Identifying, Quantifying and Tracking Microbial Contaminants, Antibiotics and Antibiotic Resistance Genes in Order to Protect Food and Water Supplies

Scott Bradford, A. Mark Ibekwe, and Brendan Headd

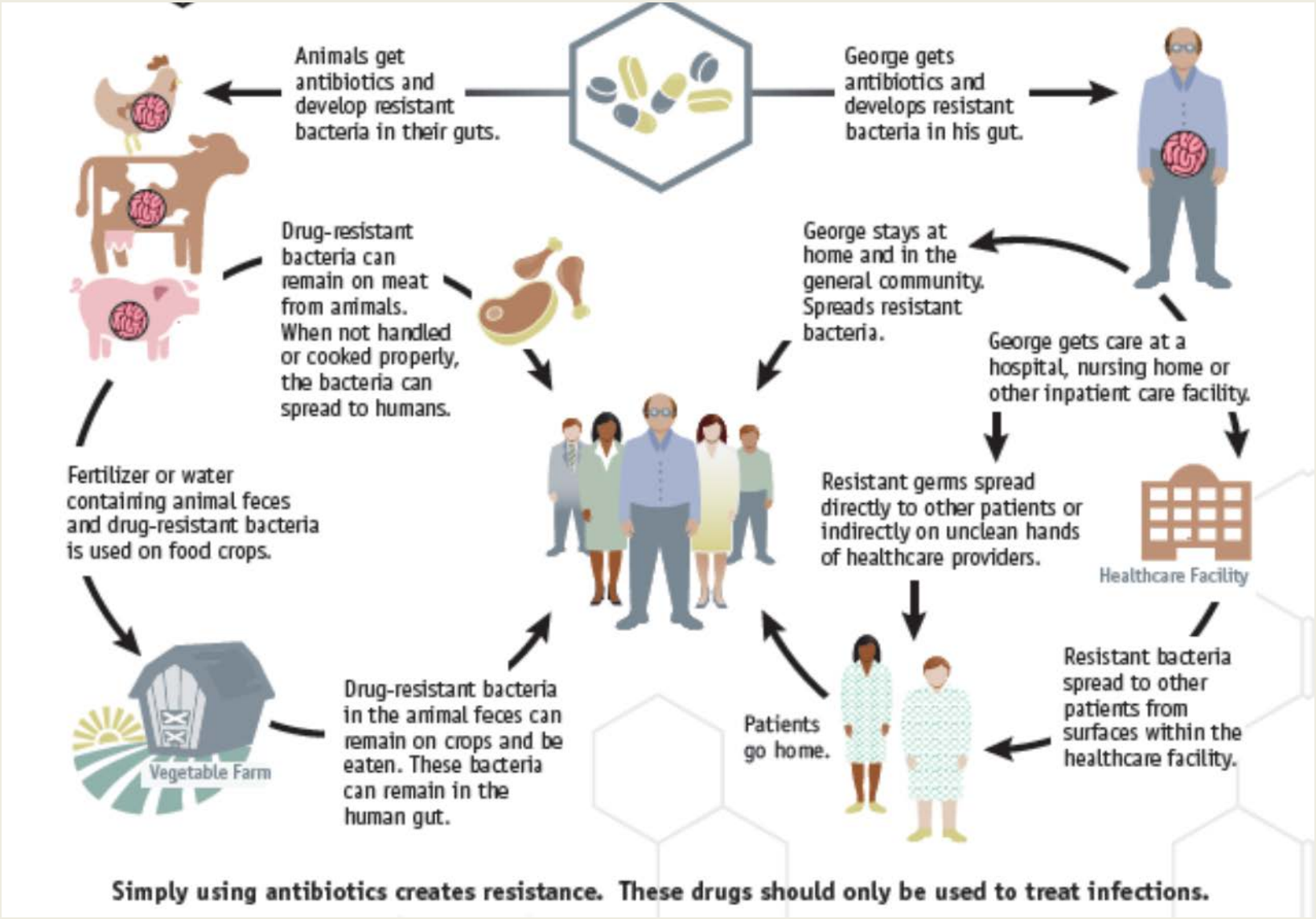
U.S. Salinity Lab, Agricultural Research Service, USDA, Riverside, CA

Antibiotic Resistance and Public Health

- In the United States each year (according to the CDC):
  - 2 million people are infected with Antibiotic Resistant Bacteria (ARB).
  - At least 23,000 die from the resulting infections.
  - Most infections occur in the community, while most deaths occur in healthcare facilities.
- The use of antibiotics is the most important factor leading to ARB around the world (CDC).
  - ARB arise naturally, but misuse speeds up the process.
  - Up to 50% of antibiotics are not optimally prescribed (CDC).
  - In some US states, the number of prescribed antibiotic treatments exceeds the population (Gross, 2013; Ventola, 2016).

