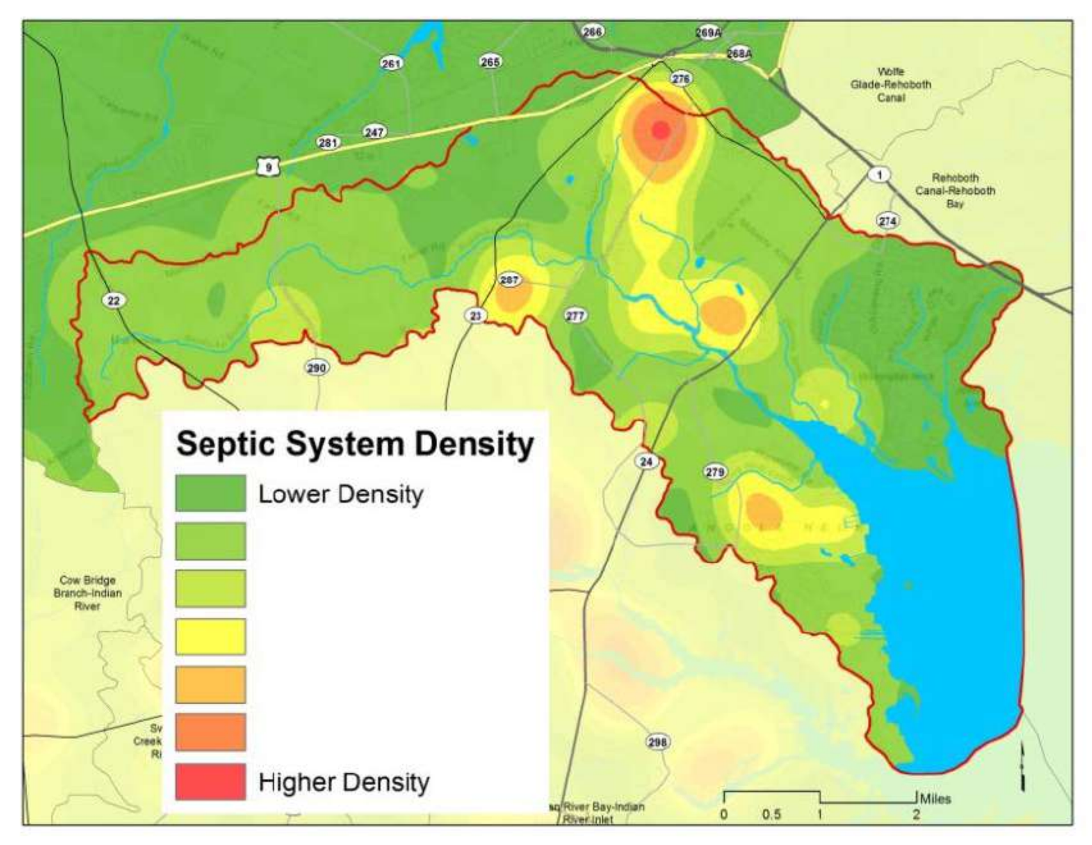


Note: Proposed Development Project areas represent full project areas, which might include some areas left undeveloped such as forests, waters, and wetlands.

From: https://www.inlandbays.org/wp-content/uploads/State-of-Love-Creek-Report-FINAL-.pdf

