Determining Sources of *E. coli* using rep-PCR in a North Coastal Massachusetts Watershed

COASTAL ZONE MANAGEMENT

Todd Callaghan

Paul Somerville

DIVISION OF MARINE FISHERIES

Jeff Kennedy

Dave Roach

DEPARTMENT OF ENVIRONMENTAL PROTECTON

Jean Tang

Ron Stoner

Oscar Pancorbo

DEPARTMENT OF FOOD AND AGRICULTURE

Scott Soares

Rep-PCR OVERVIEW

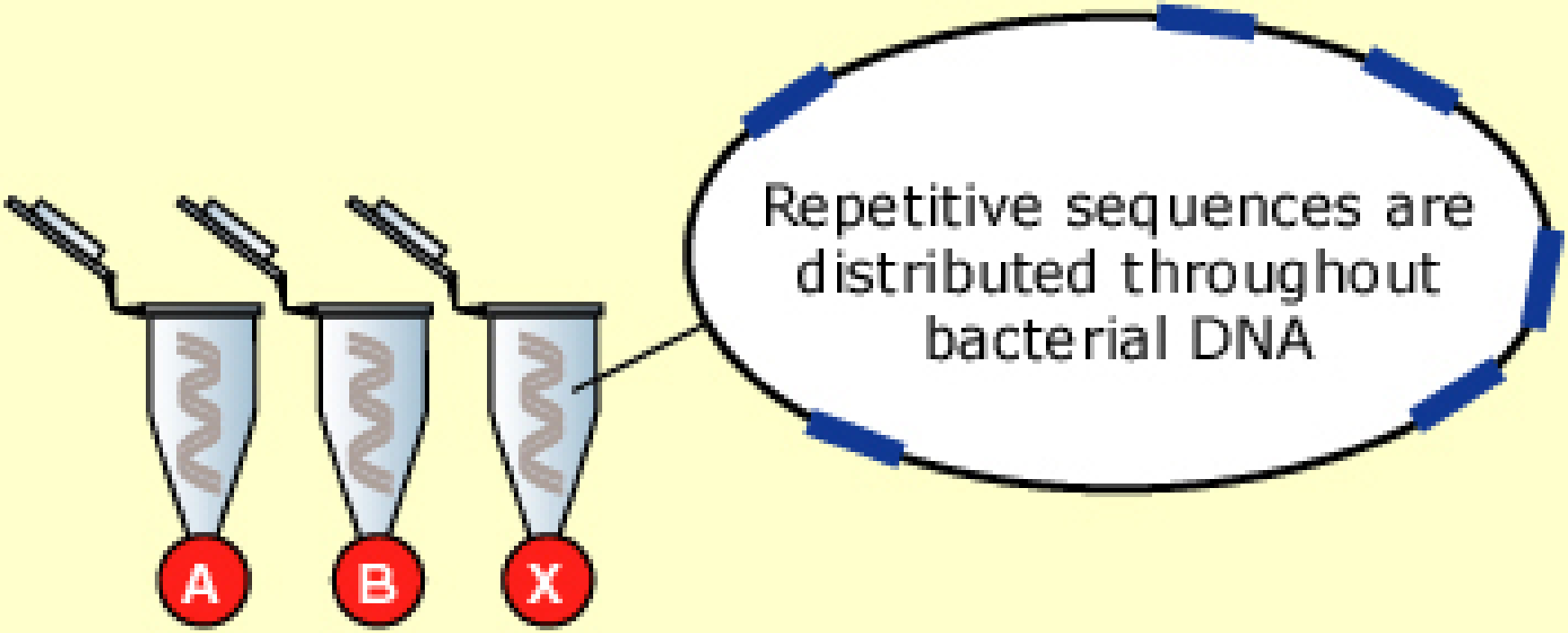
rep-PCR DNA FINGERPRINTING

- PCR amplification of the DNA between adjacent repetitive extragenic elements in bacteria, such as in *E. coli*

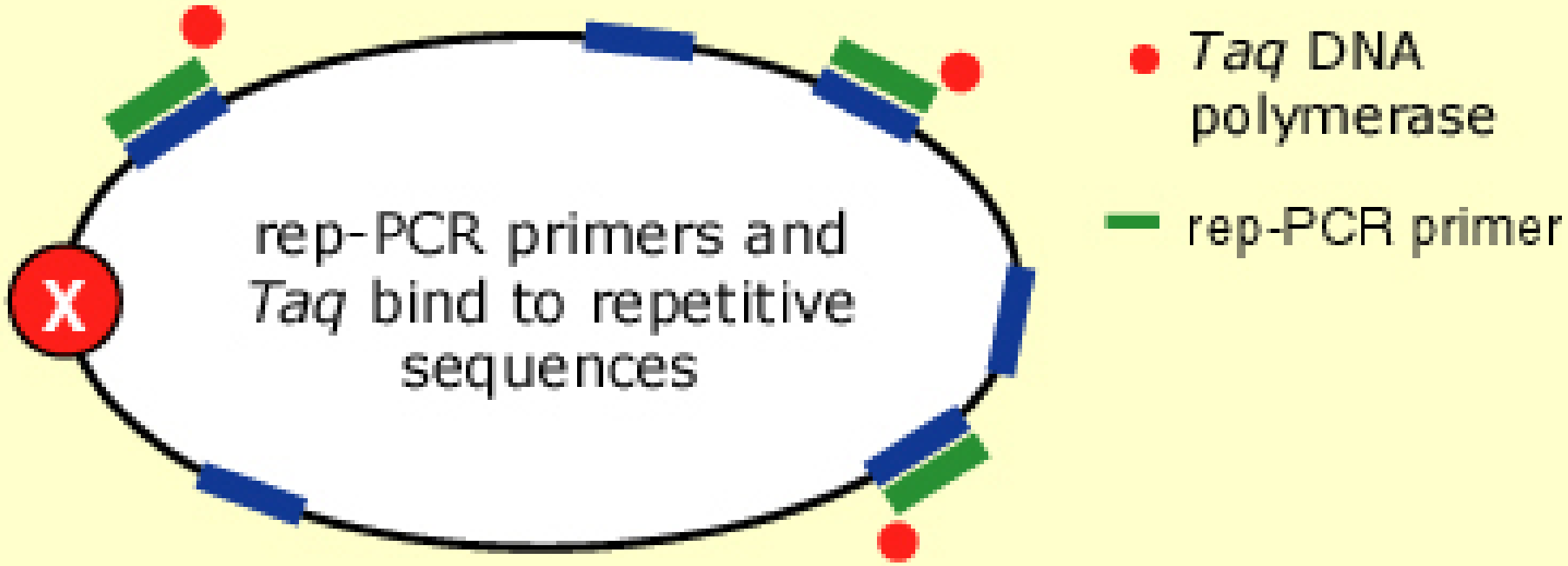
- Generates strain-specific DNA fingerprints that can be analyzed with computer software

- Does not require DNA isolation/ purification (i.e., can use whole-cell suspension); simple high-throughput analysis