ARB Occurs in Pristine Soils

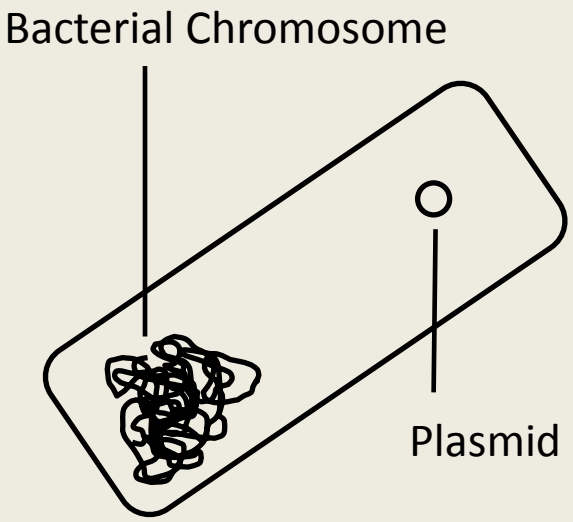
ARB Increases with Antibiotic Use

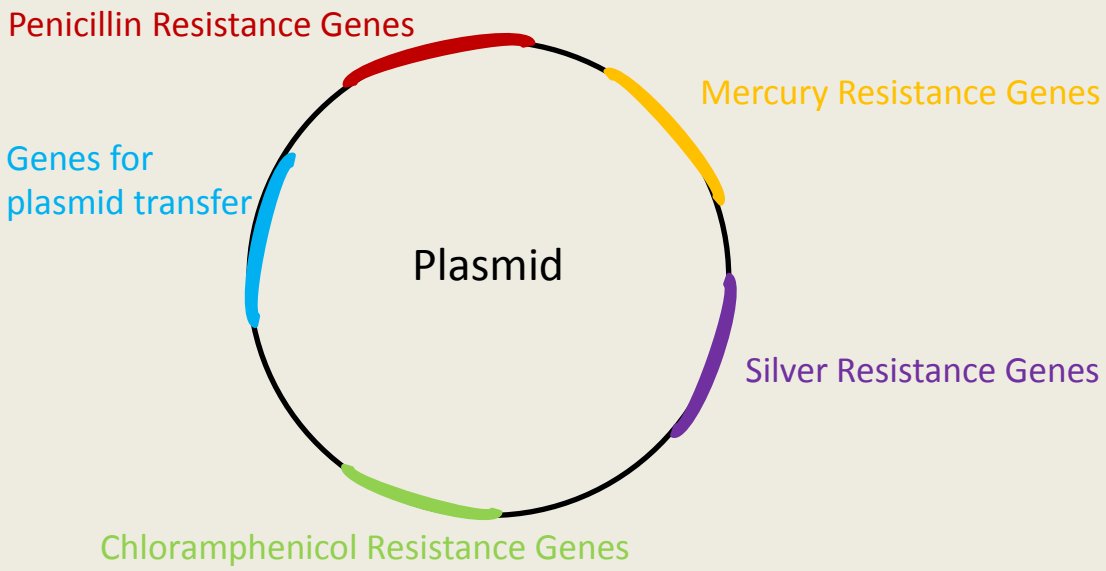


From: www.cdc.gov/drugresistance/about.html

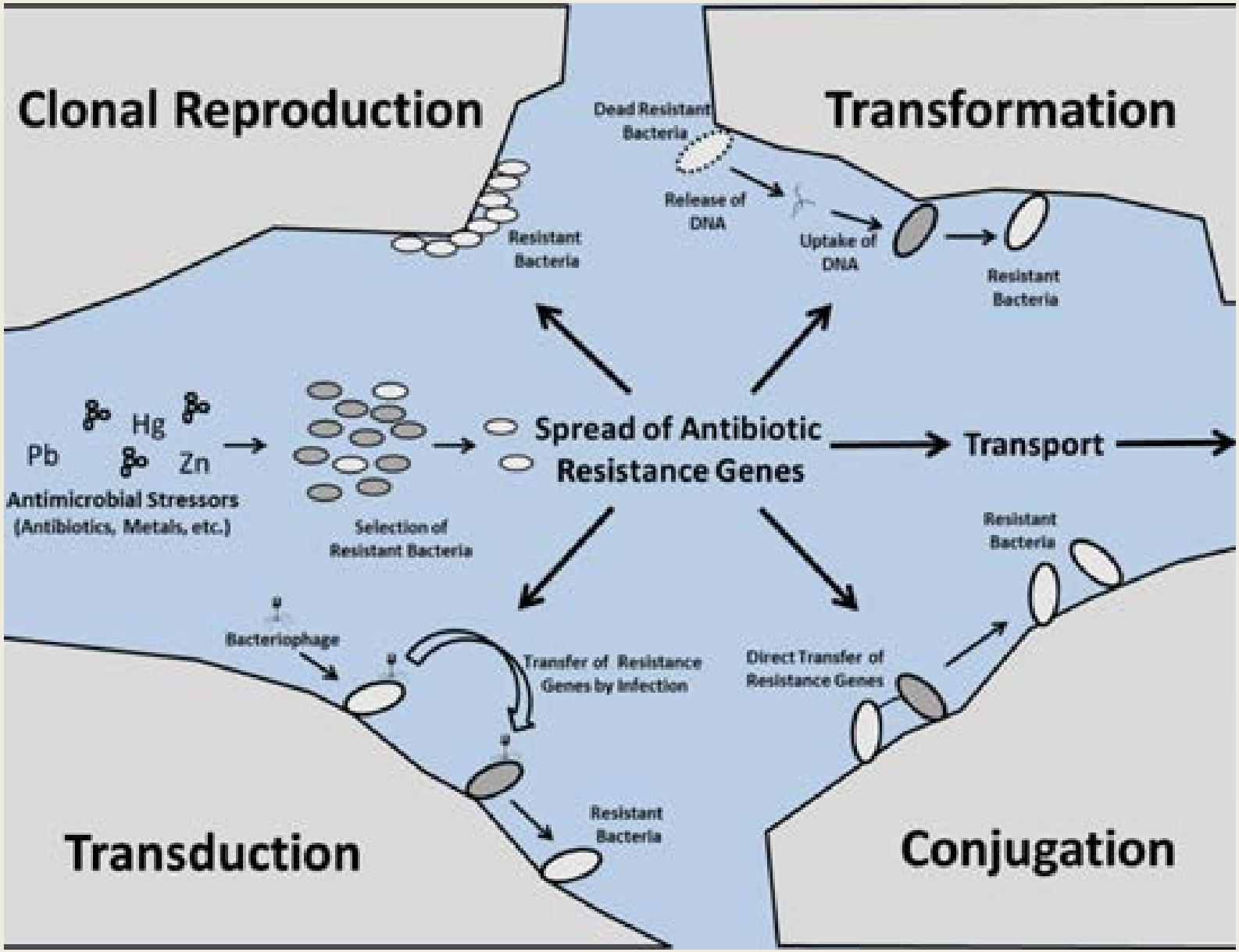
Environmental Stressors and ARB

- ARB arise naturally due to stressors in the environment
  - Antibiotic resistance genes (ARGs) are often found on plasmids.
  - These plasmids contain resistance genes to antibiotics, heavy metals, and other pollutants that allow bacteria to survive.
  - The presence of one of these stressors can ensure the survival and dissemination of all the other resistance genes on the plasmid through Horizontal Gene Transfer (HGT).