Love Creek Watershed

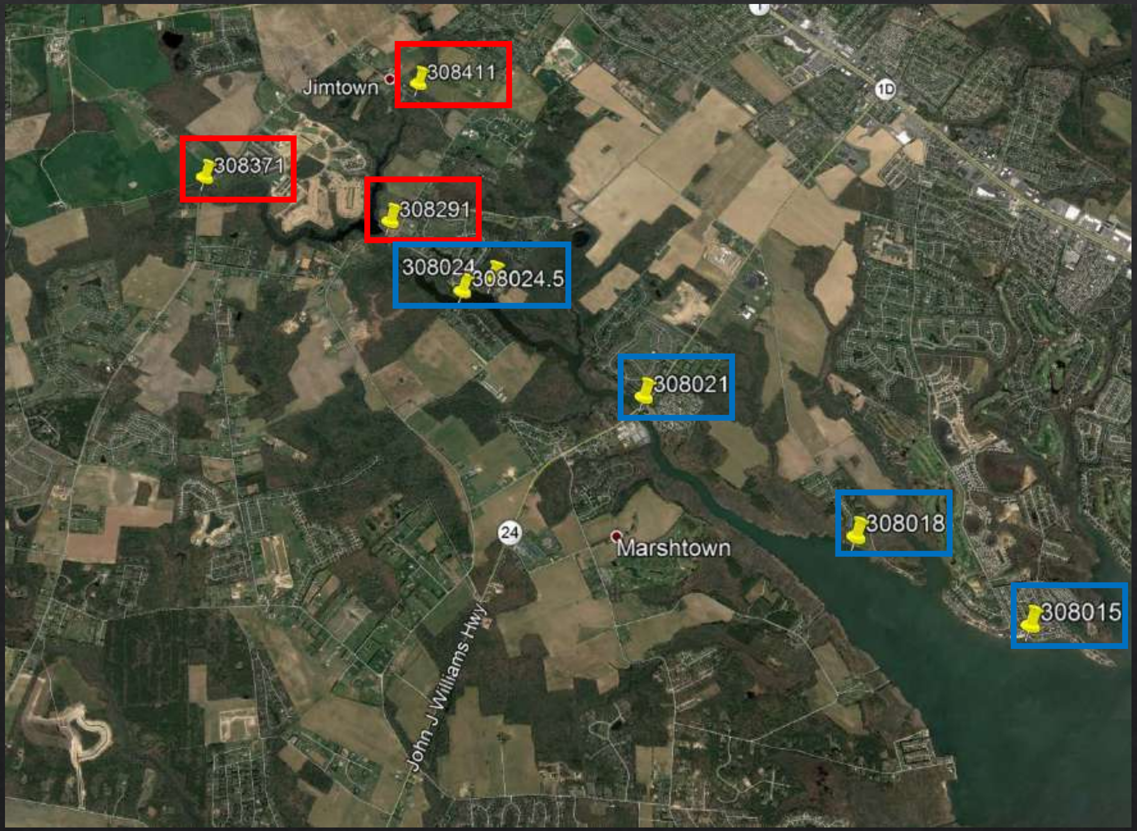
- Nutrient input sources
  - Agricultural and residential fertilizers
  - Stormwater runoff
  - Wastewater disposal including septic systems
- Currently listed as impaired under the CWA for both bacteria and nutrients



From: https://www.inlandbays.org/wp-content/uploads/State-of-Love-Creek-Report-FINAL-.pdf