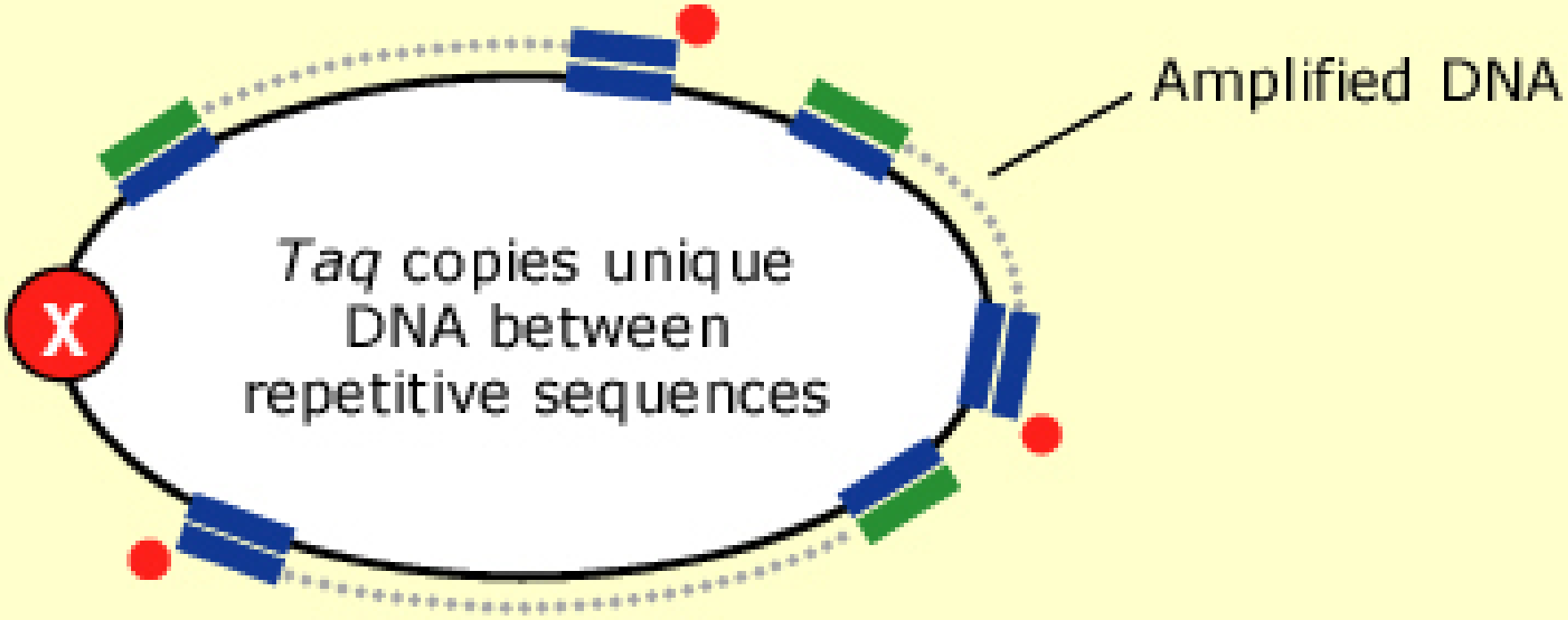
Rep-PCR FINGERPRINTING STEPS



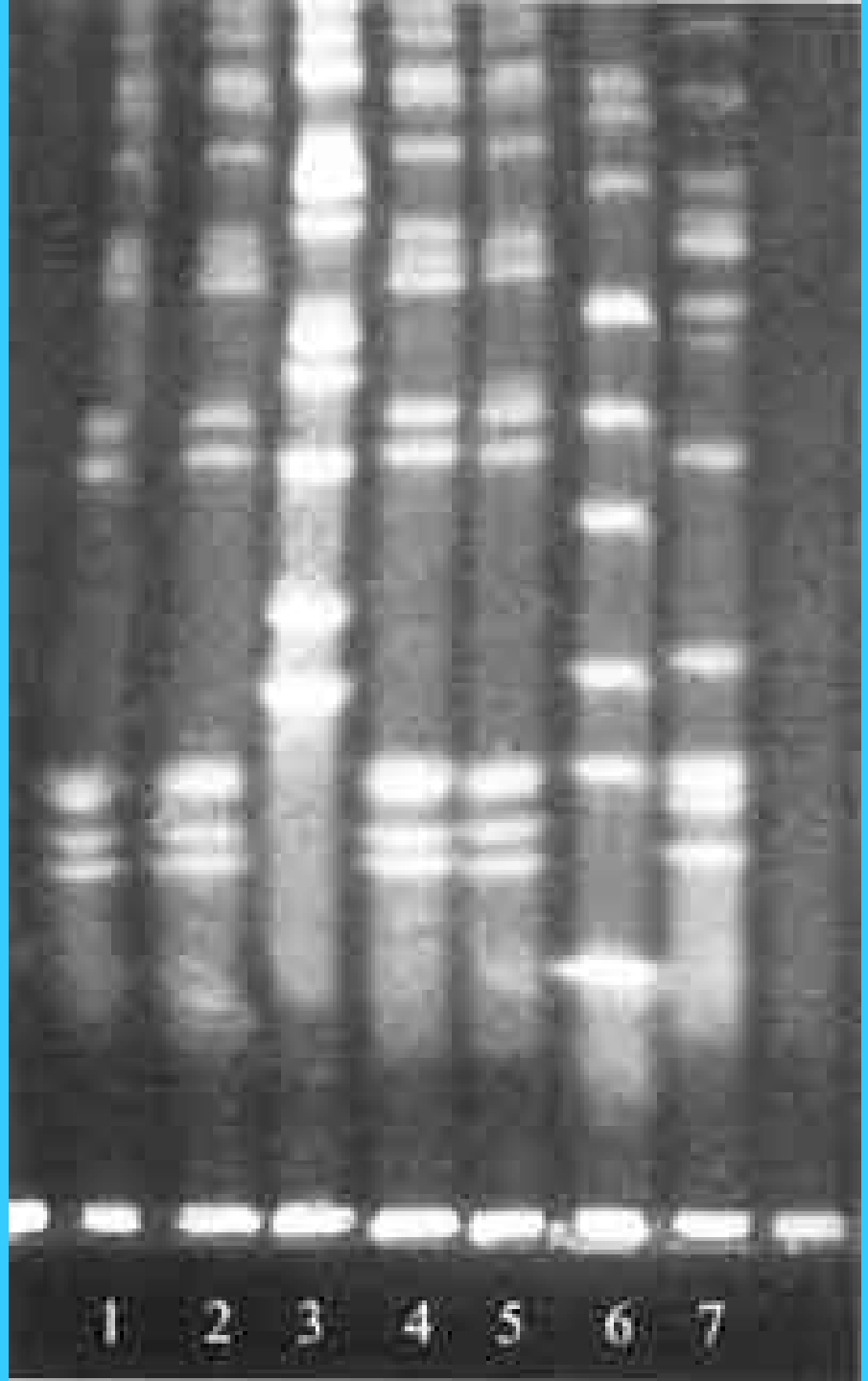
Rep-PCR FINGERPRINTING STEPS



Rep-PCR FINGERPRINTING STEPS
(30 PCR AMPLIFICATION SEQUENCES)