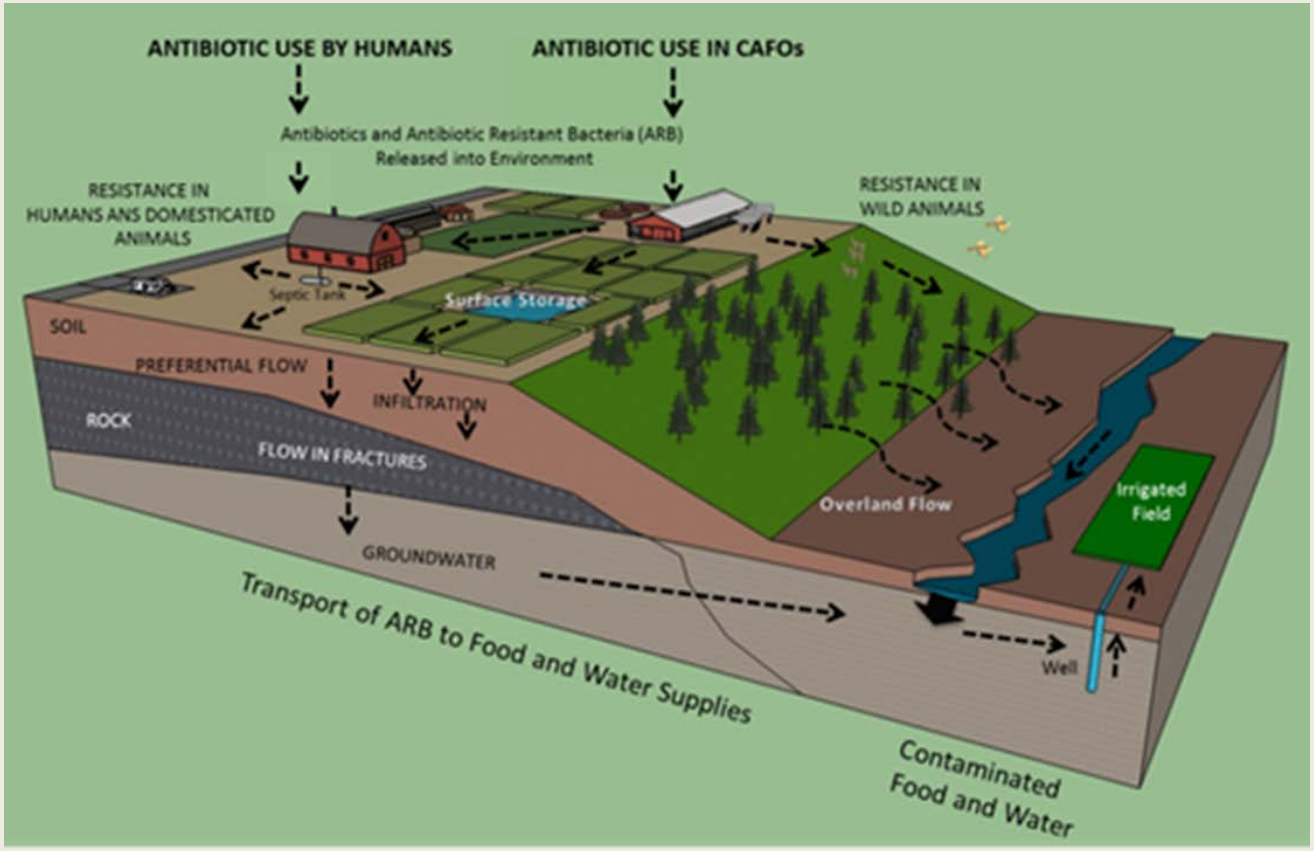


Modes of Antibiotic Gene Transfer



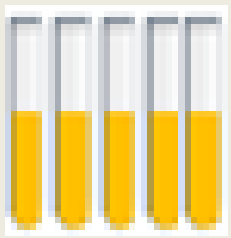
Environmental Fate of ARB

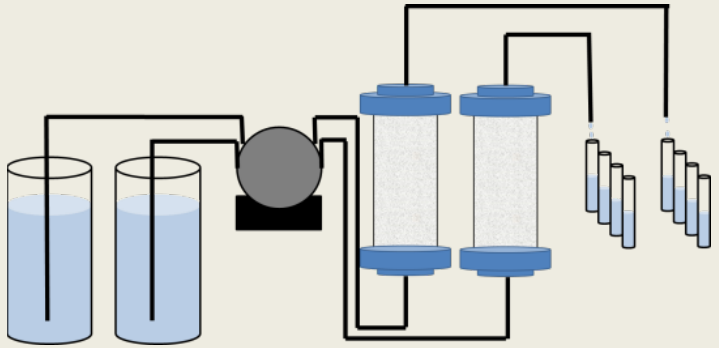
- Factors that influence retention and release of ARB in the nature:
  - Water chemistry, flow velocity, surface properties of bacteria



- What role does HGT play in the spread of ARB/ARGs in nature?
- Under what physiochemical conditions does HGT occur in nature?

