

Microbial Source Tracking *E. coli* at Two Beaches in the Duluth-Superior Harbor

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Beach Issues

- Beach advisories are being posted due to high *E. coli* levels
 - **Don't know source of contamination**
- In 2006, the local wastewater treatment plant (WLSSD) only chlorinated their effluent when harbor water at the Blatnik Bridge failed to meet fecal coliform water quality standards.
 - **Don't know if fecal coliforms in harbor water are all from treated wastewater**

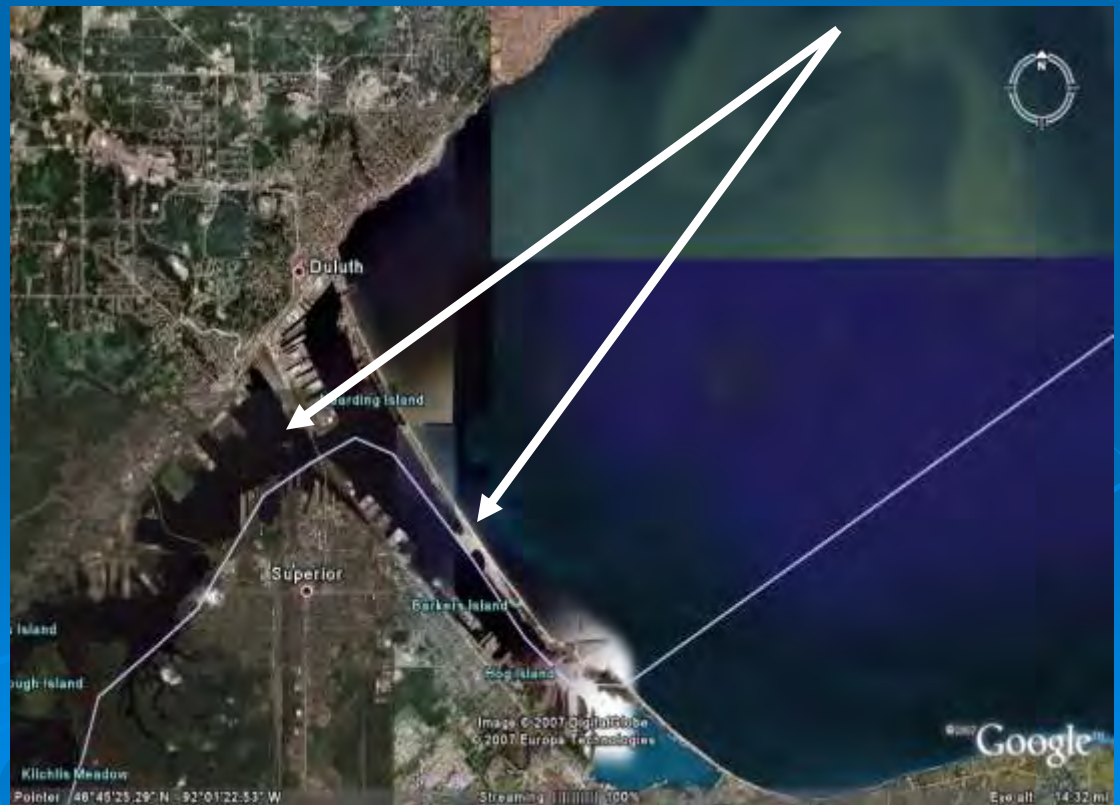


Objectives

Using a library based microbial source tracking technique...

- Identify the most probable sources of *E. coli* in sand, water, and sediment at two public beaches in the Duluth-Superior harbor.
- Determine if the sources of *E. coli* at these two beaches change seasonally

Duluth, Minnesota



A satellite map of the Superior, Wisconsin area. The map shows a mix of urban development, green spaces, and water bodies. A large, dark, irregularly shaped area in the upper left is labeled 'WLSSD'. A road bridge crossing a waterway is labeled 'Blatnik Bridge'. A large, dark, irregularly shaped area in the lower right is labeled 'Southworth Marsh'. A large, dark, irregularly shaped area in the lower right is labeled 'Superior WI, waste water'. A road in the lower left is labeled 'Interstate Isle'. A red location pin is visible in the lower right. The map is overlaid with white and yellow arrows pointing to the labeled areas.