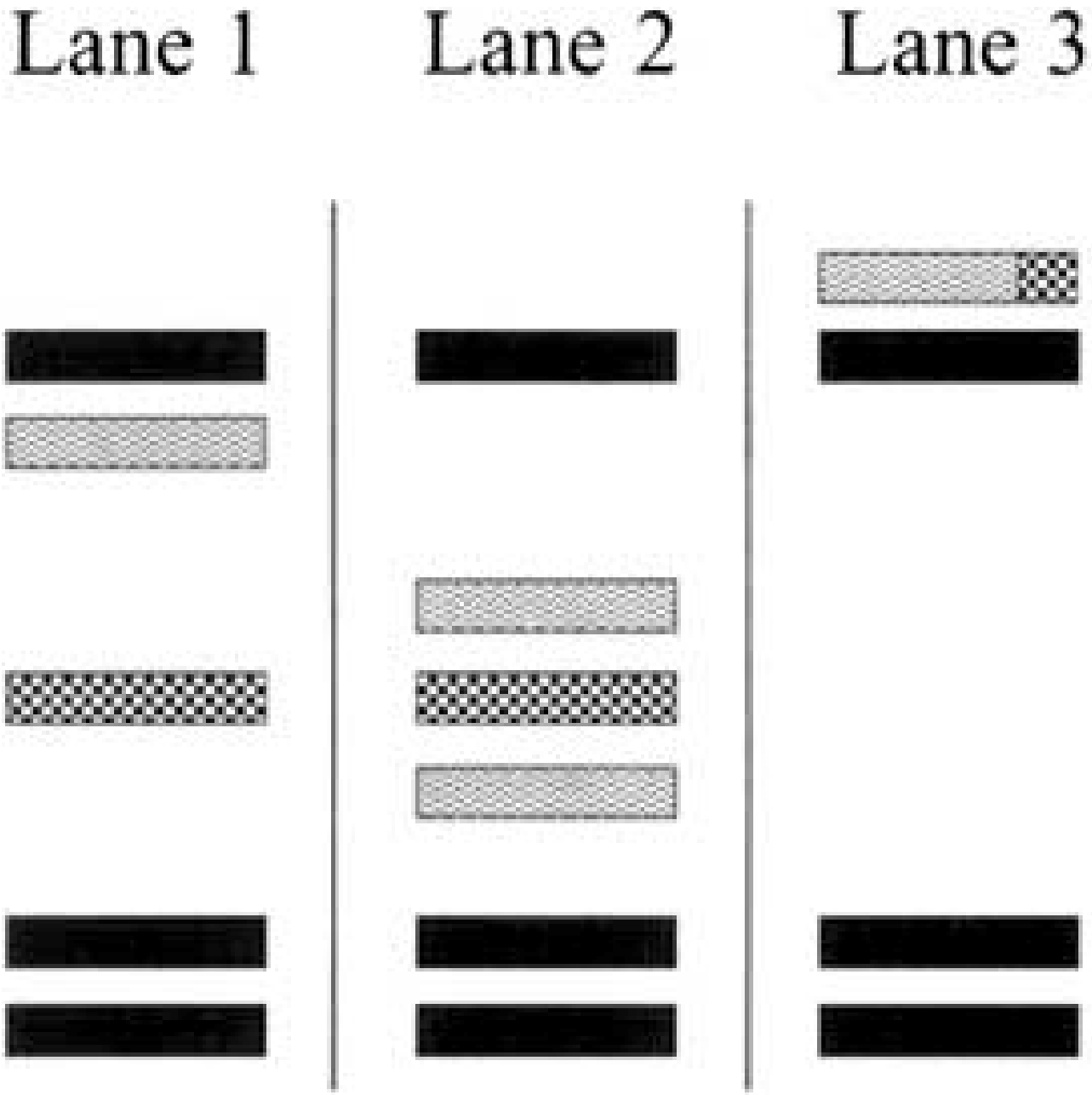


rep-PCR DNA FINGERPRINT PATTERN ON GEL



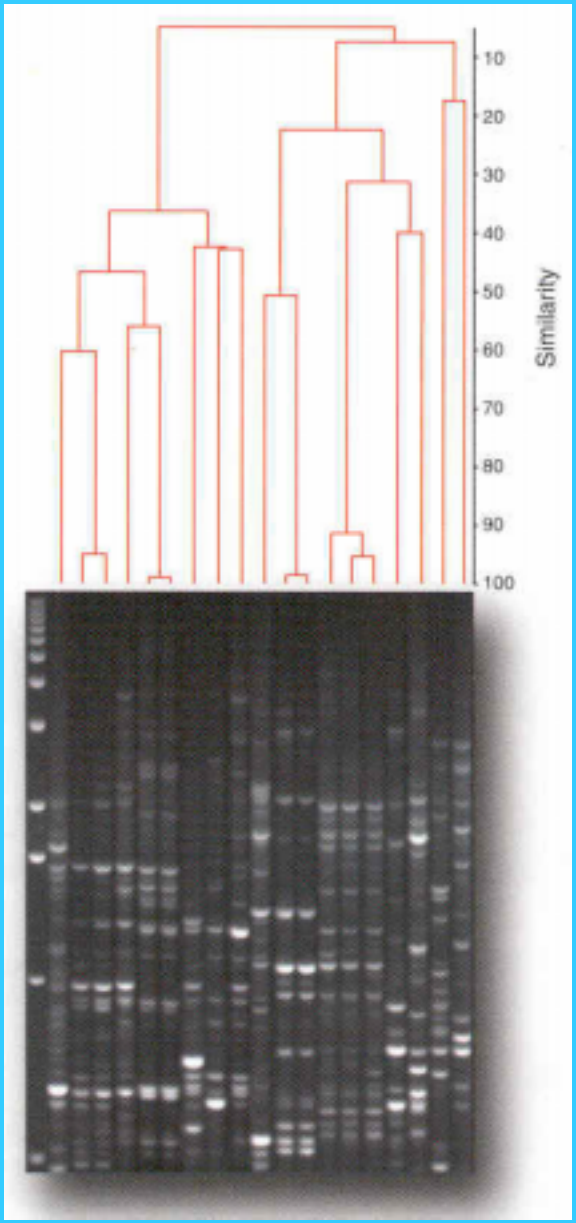
Bacterial BarCodes, Inc.

COMPARE Rep-PCR DNA FINGERPRINT PATTERNS (Gel Bands)



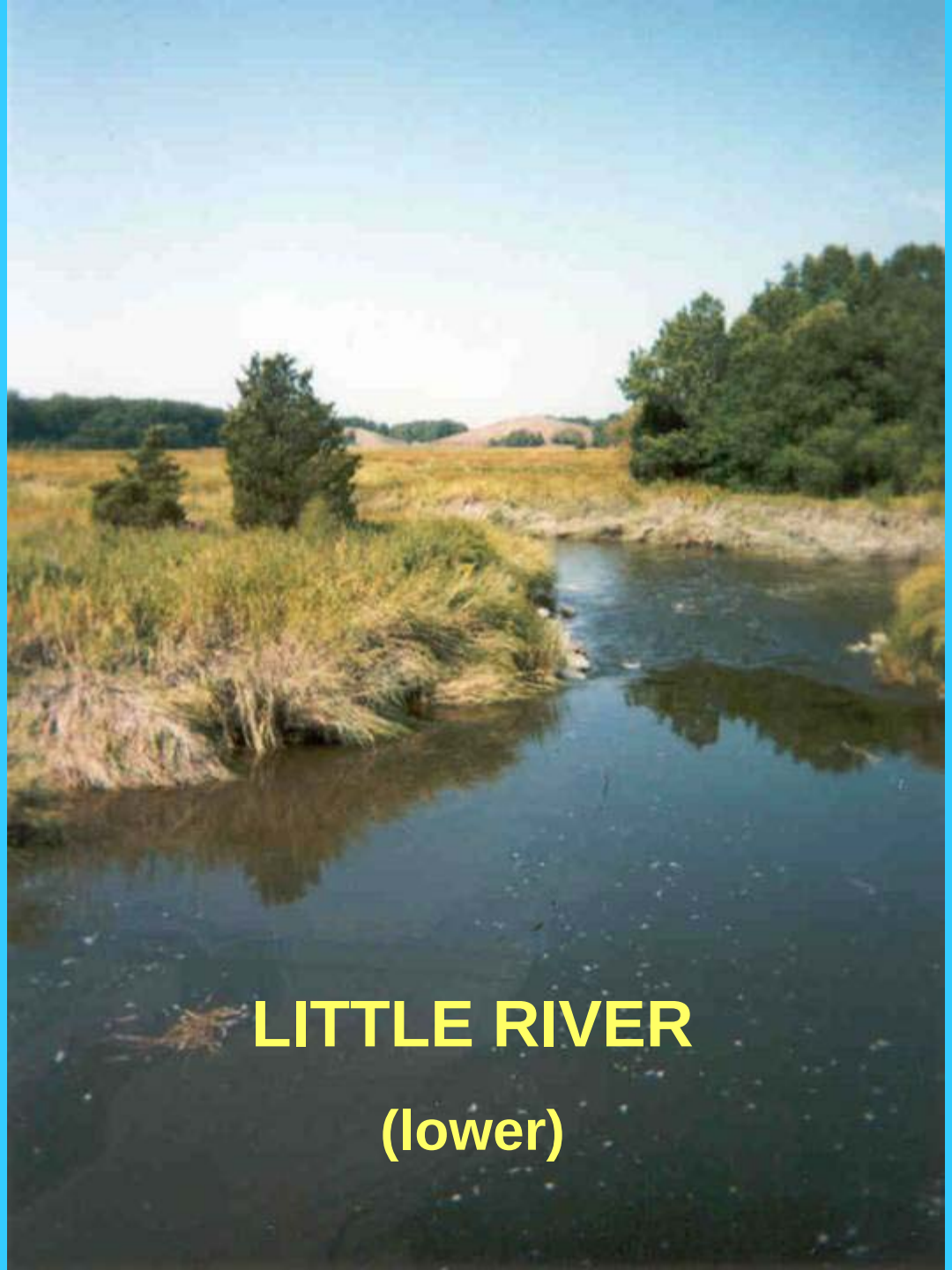
Bacterial BarCodes, Inc.