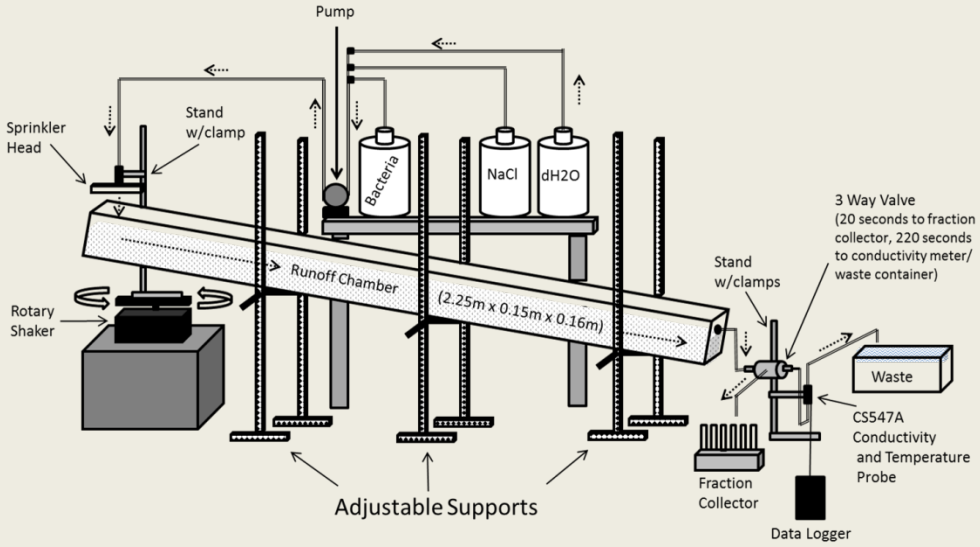
Batch and Column Experiments





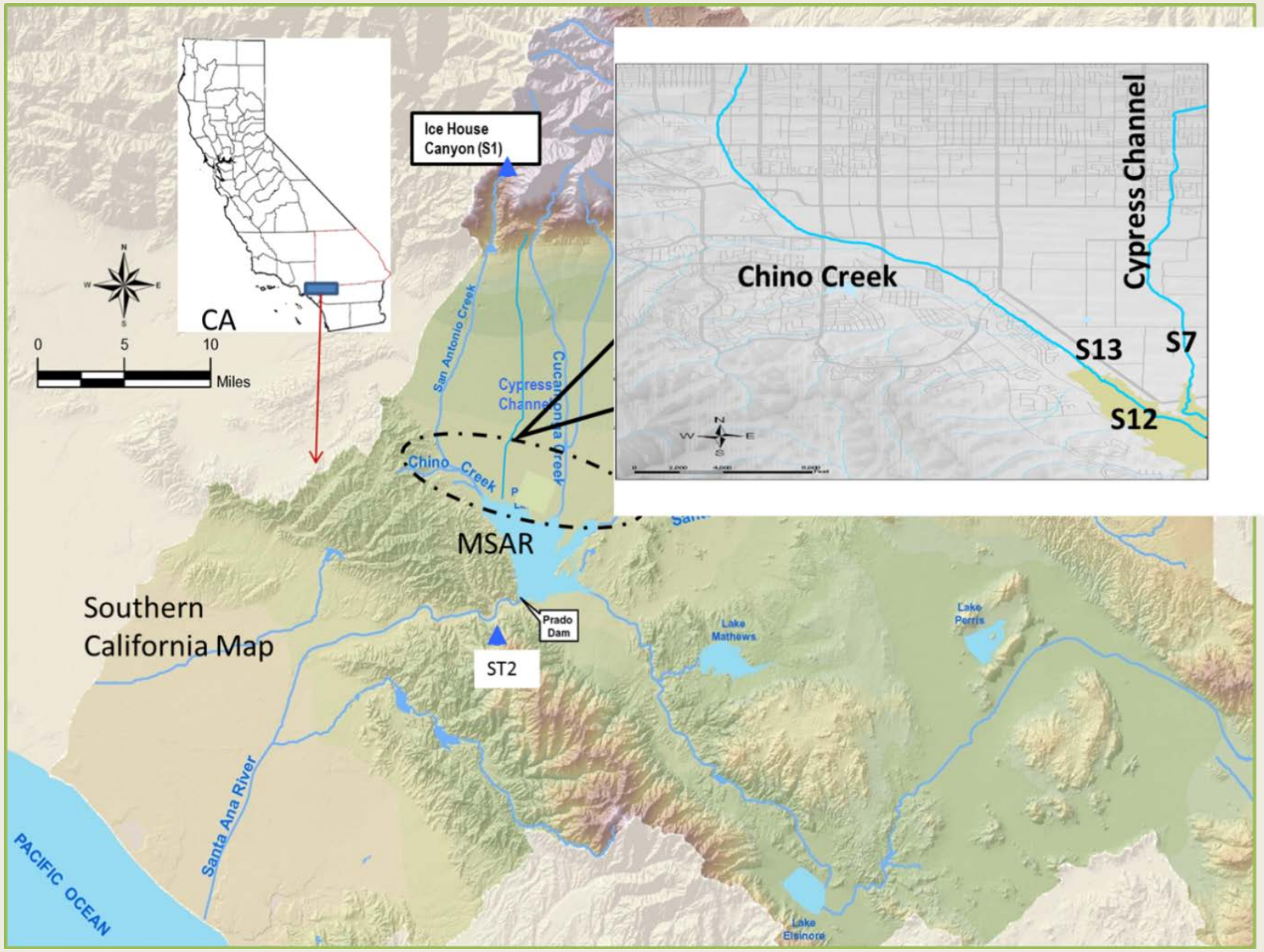
- Goal: Mechanistically study and model the transport, retention, and release of ARB and ARGs.
- Method: Idealized batch and column systems with fluorescent bacteria and selected antibiotic and metal resistant plasmids.
- Outcome: Model and identify environmental stressors and physicochemical conditions required for HGT and transport of ARB.