WLSSD

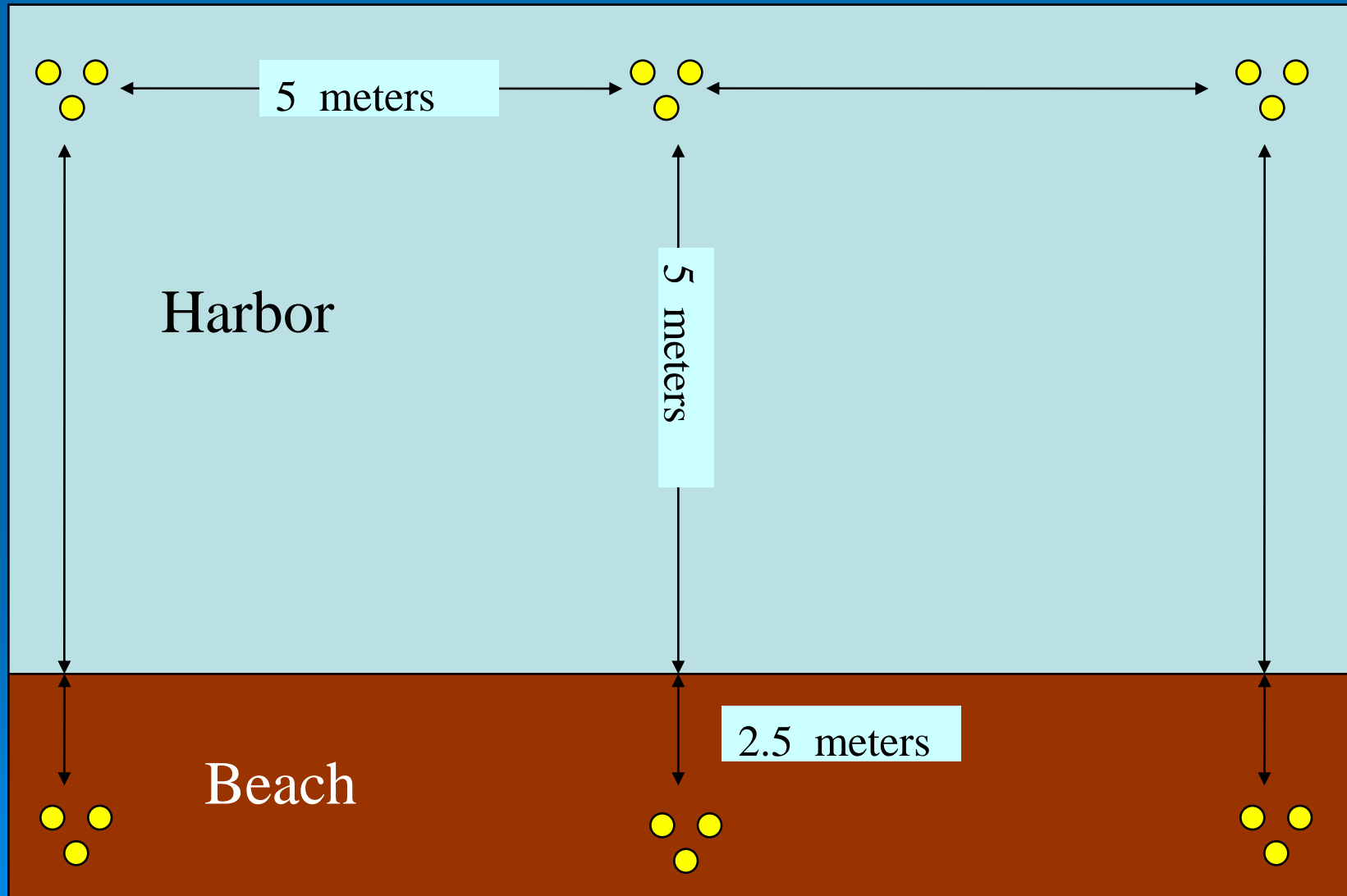
Interstate
Isle

Blatnik
Bridge

Southworth
Marsh

Superior
WI, waste
water

Sampling Scheme



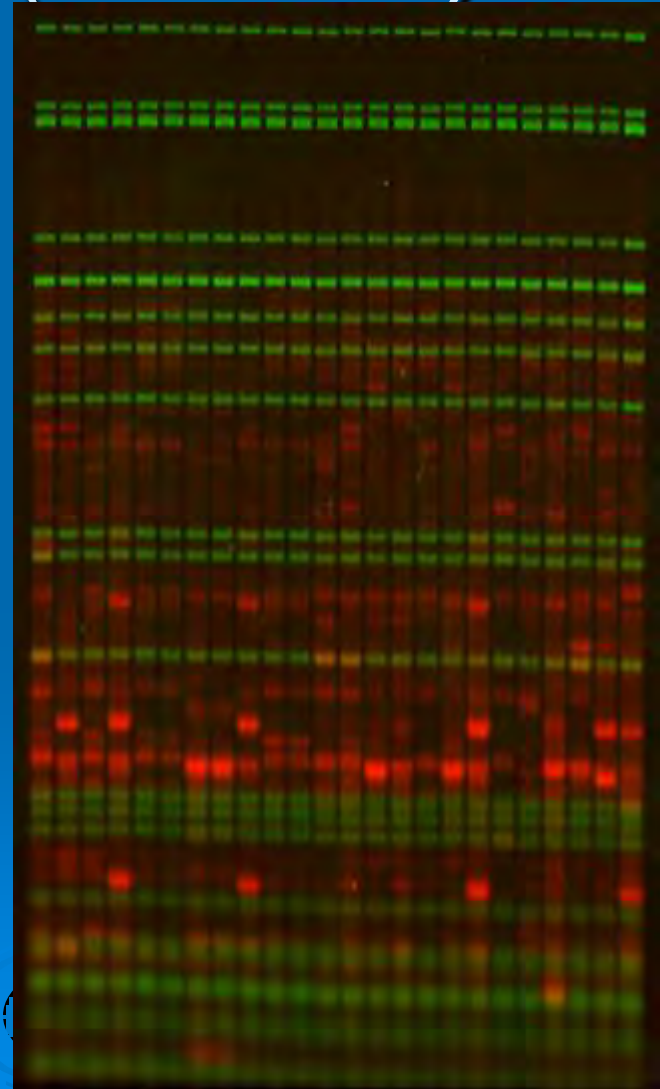
Host Collection

- Collect fecal swabs from bird cloacae
- Freshly voided fecal material
- Isolate *E. coli* from treated wastewater



Horizontal Fluorophore Enhanced rep-PCR(HFERP)

- BOX A1R primer
- Gel excited at 523nm
- DNA fingerprints were analyzed with BioNumerics (ver. 4.5) software package.