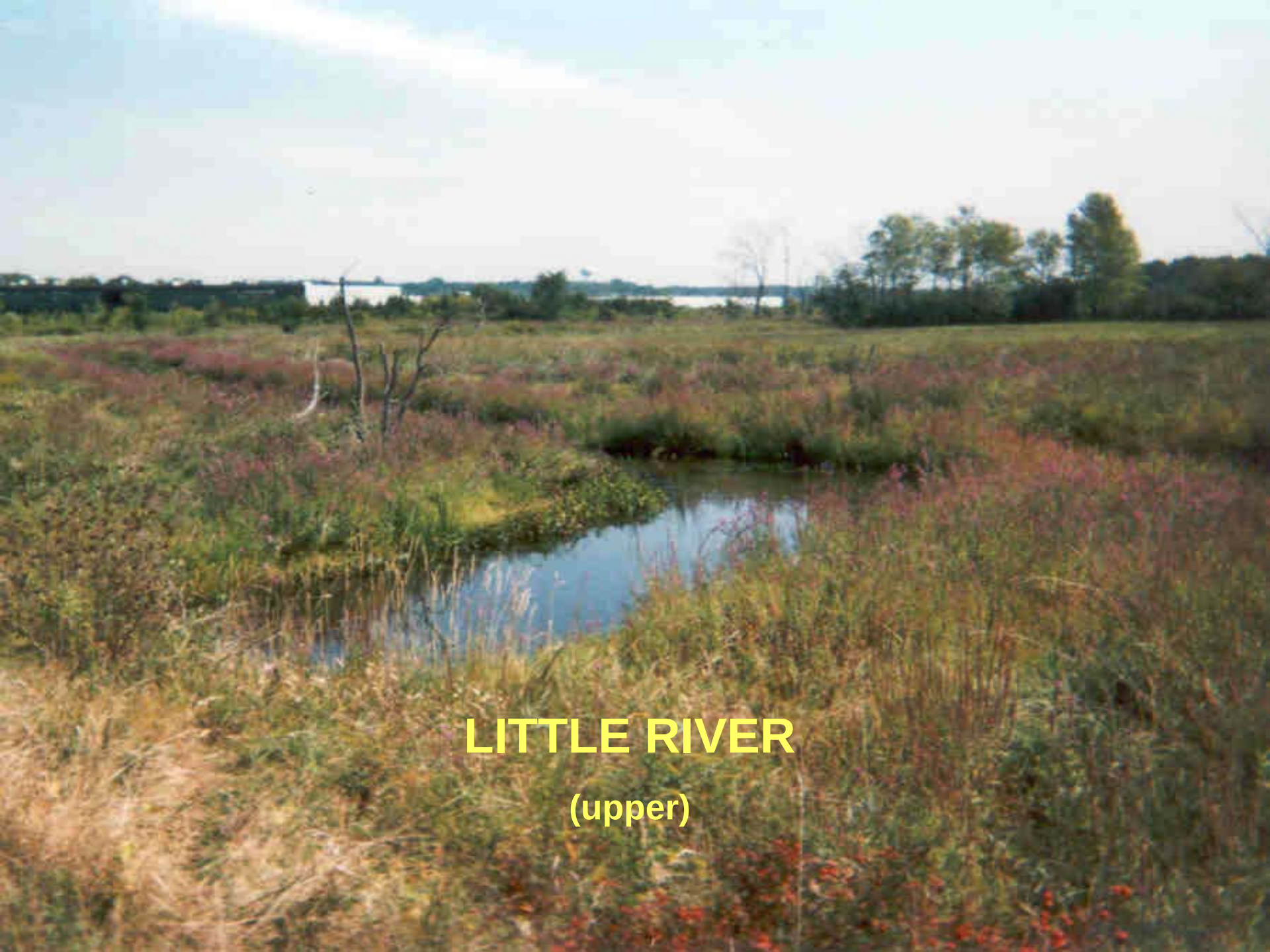
CLUSTER ANALYSIS OF Rep-PCR DNA FINGERPRINTS