Runoff Chamber Experiments



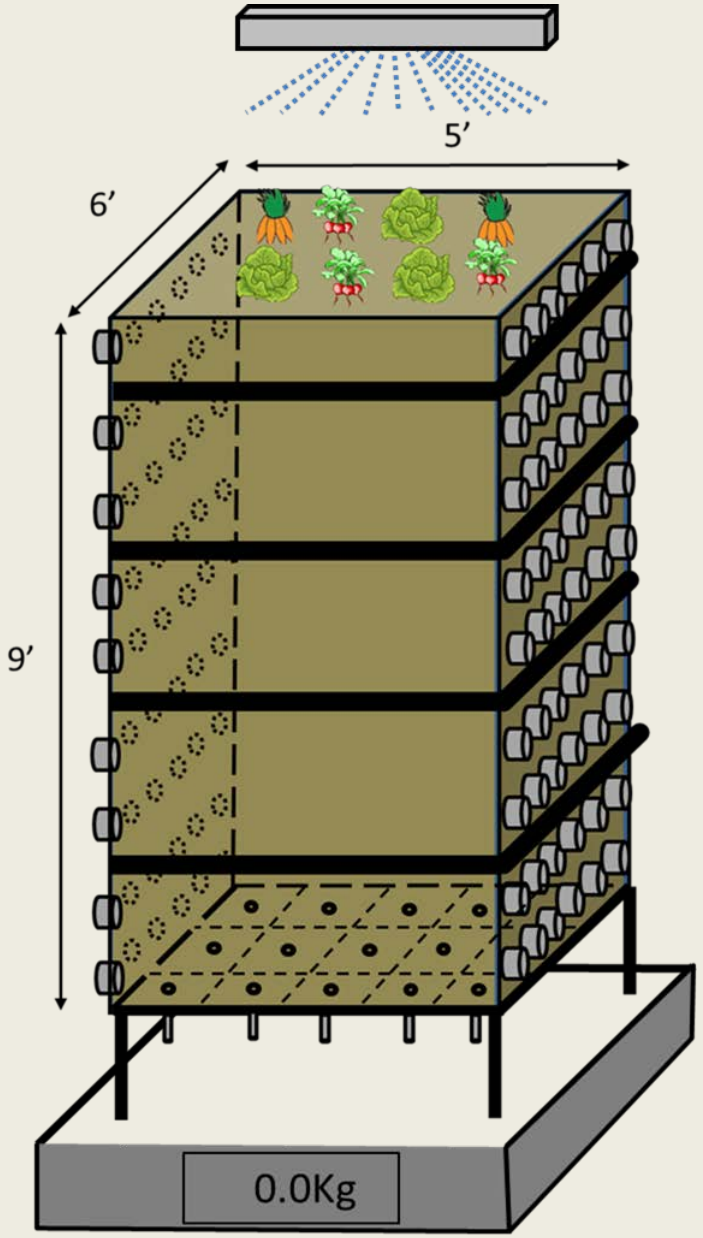
- Goal: Investigate factors that influence the development, spread and mitigation of ARB, ARGs, and pathogenic *E. coli* and *Salmonella* in sediment/runoff water from the Santa Ana River watershed.
- Method: Natural sediment and microbial communities spiked with stressors and pathogens in runoff chamber.
- Outcome: Modeling and isolation/identification/quantification of environmental pathogenic bacteria and ARGs.

Sample Locations - Runoff Chamber



Lysimeter Experiments

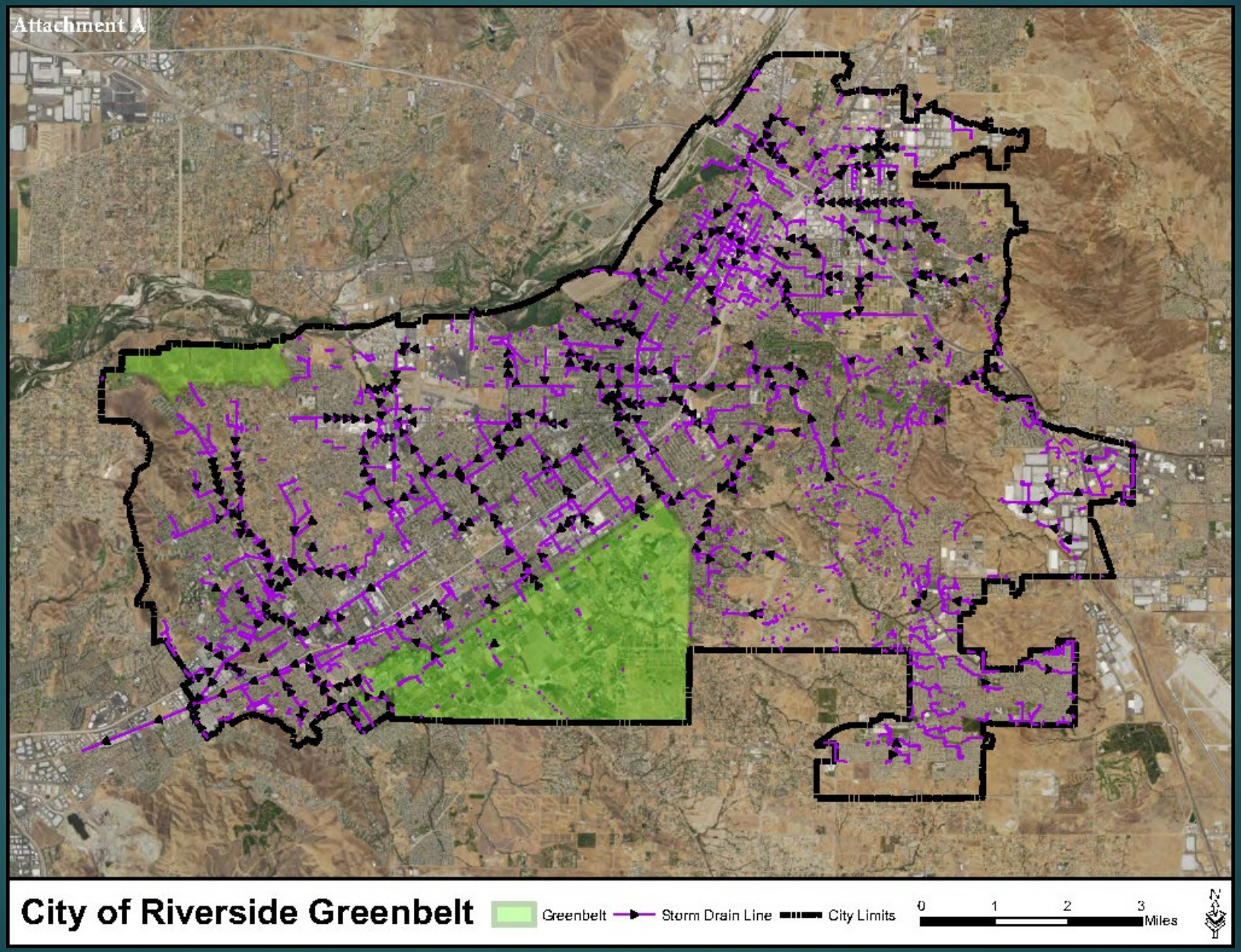
- Goal: Investigate the influence of environmental stressors on the development, spread, and mitigation of ARB and ARGs in the root zone
- Method: Crops in unsaturated soil with *in situ* and injected microbes and stressors
- Outcome: Modeling and Identification of HGT in the root zone and uptake of ARB in plants