Duluth *E. coli* Host Library

Animal	# of animals	Unique isolates
Beaver	10	10
Deer	30	52
Duck	36	71
Terns	56	91
Gulls	141	247
Geese	78	139
Treated wastewater		340
Total	353	955

Jackknife analysis

	Waterfowl	Wildlife	Treated Wastewater
Waterfowl	87%	19	33
Wildlife	1	71%	1
Treated Wastewater	12	10	66%

Statistics

➤ Source identification

➤ ID bootstrap analysis (at least $P=0.90$), using a BioNumerics script

➤ <http://www.applied-maths.com/bn/scripts/bnscripts.htm>

➤ Dendrograms

➤ Pearson's product moment correlation coefficient

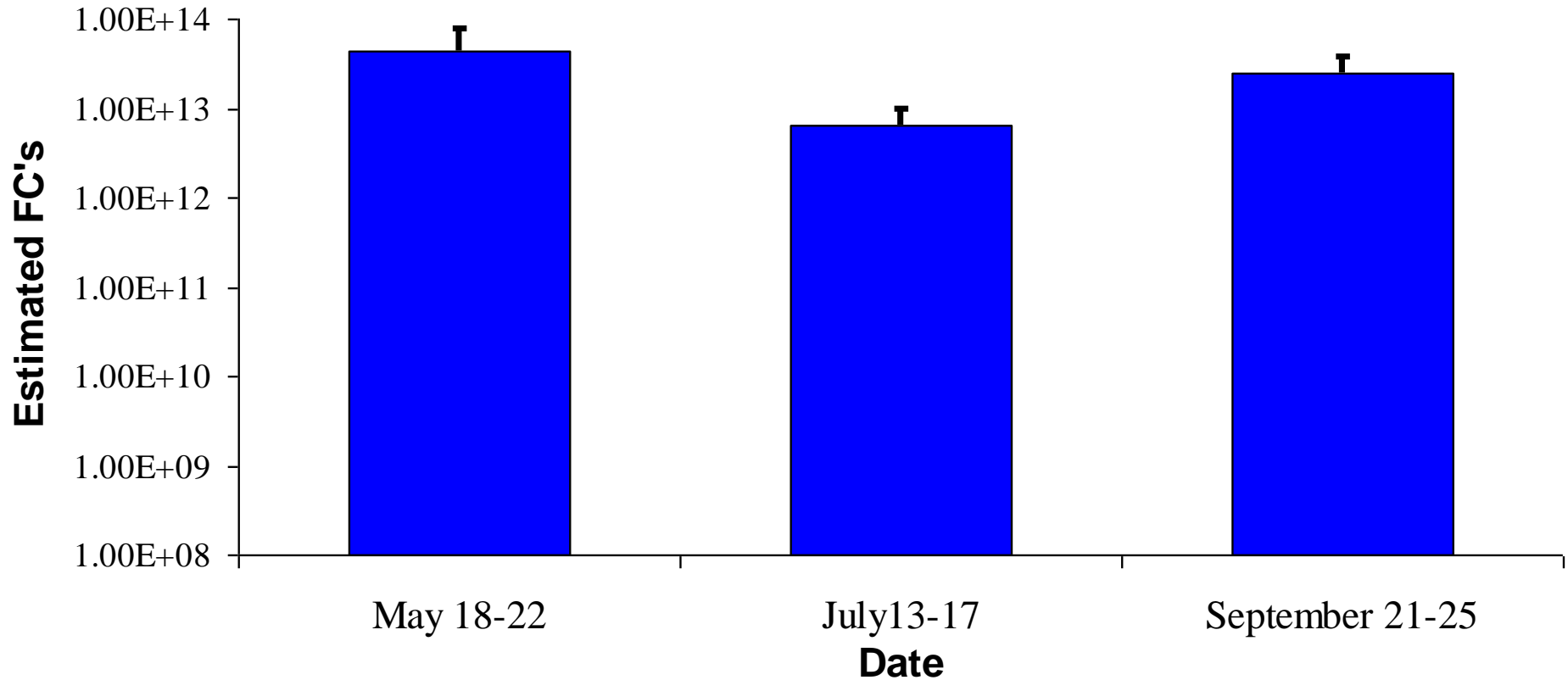
➤ Unweighted pair group method with arithmetic means (UPGMA) clustering method.