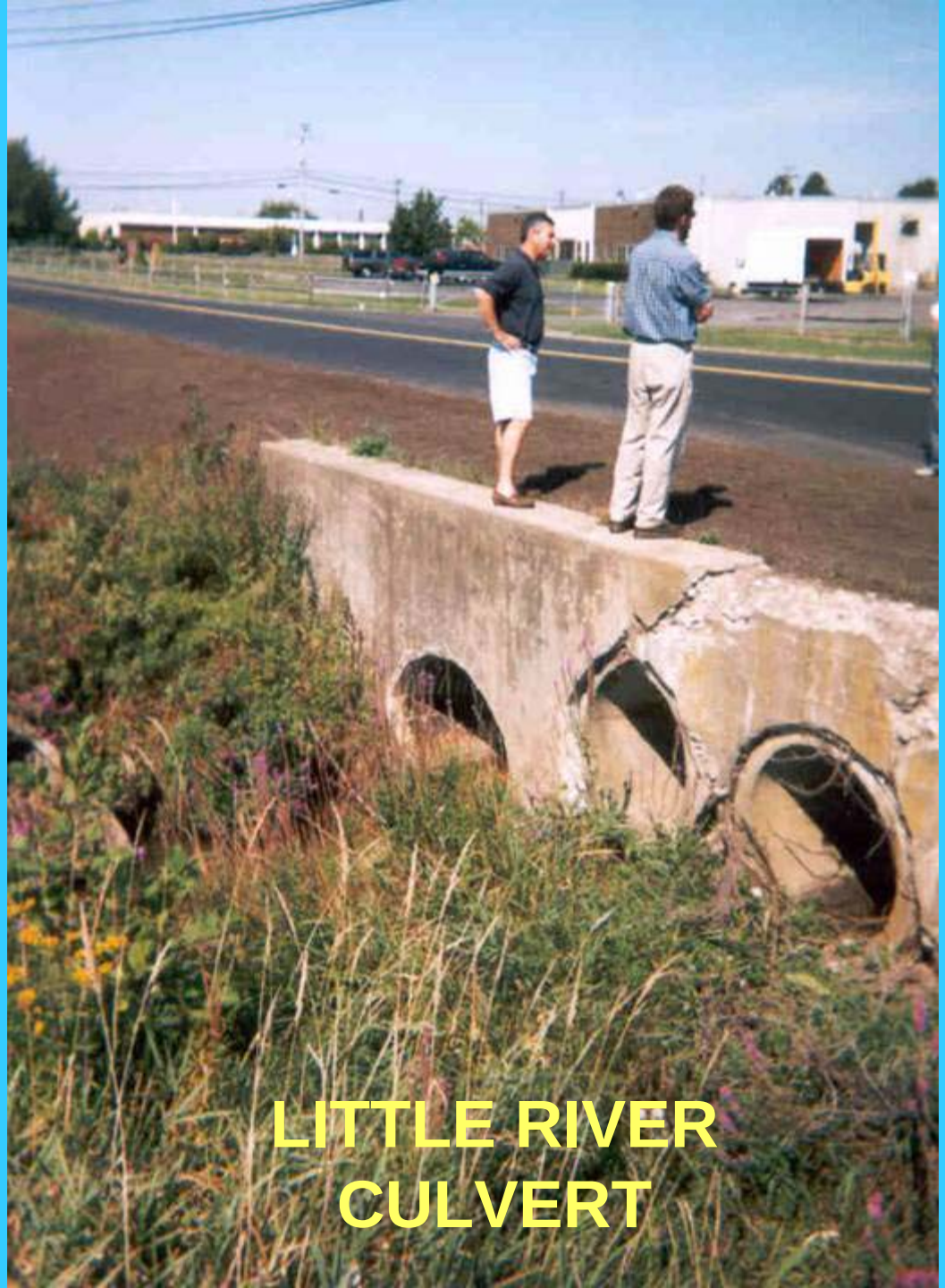


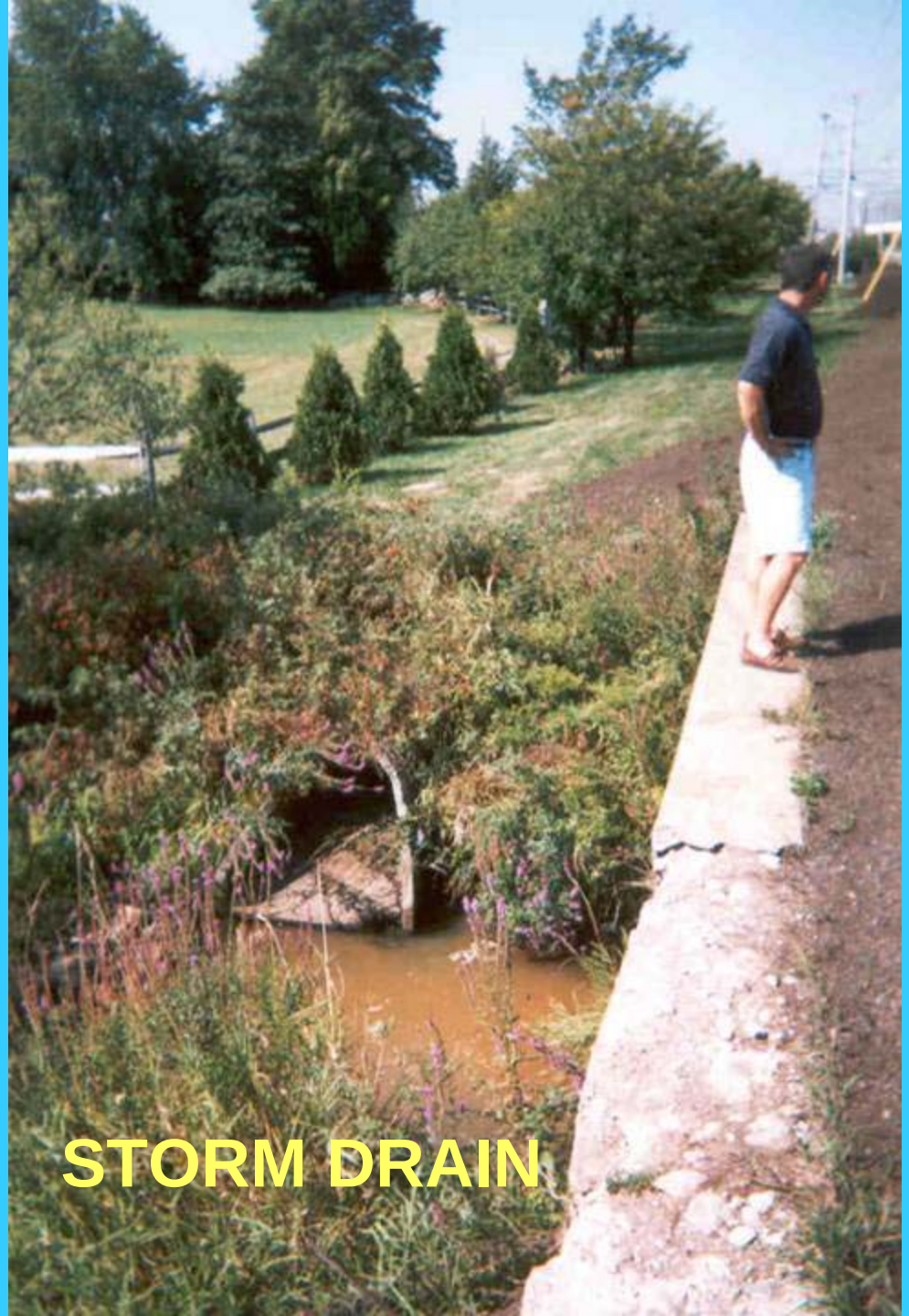
DENDROGRAM SHOWS % SIMILARITY OF BACTERIAL ISOLATES

STUDY SITE


LITTLE RIVER
(lower)

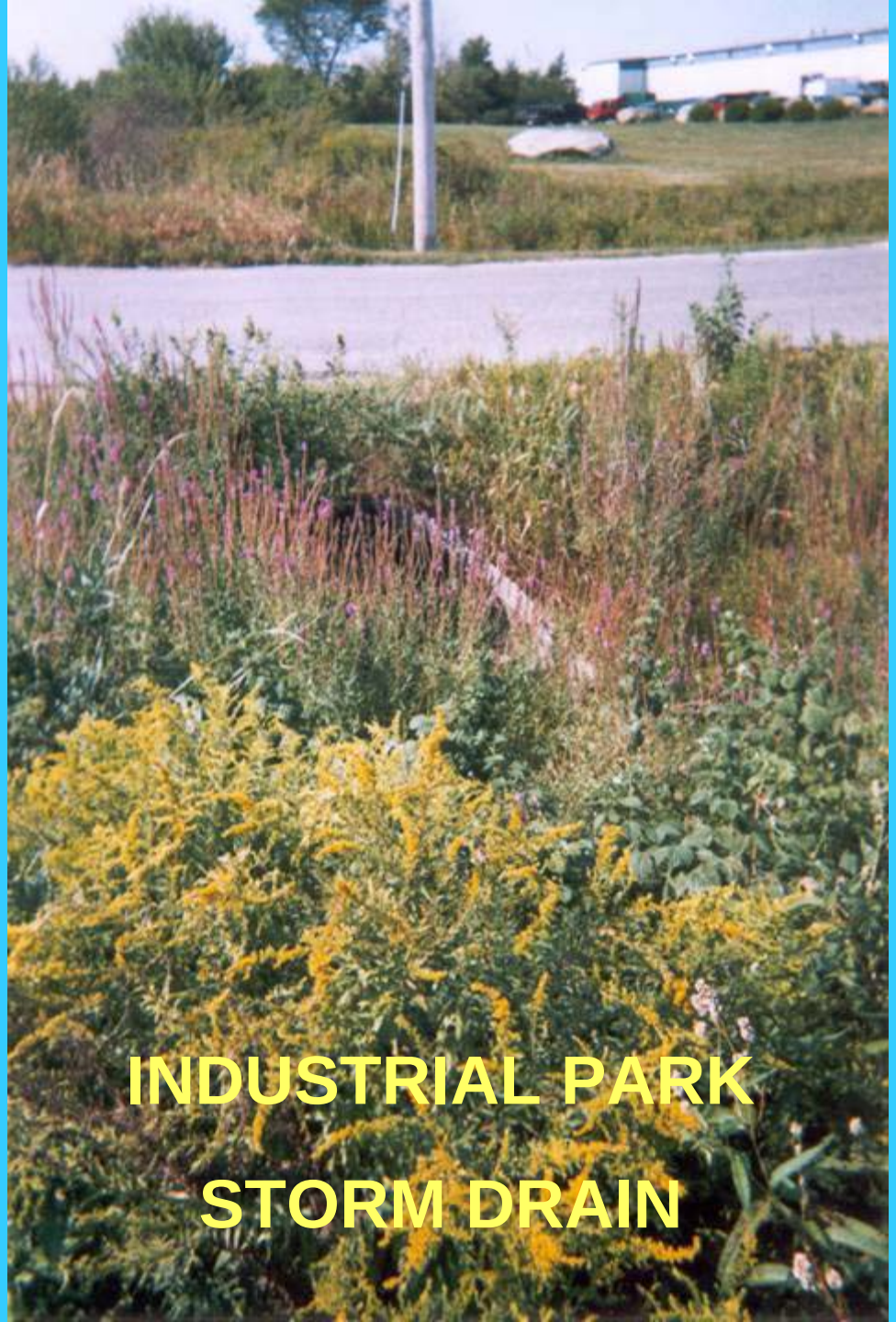

LITTLE RIVER
(upper)