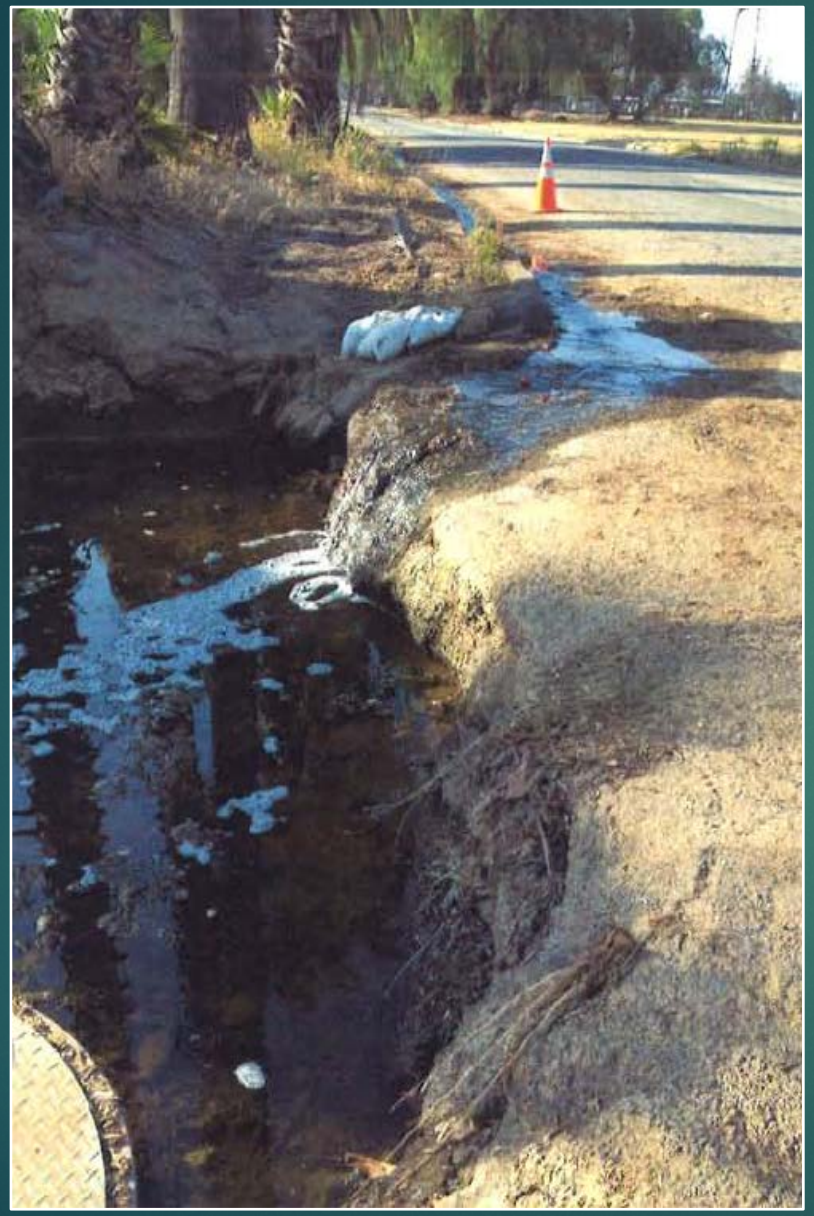


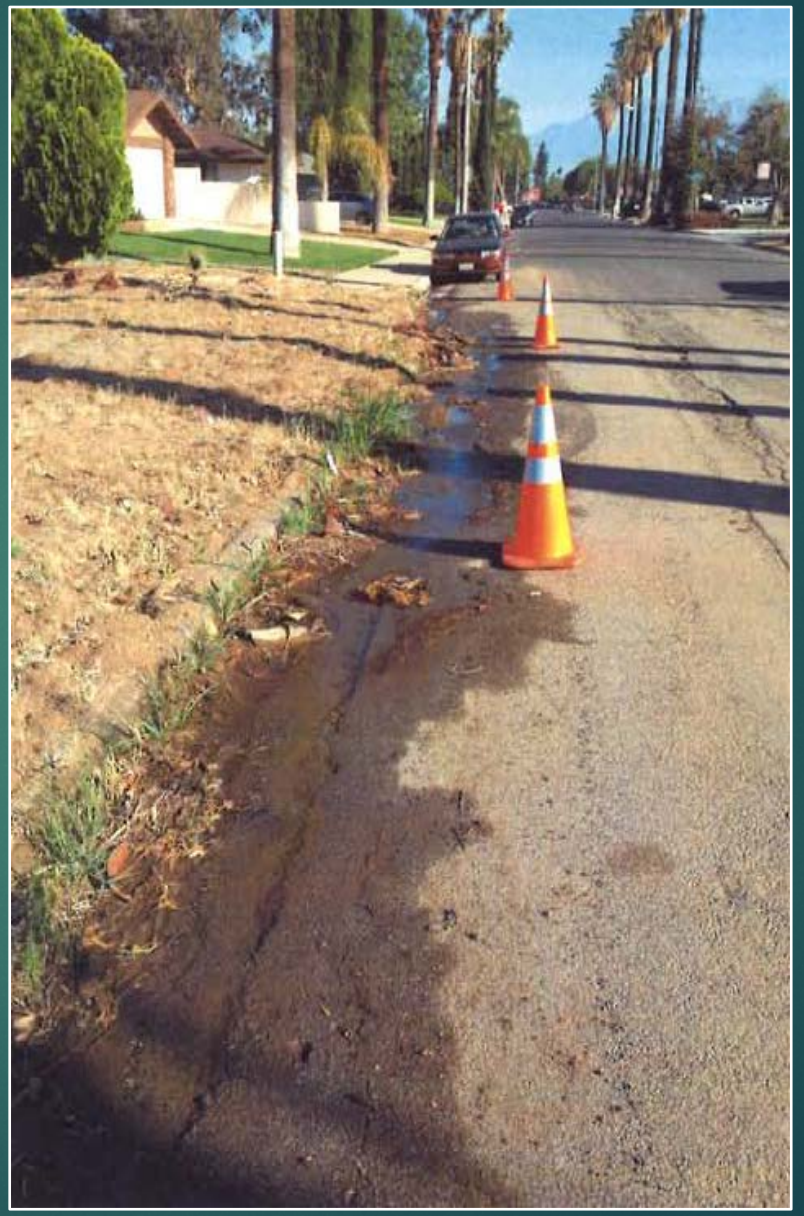
Summary

- Antibiotic resistance is a growing problem around the world.
- The physiochemical factors that lead to the spread of ARB via HGT in the environment are not well studied.
  - Batch and column experiments will reveal which physiochemical factors most influence HGT.