Study Area

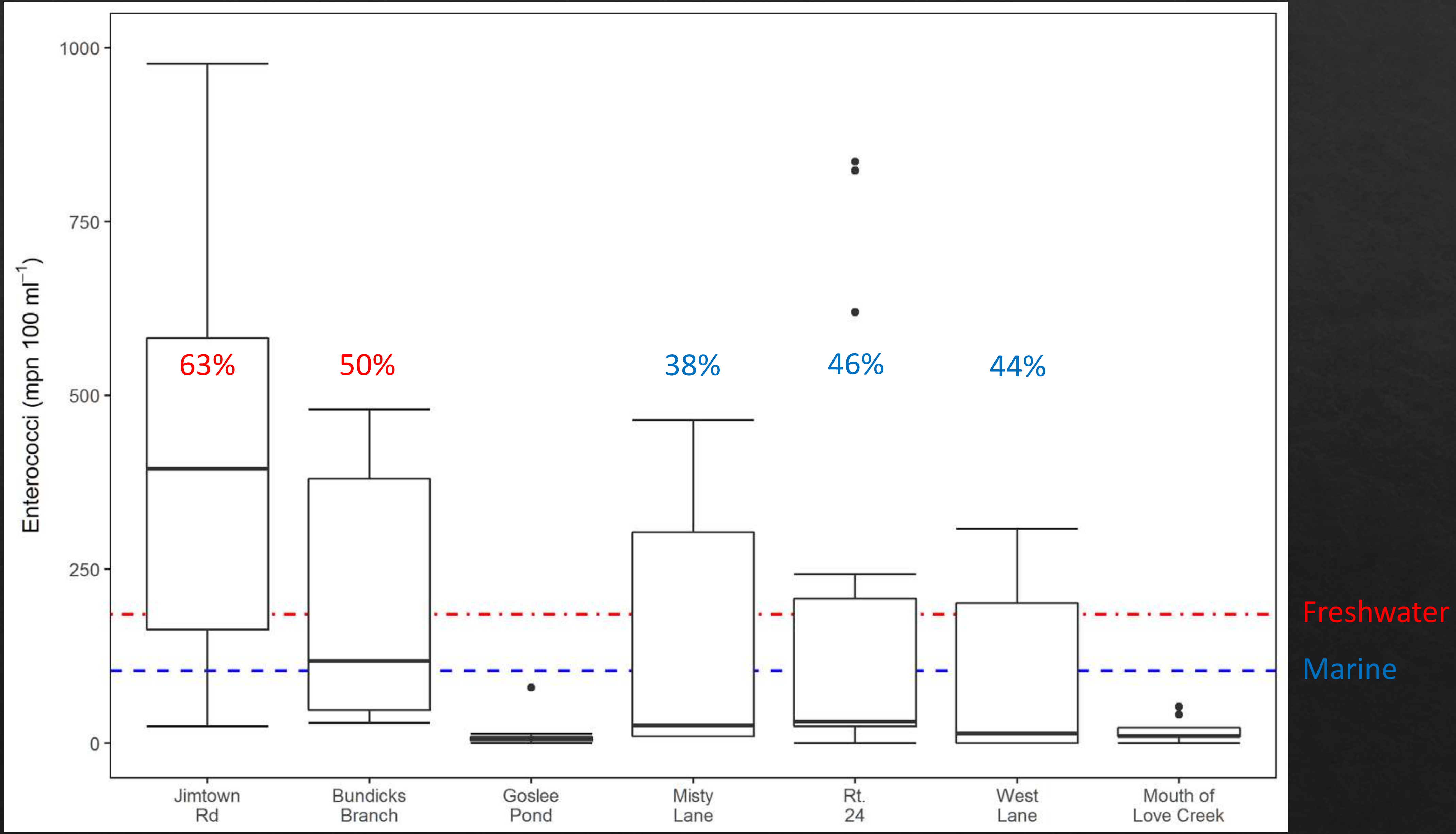
- Tidally driven till Goslee Pond at Robinsonville Road (308291)
- Monthly Samples March to October
  - 7 Sites along within the watershed
  - 3 Non-tidal
  - 4 Tidal
  - 2 replicates at RB34
- One sampling event after major rainfall