LITTLE RIVER
CULVERT

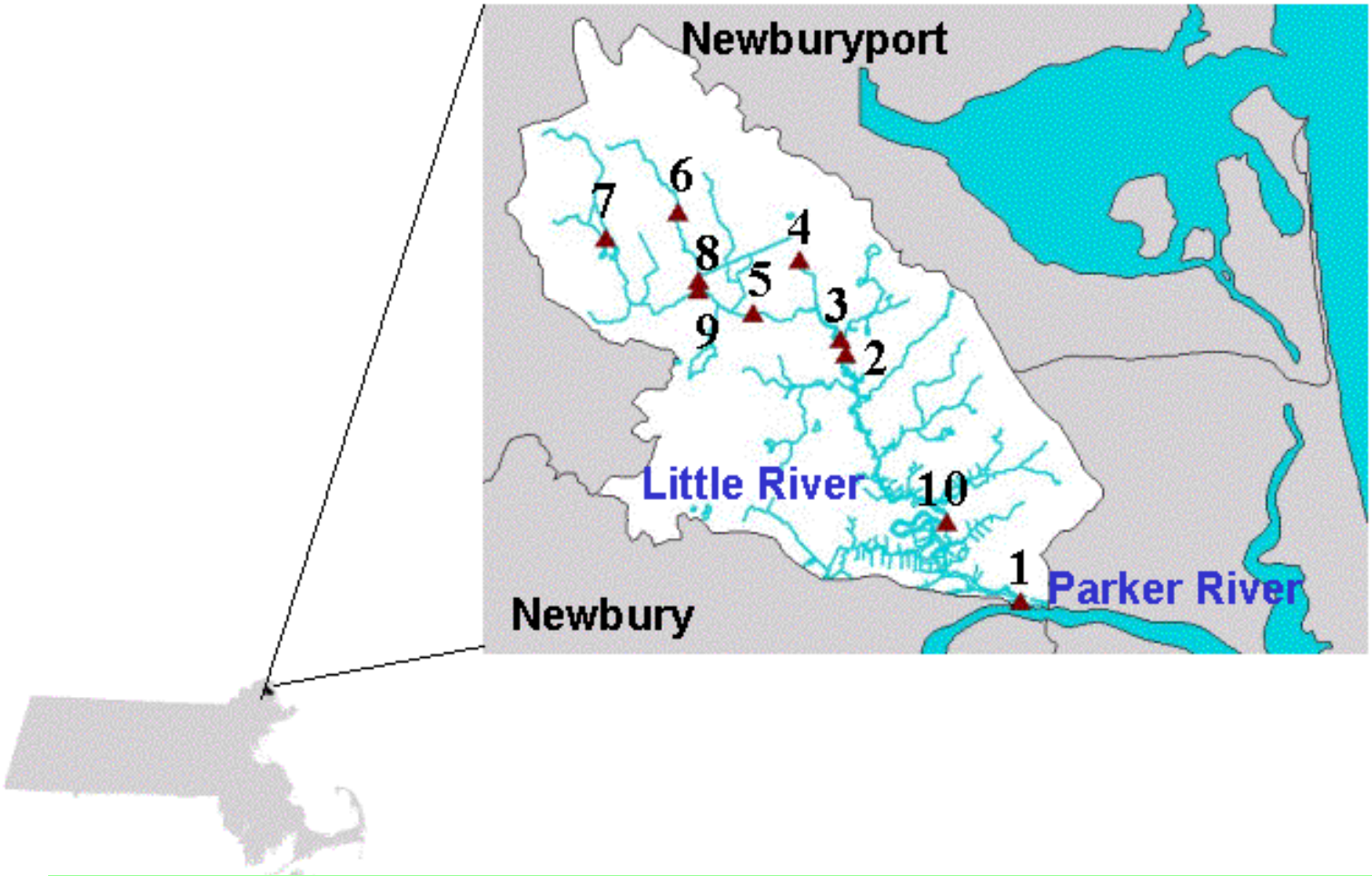

STORM DRAIN

STORM DRAIN

INDUSTRIAL PARK


INDUSTRIAL PARK
STORM DRAIN

COW PASTURE



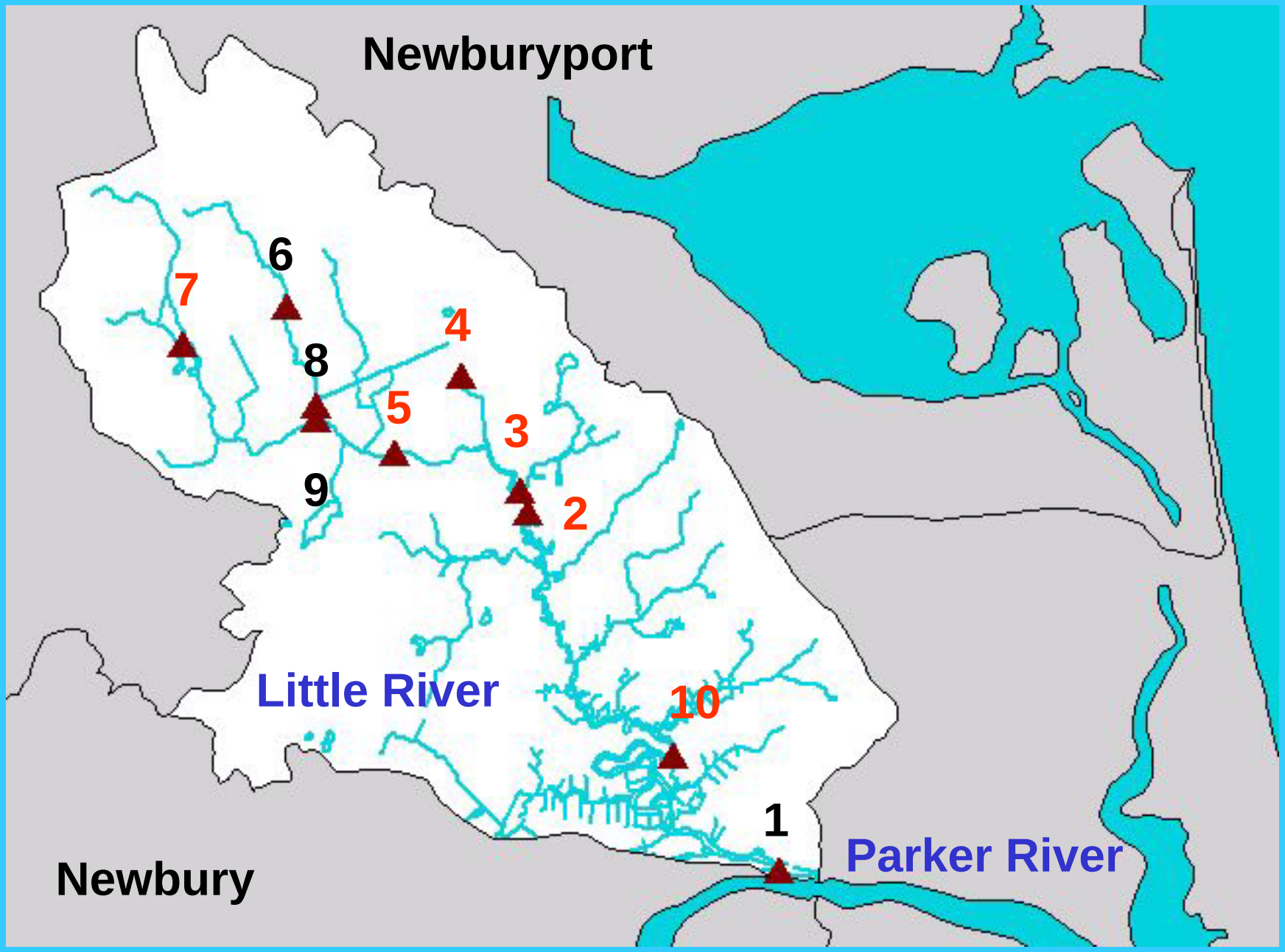
Study area along the Little River in Newburyport, MA. Triangles 2-10 are water quality sampling sites.

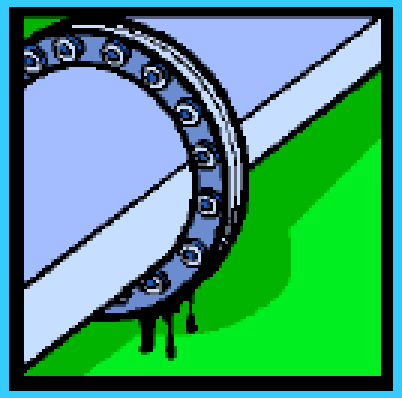
Site 1 is an oyster bed.

Historical Geometric Mean Fecal Coliform Counts (#/100ml) at Select Study Sites

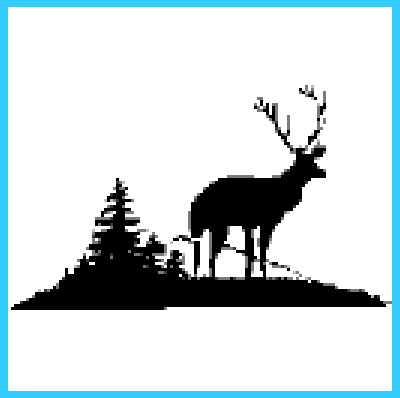
*Source: MA DMF

Site	1992	1993	1994	1995	1996	1997
2	108	532	532	--	--	79
3	94	1689	--	--	565	1470
4	1587	1335	330	--	390	117
5	347	180	223	--	34	58
6	87	50	--	--	--	--
7	61	764	133	--	149	33
10	278	220	742	--	270	78