Potential Sources

- Estimated the fecal coliform input by treated waste water for the 5 days before a sample
- Counted birds the day before a sample (two days before the May sample)

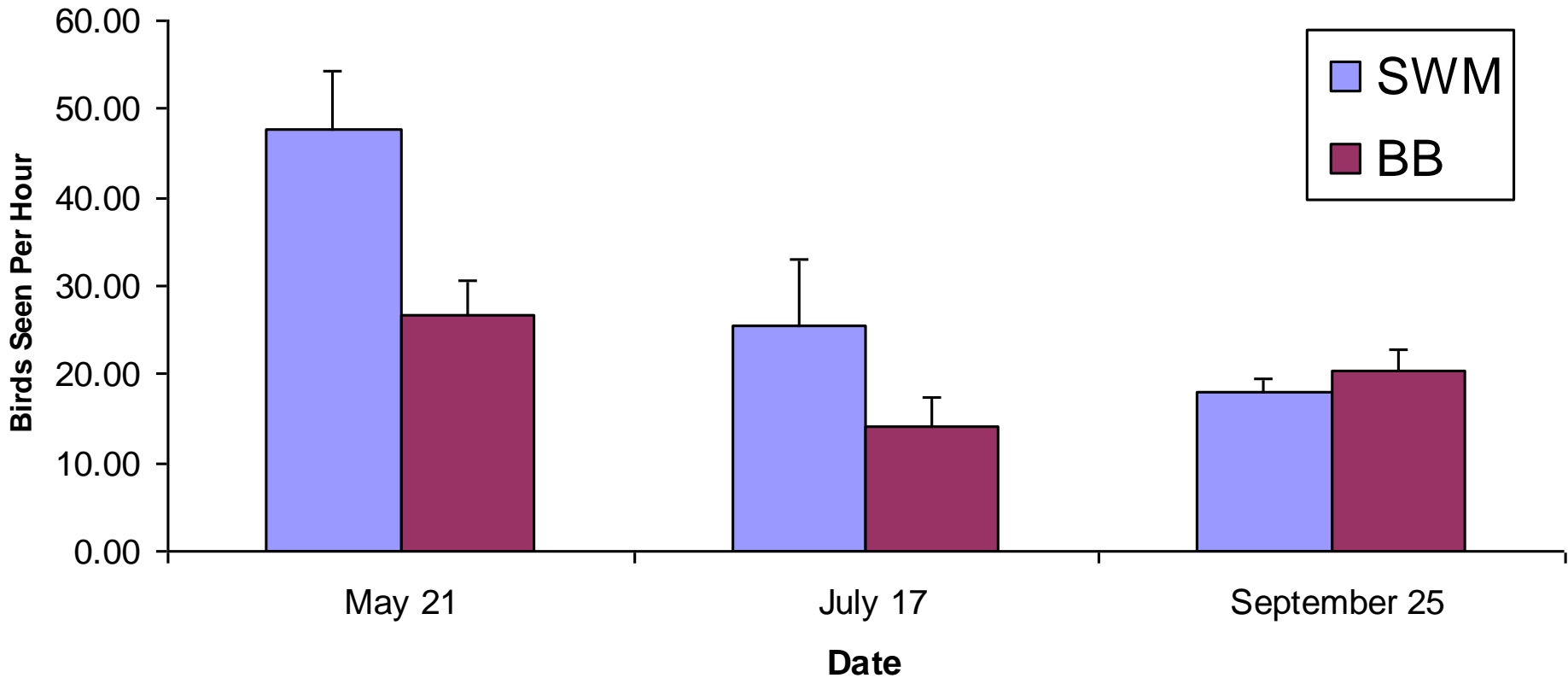


Estimated FC Discharged by WLSSD 5 Days Before a Sample in 2006