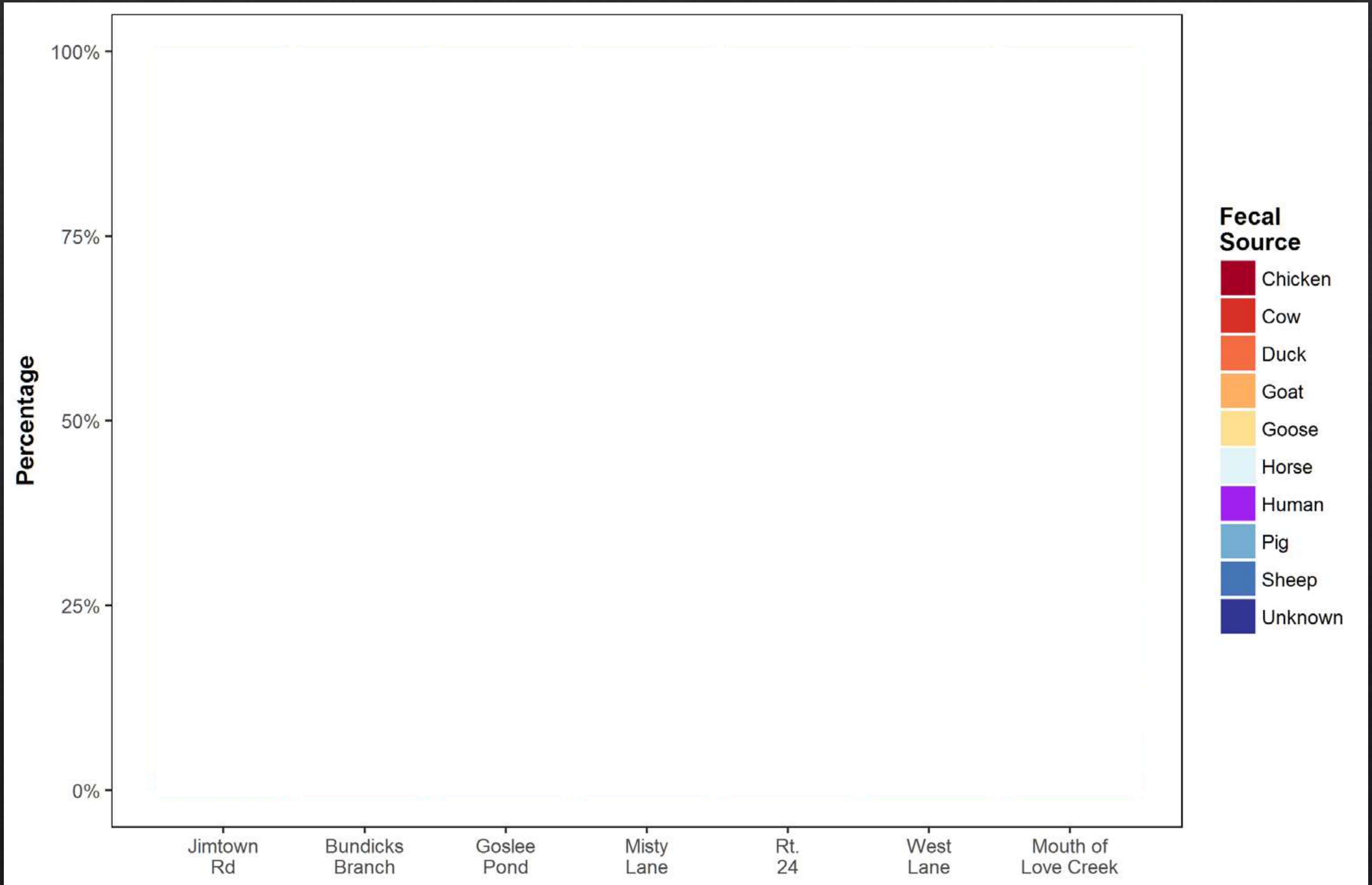
Sampling

- Nutrients
  - DOC/TOC, NO_X, Orthophosphate, NH_3, Total N and P, TSS, TDS, Turbidity
- Bacterial
  - Enterococci via Enterolert
  - Microbial community via Illumina MiSeq
- Fecal Samples
  - Chicken, Cow, Duck, Goat, Goose, Horse, Human, Pig, Sheep

Enterolert


Enterococci (mpn 100 ml^{-1})
1000
750
500
250
0
63%
50%
38%
46%
44%
Jimtown Rd
Bundicks Branch
Goslee Pond
Misty Lane
Rt. 24
West Lane
Mouth of Love Creek
Freshwater
Marine