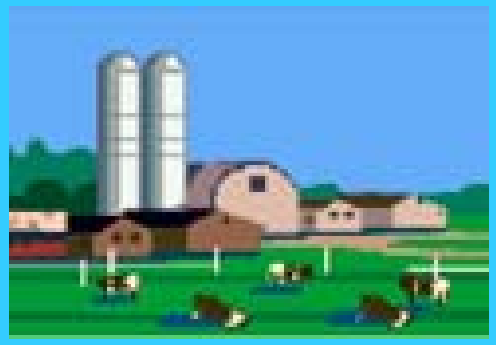

Newburyport
6
7
4
8
5
3
9
2
Little River
10
1
Parker River
Newbury



DOMESTIC



WILDLIFE

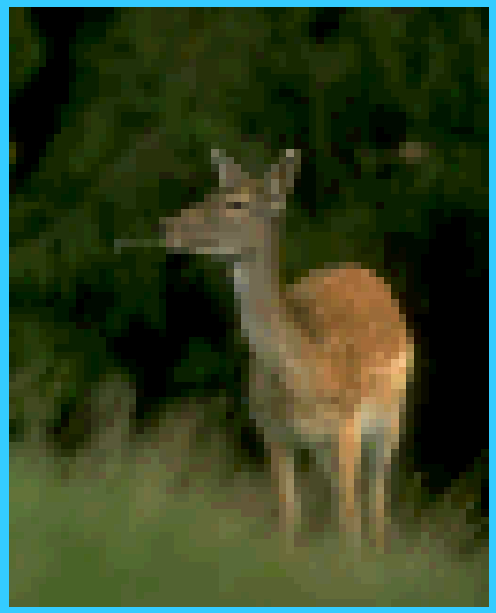


AGRICULTURAL

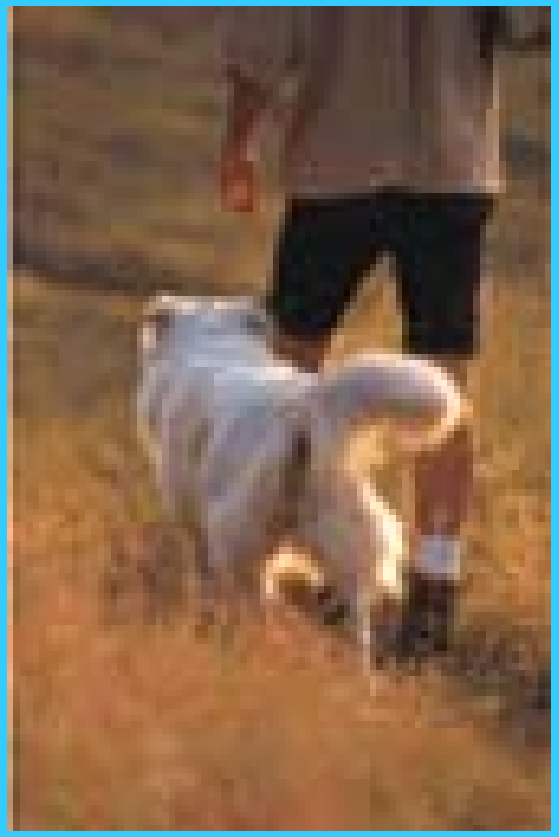
POTENTIAL SOURCES

BIRDS

AQUATIC MAMMALS



TERRESTRIAL MAMMALS



DOMESTIC PETS AND HUMANS

TOPSFIELD FAIR

COW

SHEEP

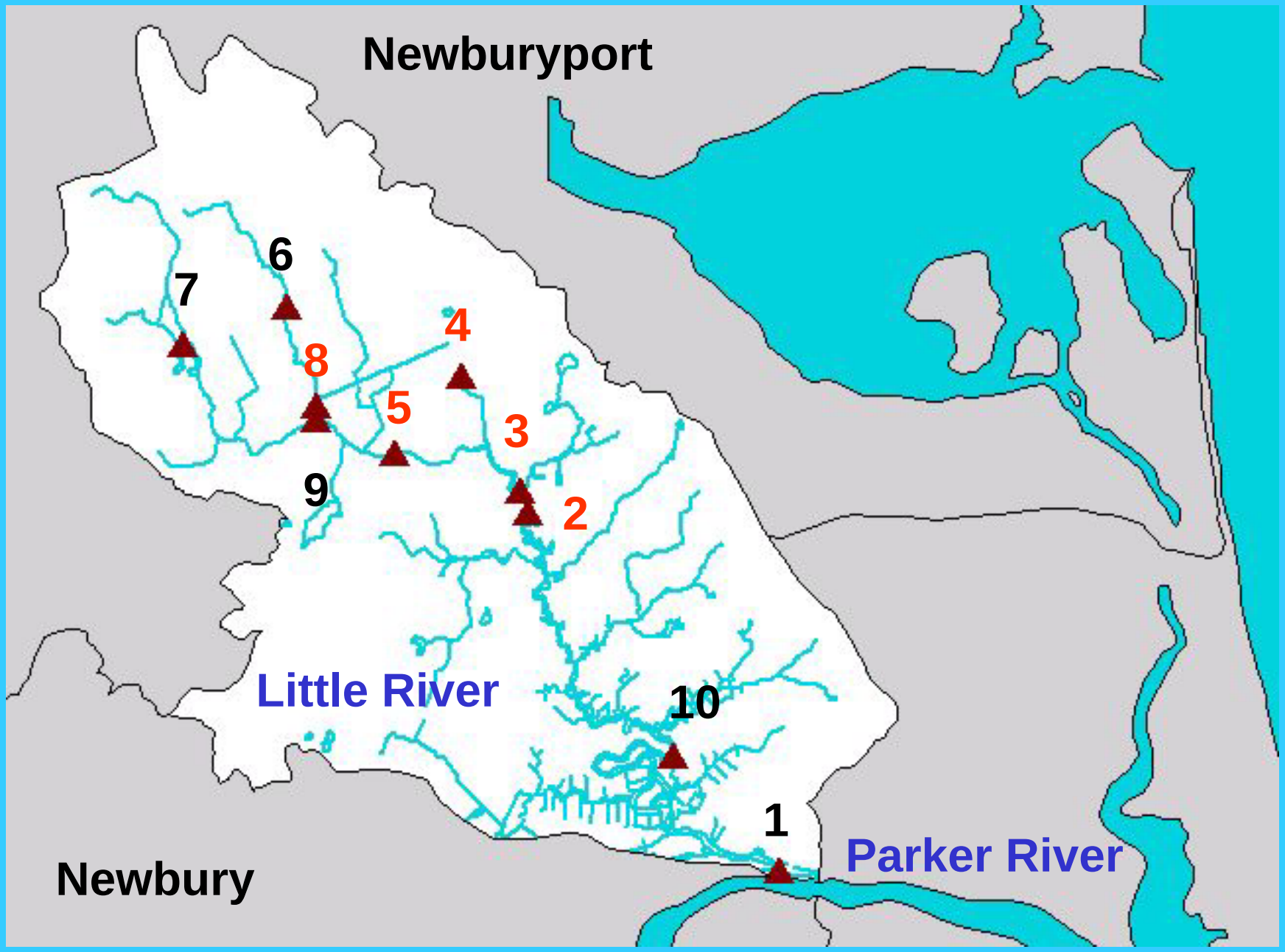
E. coli isolates in the Massachusetts DEP Wall Experimental Station library (as of 10/02).

Animal Type	Number of Animals	Total *E. coli* Isolates
Cat	15	83
Cow	12	48
Dog	20	76
Duck	19	69
Goose	20	69
Horse	18	74
Human	18	61
Pigeon	21	84
Seagull	26	99
Sheep	7	28
Total	176	691

WATER SAMPLES

Bacterial Indicator Concentrations in Water Samples from the Little River, MA. *Estimated values because of colony swarming

Collection Date	Sample Site	Fecal Coliform Concentration (CFU/100 mL) DEP	Fecal Coliform Concentration (CFU/100 mL) DMF	*E. coli* Concentration (CFU/100 mL)	*Enterococcus* Concentration (CFU/100 mL)
11-20-2002	6	120	61	100	400
11-20-2002	5	800	532	860	860
11-20-2002	4	1,200	347	330	540
11-20-2002	3	600	900	600	230
11-20-2002	2	1,100	900	900	460
11-20-2002	7	32	87	52	26
11-21-2002	2	210*	347	350	26
11-21-2002	3	280*	347	230	230
11-21-2002	4	71*	35	73	110
11-21-2002	5	220*	347	350	210
11-21-2002	8	330*	532	330*	170
11-21-2002	9	65	46	63	90
11-25-2002	10	130	133	130	130
11-25-2002	2	N/A	46	230	210
11-25-2002	3	130	57	84	26
11-25-2002	4	35	46	42	68
11-25-2002	5	52	57	58	140