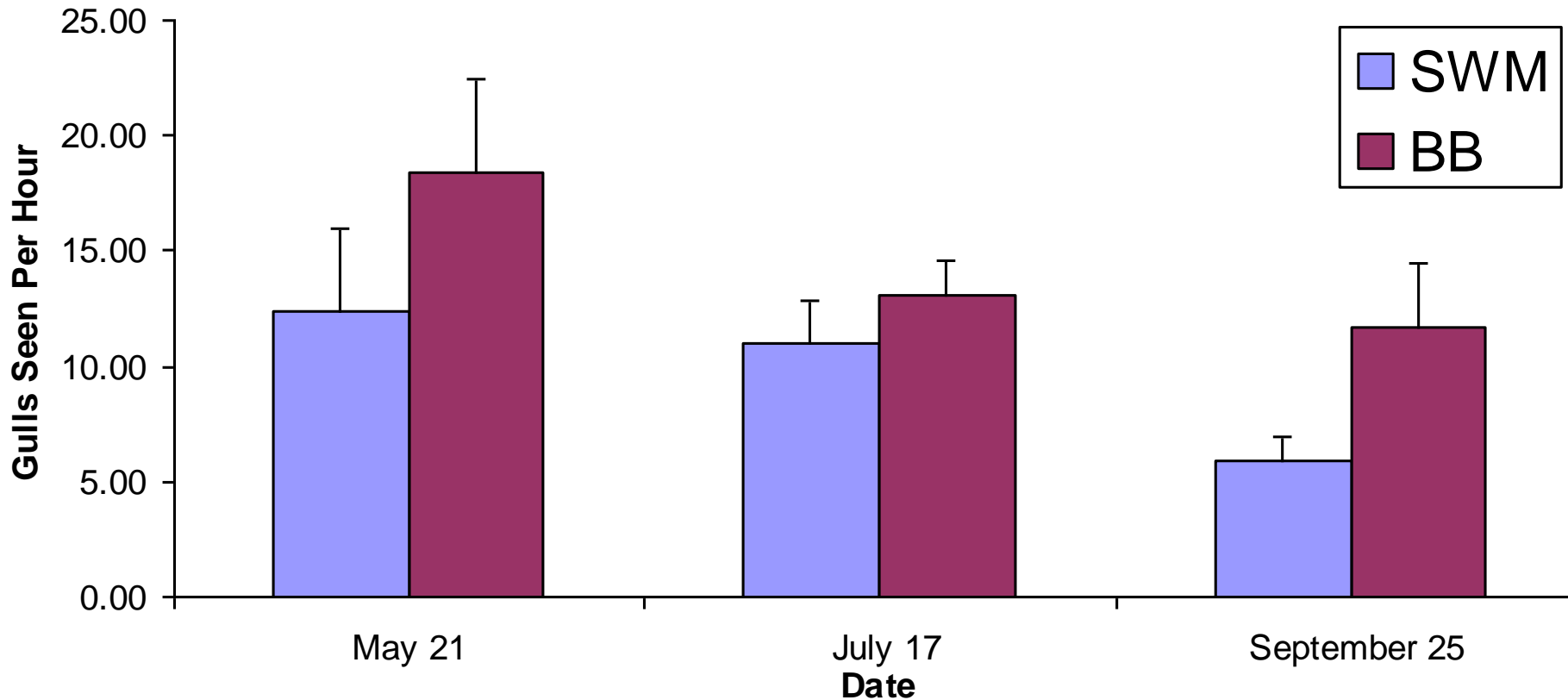
Not significantly different ($p > 0.05$)

Total Bird Counts in 2006



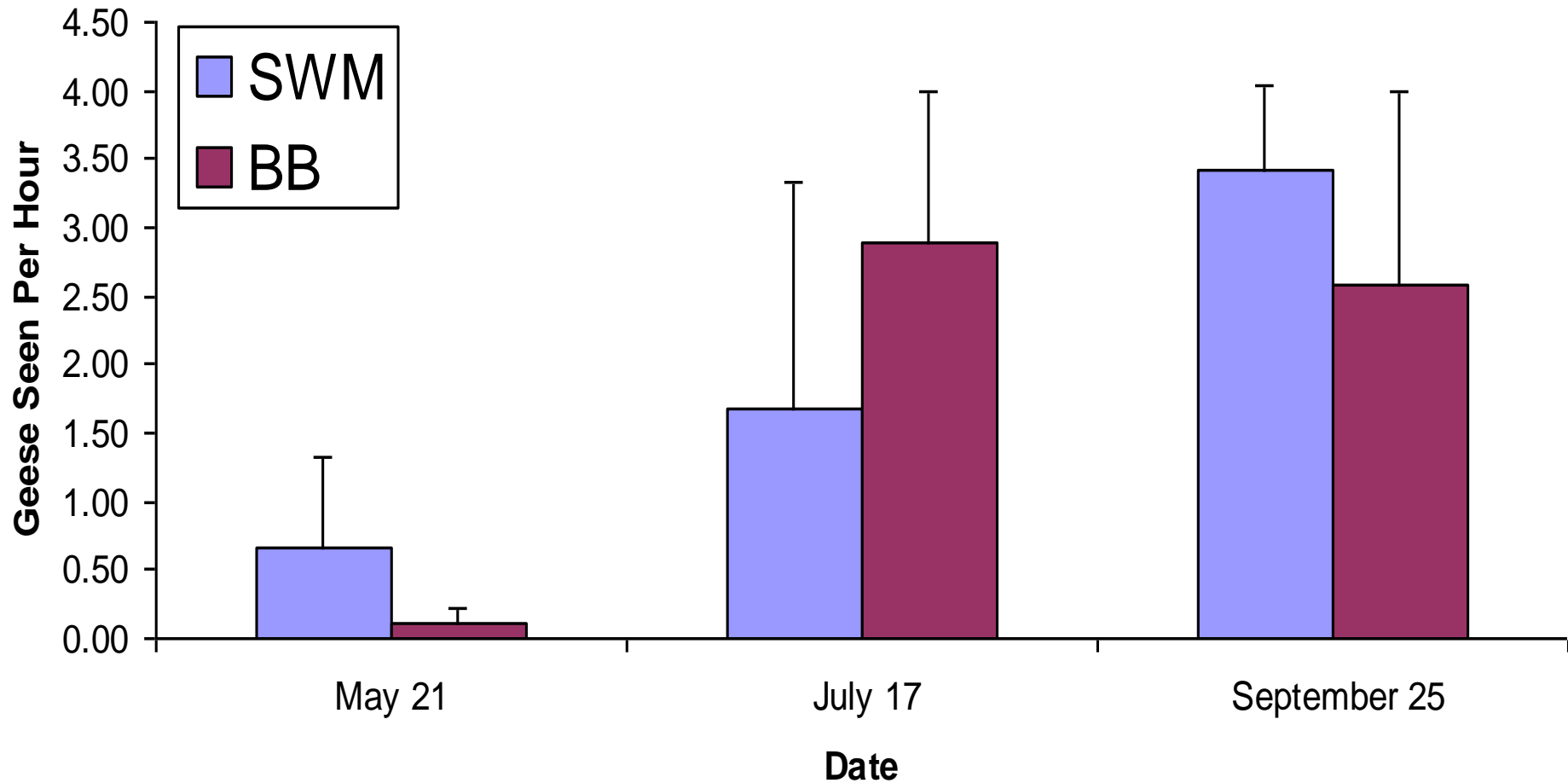
- The 10,000 nesting pair of ring-billed gulls near the Blatnik Bridge were not included.
- There were more total birds in May than July and September ($p < 0.05$)