March



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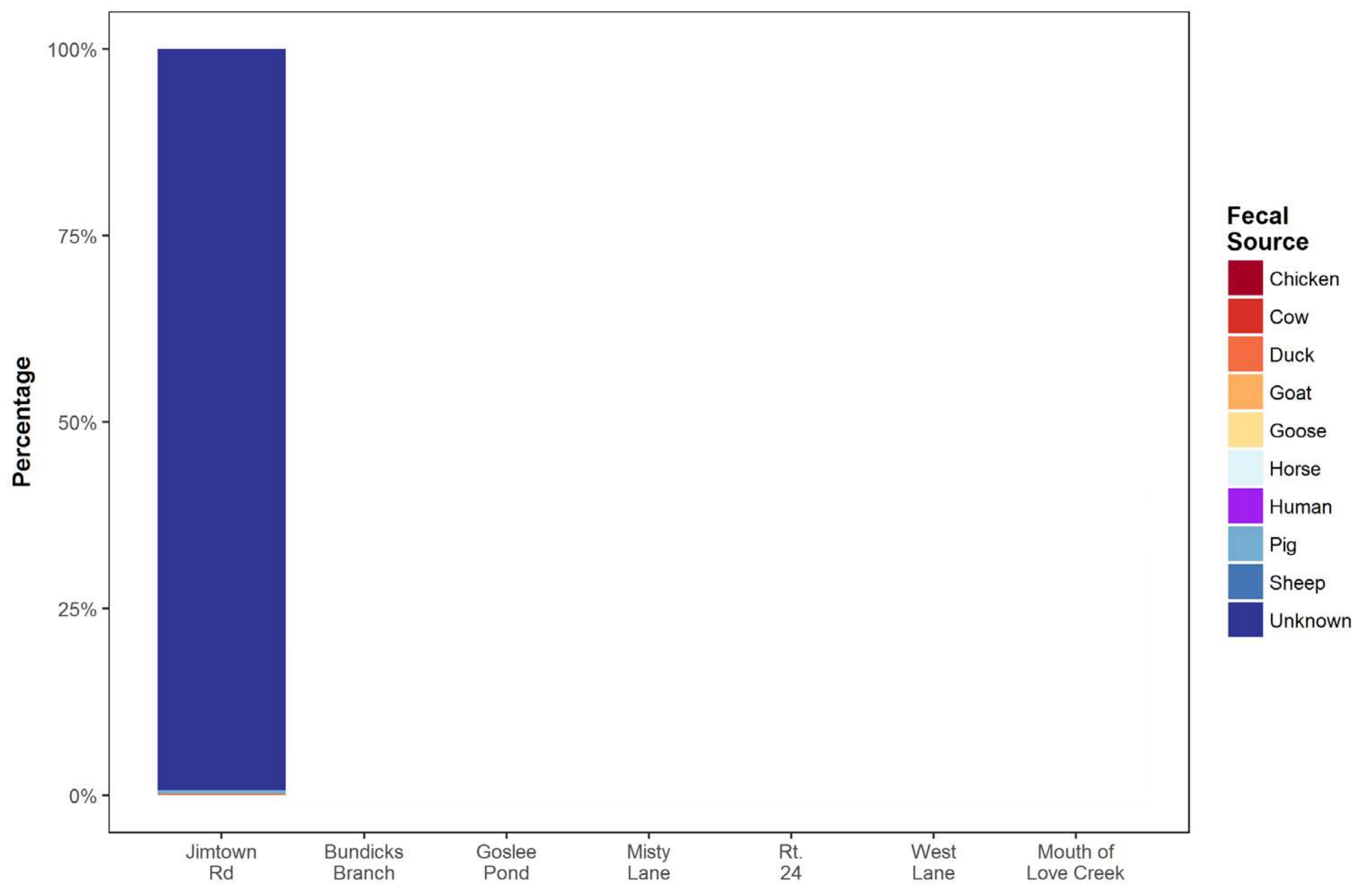
25%
0%
Percentage
Jimtown Rd
Bundicks Branch
Goslee Pond
Misty Lane
Rt. 24
West Lane
Mouth of Love Creek
Fecal Source
Chicken
Cow
Duck
Goat
Goose
Horse
Human
Pig
Sheep
Unknown