  - Runoff chamber experiments will reveal the most common ARB and ARGs in the Santa Ana River watershed.
  - Lysimeter experiments will reveal under what physiochemical conditions HGT occurs in the root zone and possible uptake by plants.

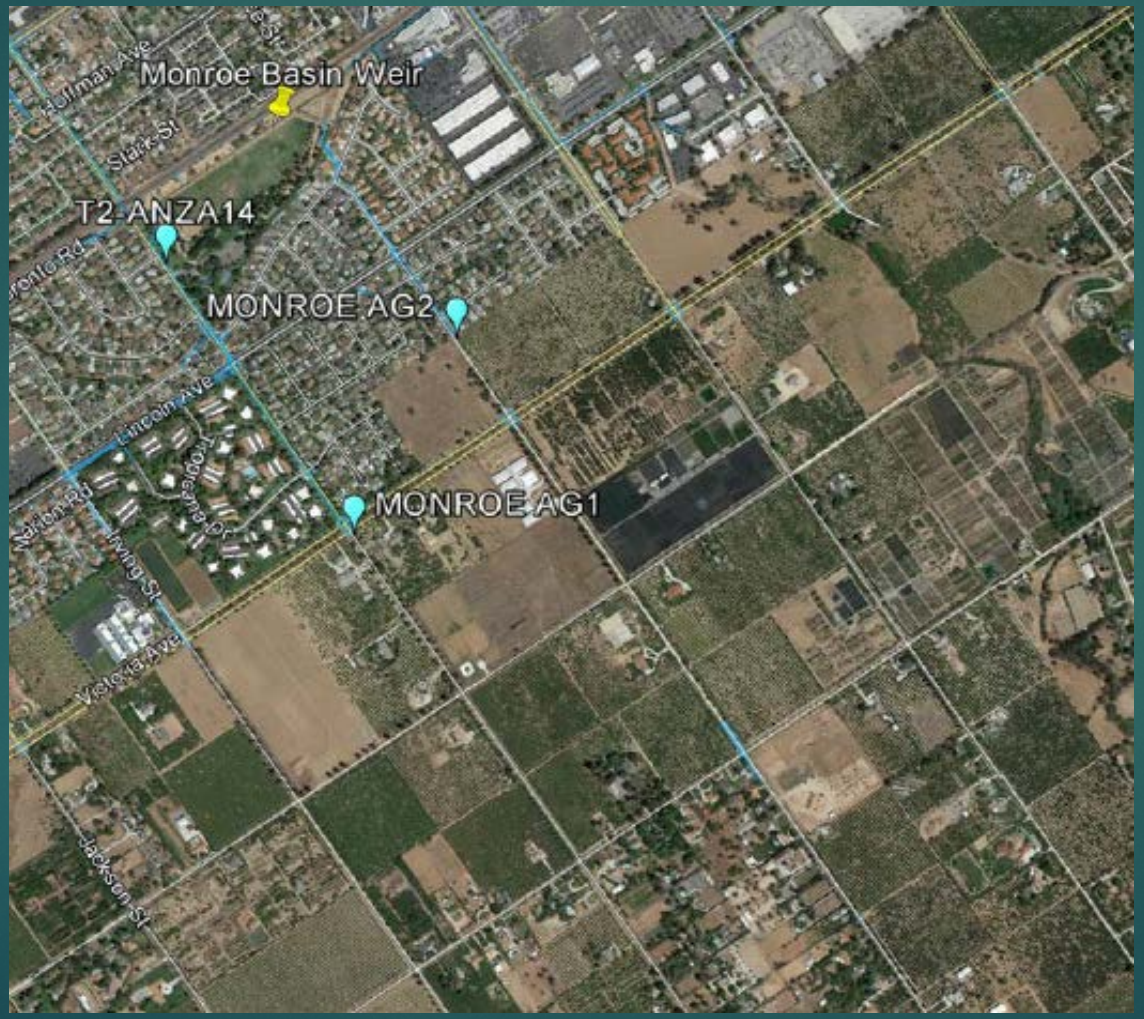
Tier 2 Source Investigation in the Arlington Greenbelt


Attachment A
City of Riverside Greenbelt
Greenbelt
Storm Drain Line
City Limits
0
1
2
3
Miles
N