Ring-Billed Gulls Seen Over Land in 2006



- The 10,000 nesting pair of ring-billed gulls near the Blatnik Bridge were not included
- There were more gulls at Blatnik Bridge than Southworth Marsh
- The number of gulls between seasons were not different ($p > 0.05$)

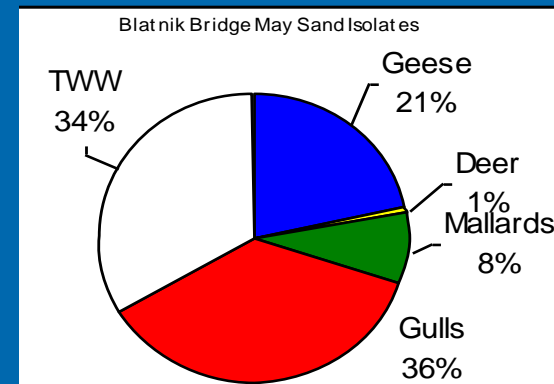
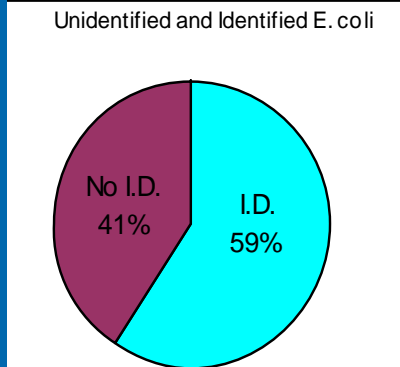
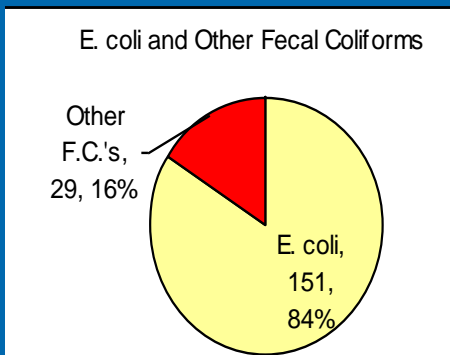
Canada Goose Counts for 2006



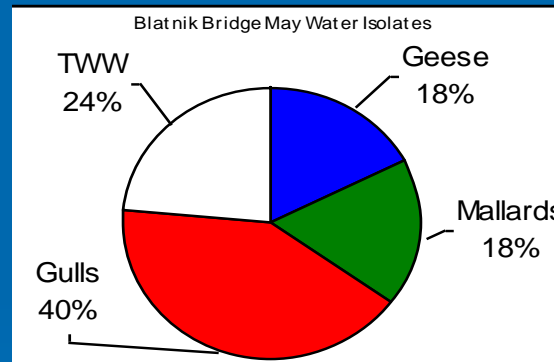
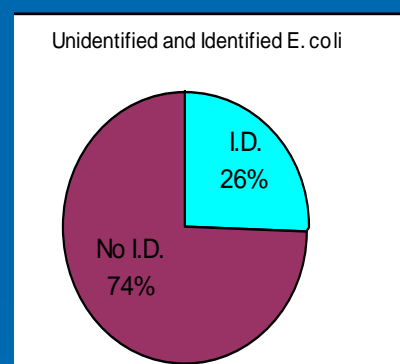
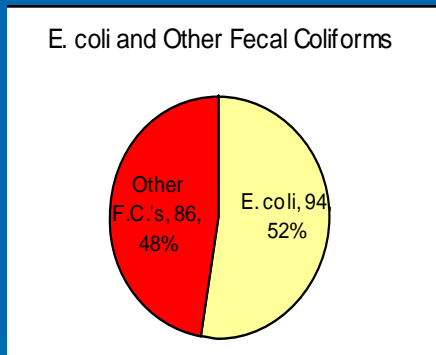
➤ When sites were combined, there were more Canada geese in July and September than May ($P < 0.05$)