April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Misty Lane
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West Lane
Mouth of Love Creek
Fecal Source
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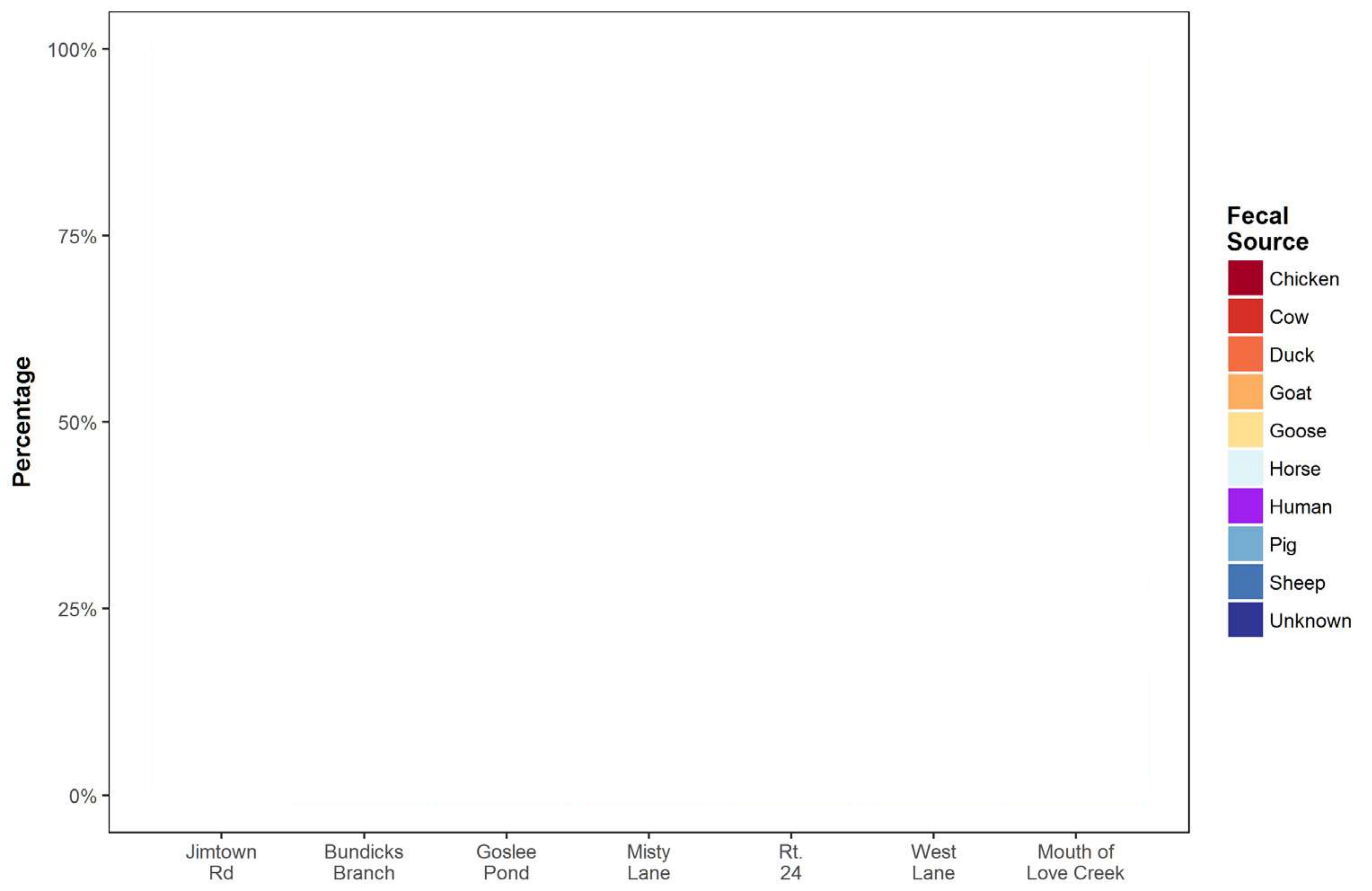