Newburyport
6
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Little River
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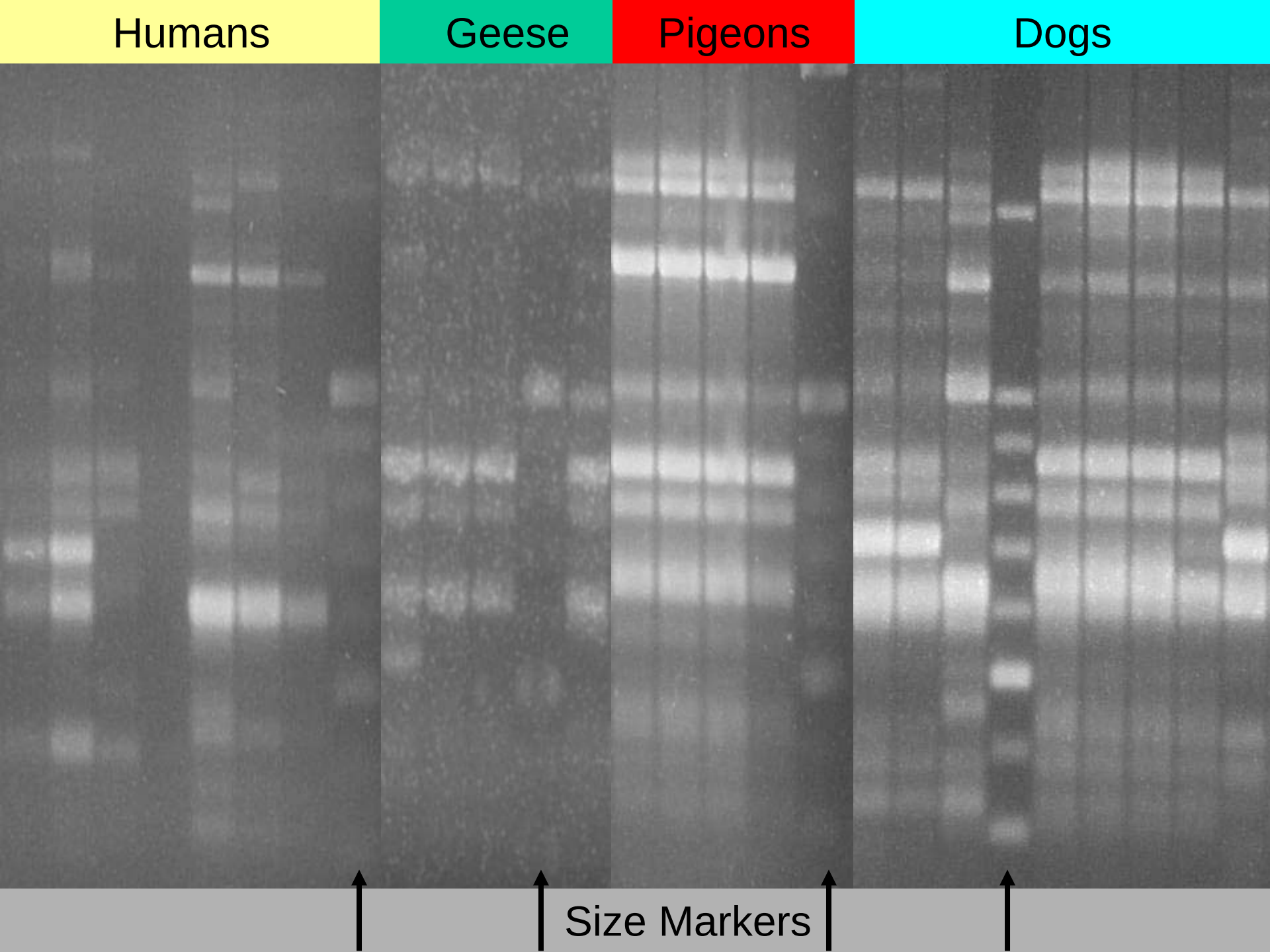
Parker River
Newbury

ANALYSIS


Humans
Geese
Pigeons
Dogs
Size Markers

Preliminary Data

November 20, 2003

- **16 *E. coli* isolates from Sites 3, 5**
- **Only 3 (19%) match DNA fingerprints in library at ≥ 80%.**

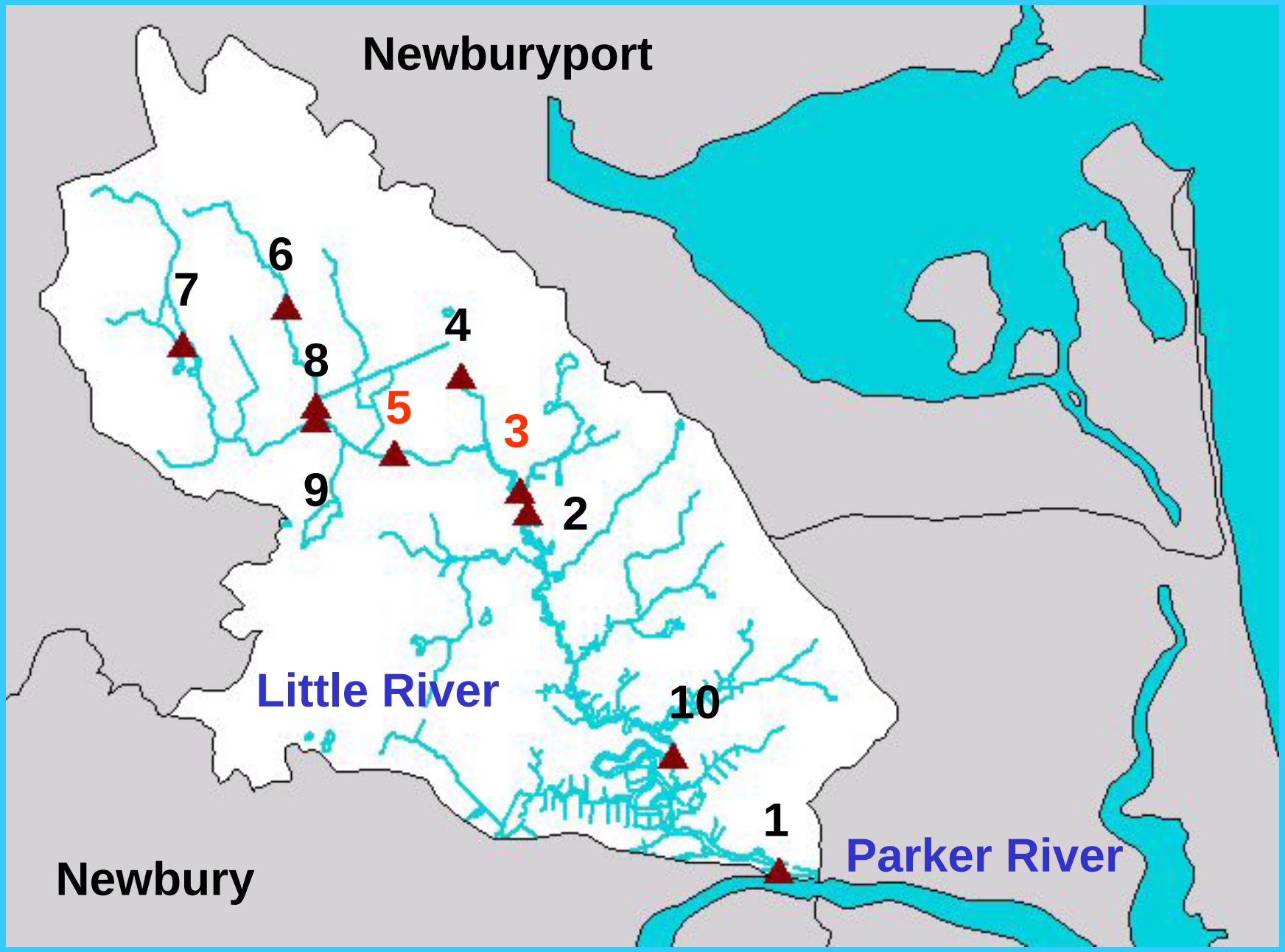
- **Sample site 5**

1 isolate matched sheep at 83%

- **Sample site 3**

1 isolate matched goose at 88%

1 isolate matched sheep at 80%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Lessons Learned

- **Collecting samples for library can be time consuming and politically thorny**

- **Necessary to Isolate > 60 *E. coli* colonies for each species-specific strain in library**

- **Wildlife may be larger contributor than initially thought.**