Blatnik Bridge - May

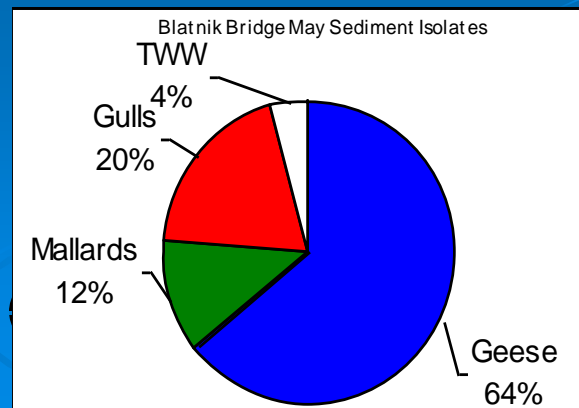
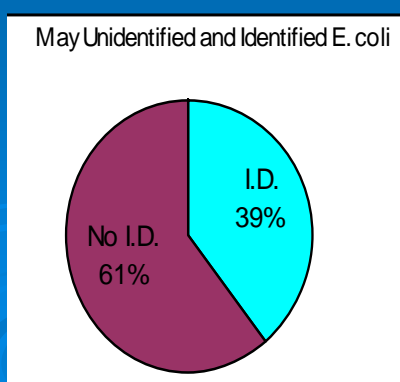
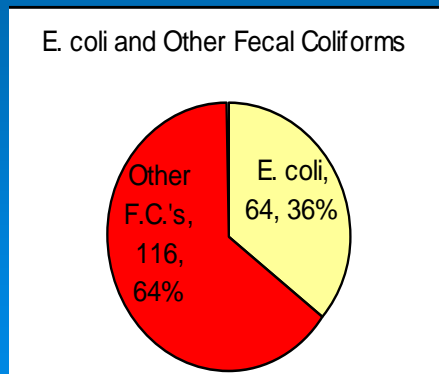
Sand