Cow
Duck
Goat
Goose
Horse
Human
Pig
Sheep
Unknown

May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Horse
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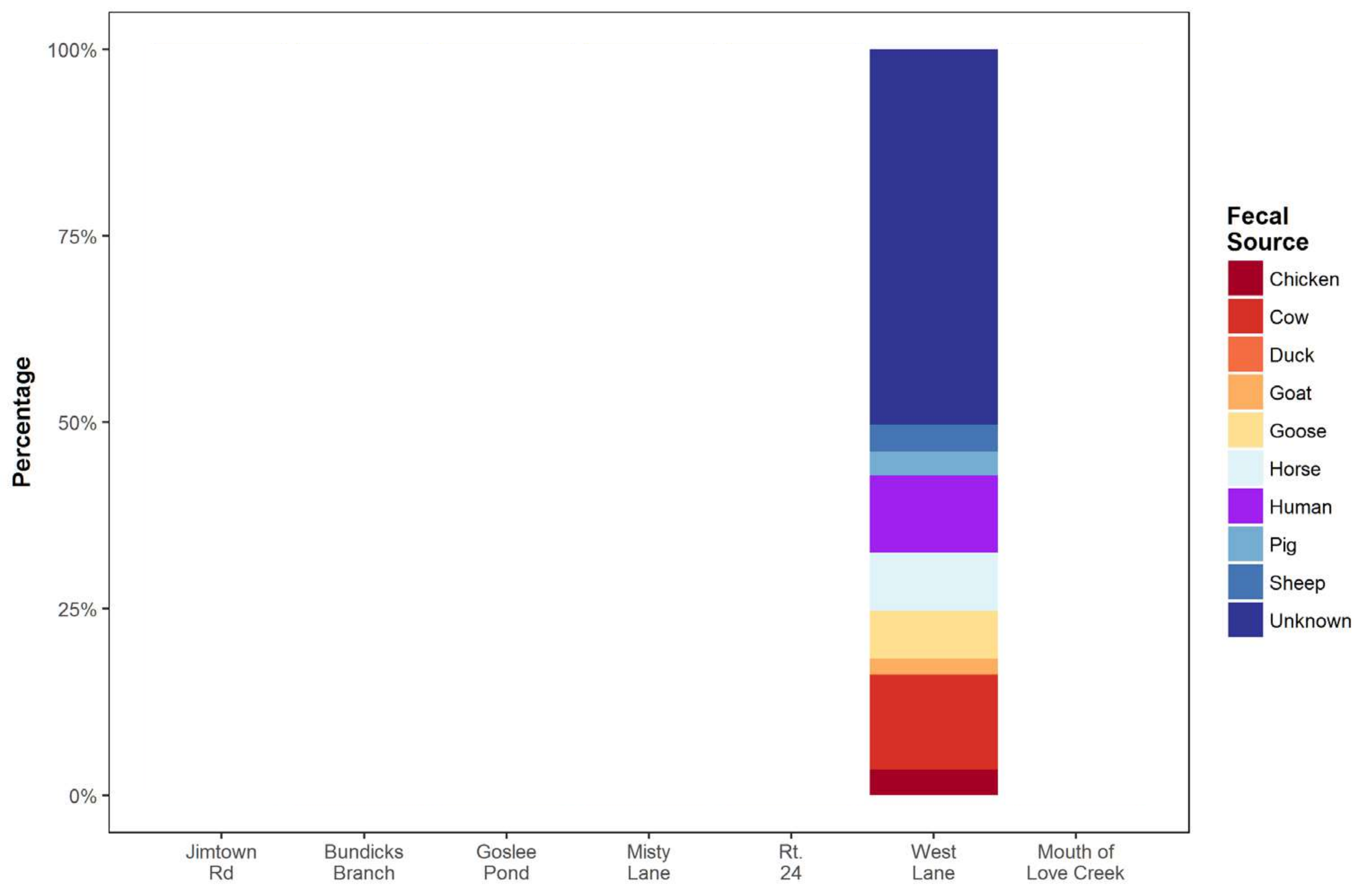
Sheep
Unknown