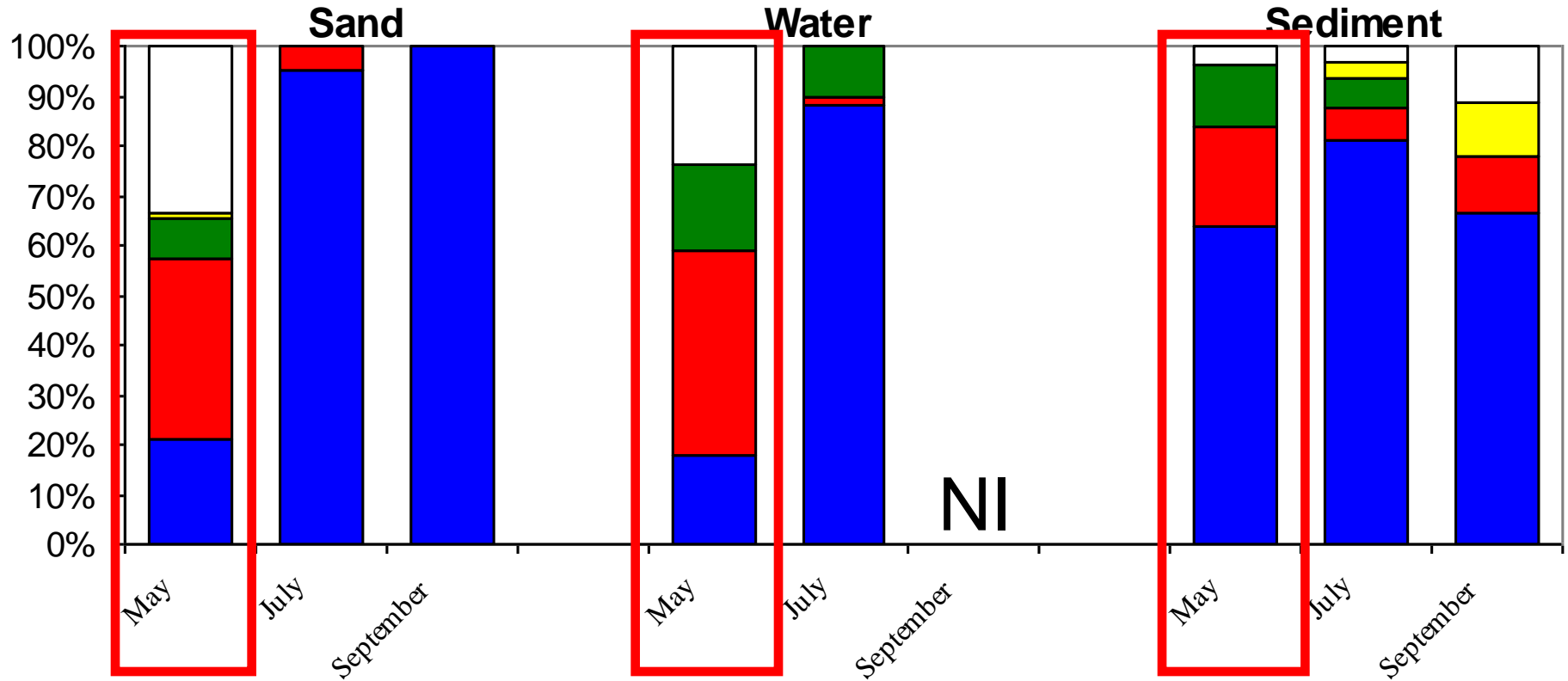
Water



Sediment

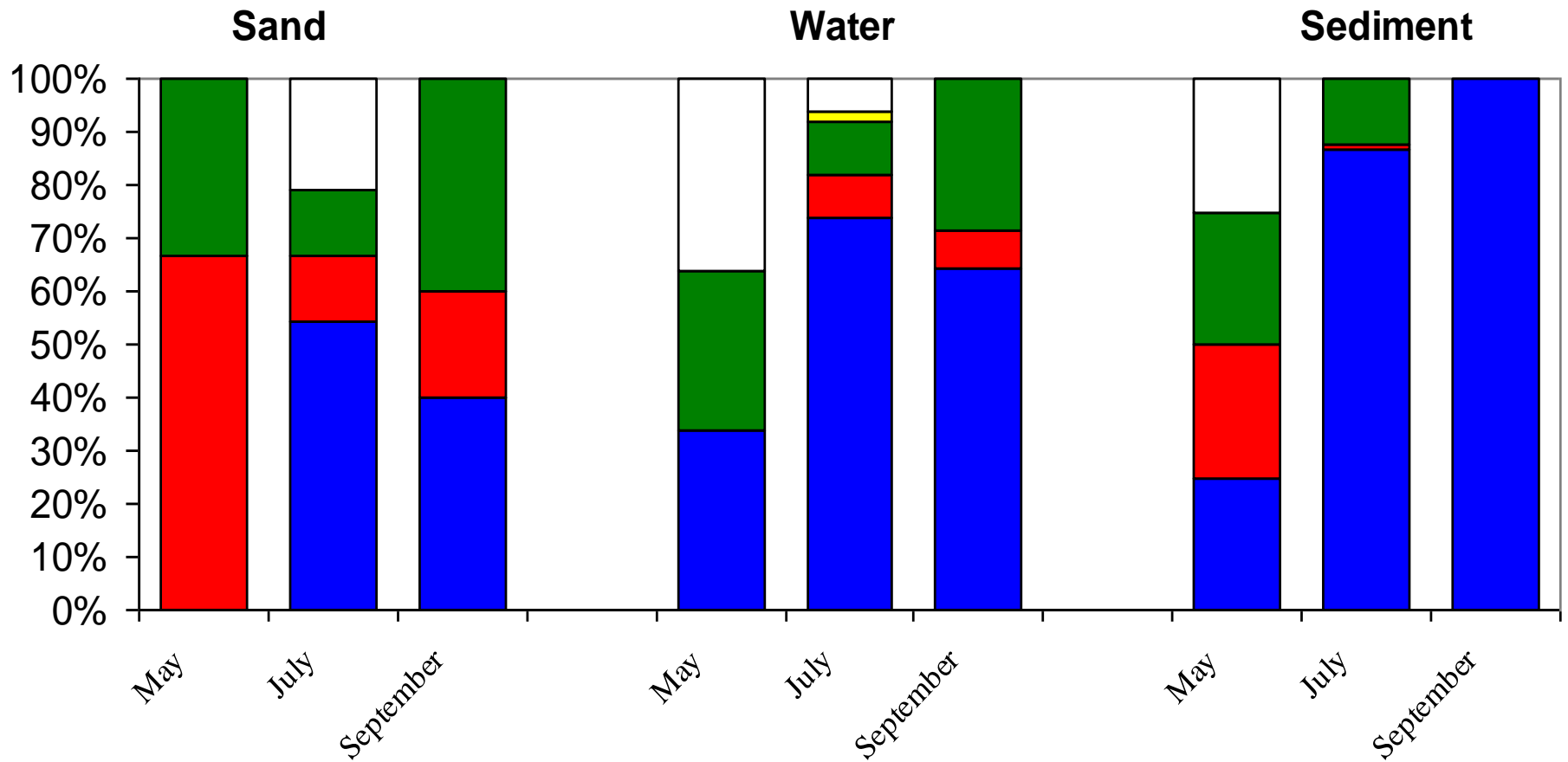


Blatnik Bridge Identified *E. coli* 2006



- Treated Wastewater
- Deer
- Mallards
- Ring-Billed Gulls
- Canadian Geese

Southworth Marsh Identified *E. coli* 2006



- Treated Wastewater
- Deer
- Mallards
- Ring-Billed Gulls
- Canadian Geese

Important Points

- Fecal coliform load from treated wastewater was not statistically different between seasons
- Gull numbers were also not different between seasons
- Goose numbers increased from May to July and September
- So did the goose's proportion of *E. coli* contamination, which decreased the proportion of *E. coli* from other sources

Conclusions

- Local waterfowl populations were a larger source of *E. coli* than treated wastewater in every season in the sand, water, and sediment of these two beaches.
- Treated wastewater relative contribution was larger in May compared to July and September, but the decrease in TWW relative contribution was probably caused by a larger contribution by waterfowl, especially geese.
- Ring-billed gulls were the most abundant bird species, but not the most important source of *E. coli* that could be identified.