June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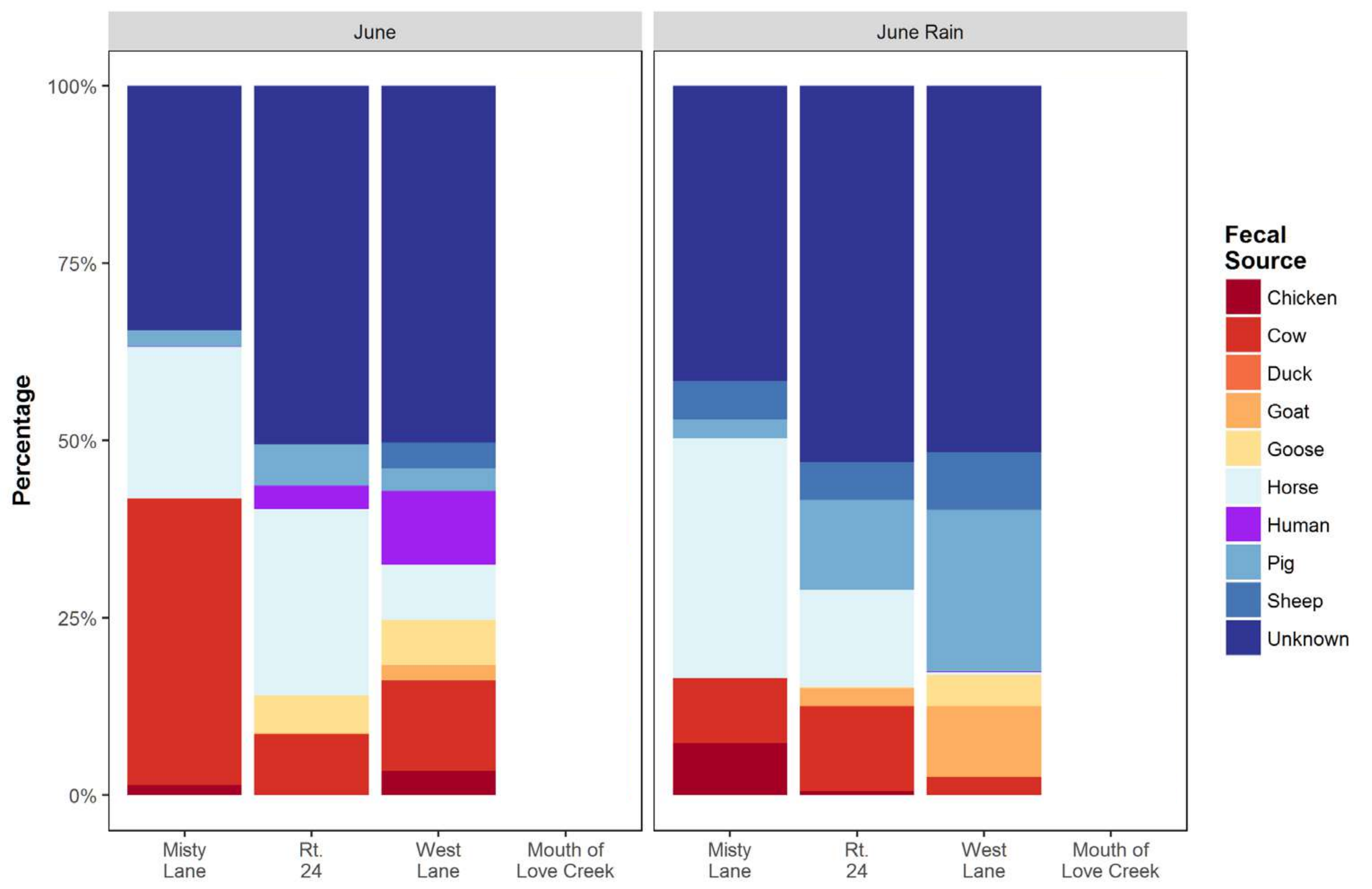
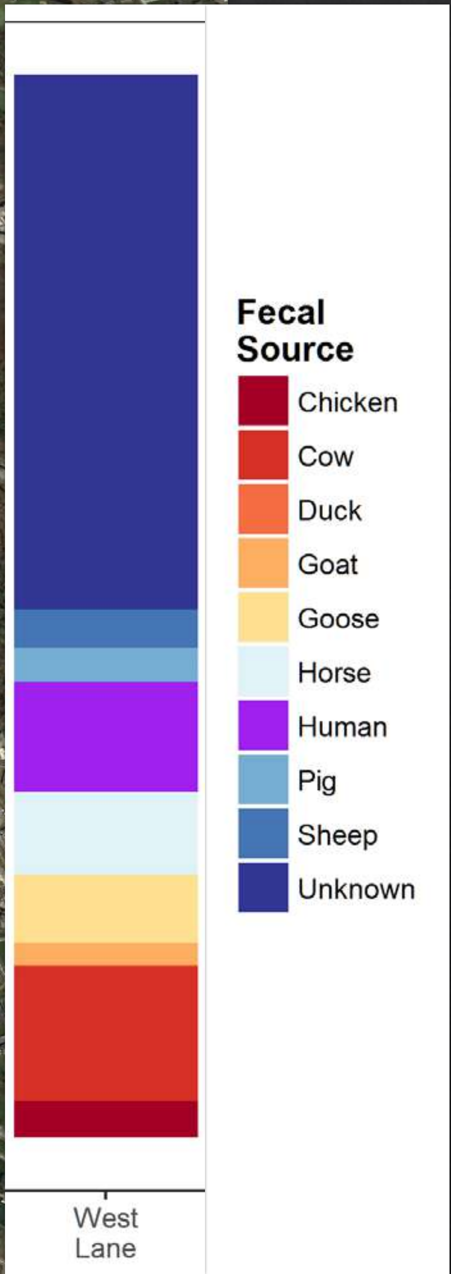
Sheep
Unknown

June Rain Event



West Lane


24
Lazy Lagoon Rd
Waterlily Ln
Rd 284
Bishop Bend
Catalina Cir
Bonaire Dr
Corolla P
Frontier Rd
W Mint Pl
West Ln
308018
East Ln
Old Landing Rd
Waterview Rd
Back Bay Ln
Marshall Rd
Google Earth


Fecal Source
Chicken
Cow
Duck
Goat
Goose
Horse
Human
Pig
Sheep
Unknown
West Lane

Conclusions

- Next Generation Sequencing techniques are able to determine potential sources of bacterial contamination
  - Ground truthing must occur to find potential sources
  - Greater fidelity can occur from fecal samples collected within the watershed

- NGS microbial source tracking is another tool to add to the monitoring toolbox

Future Analysis

- Functional analysis of the microbial community
- Emerging pathogens
- Viral source tracking
- Linkages between nutrients and the microbial community?

Acknowledgements

- DNREC Environmental Lab Section
- EPA Multipurpose Grant XXXX