Summer, 2015 Sampling Program



Summer, 2015 Sampling Program

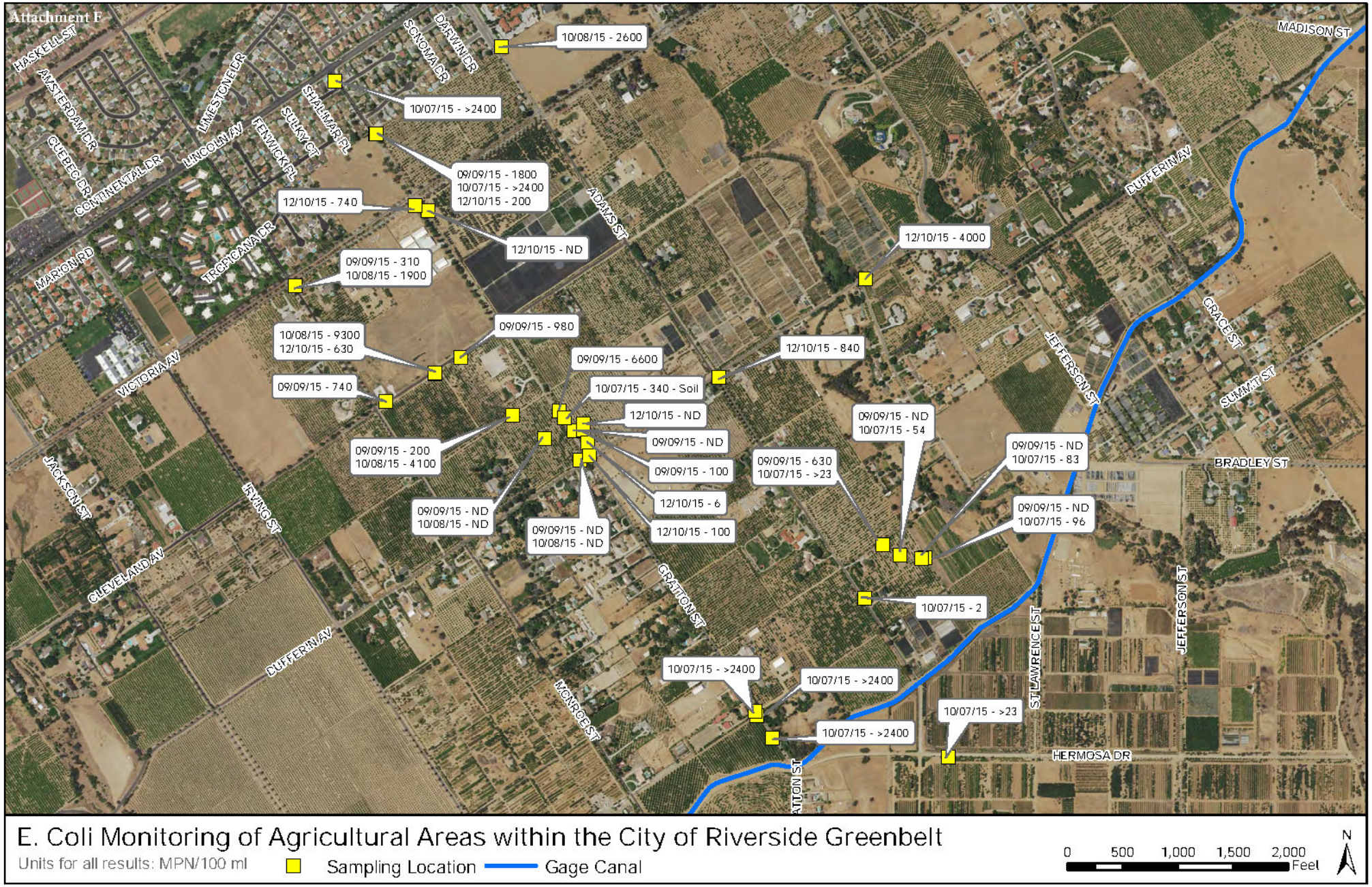
Table 3-4 Samples Collected During Dry Weather 2015					
Sampling Agency	Station Name	Sample Date	Sample Time	Flow (cfs)	Notes
District	MonroeAg01	5/27/2015	11:55 AM	0.03	▪ Bubbles forming at surface
District	MonroeAg01	6/2/2015	7:20 AM	0.025	▪ Excessive leaf litter upstream of sampling location
District	MonroeAg01	6/8/2015	7:25 AM	0.043	▪ Excessive leaf litter upstream of sampling location
District	MonroeAg01	6/16/2015	9:00 AM	0	▪ No flowing water from any direction, all inlets were dry
District	MonroeAg01	6/23/2015	8:40 AM	0	▪ All inlets are dry flow of water (south to north) along Monroe that stops about 70' from SE corner of Monroe and Victoria
District	MonroeAg01	8/17/2015	8:38 AM	0.00456	
District	MonroeAg02	6/2/2015	7:45 AM	0.05	▪ Sampled water on eastern side of Gratton St.
District	MonroeAg02	6/8/2015	7:55 AM	0.028	▪ Sampled water on western side of Gratton St. ▪ Water flows past sampling location into catch basin at corner of (SW) Gratton St. and Lincoln Ave.
District	MonroeAg02	6/16/2015	9:20 AM	0.07	▪ Sampled water on eastern side of Gratton St.
District	MonroeAg02	6/23/2015	8:15 AM	0.10	▪ sampled water on western side of Gratton St ▪ Water flows past sampling location into catch basin at corner of (SW) Gratton St. and Lincoln Ave.
District	MonroeAg02	8/17/2015	9:03 AM	0.04	▪ Sampled water on eastern side of Gratton St. ▪ Water flows past sampling location into catch basin at corner of (SW) Gratton St. and Lincoln Ave.

Summer, 2015 Sampling Program

Table 3-6 Grab Sample Results for the Monroe Stations in the 2015 Dry Season				
Station	Sample Date	Sample Time	E. coli (MPN/100 mL)	Method
MonroeAg01	5/27/2015	11:55	1600	SM9223B
MonroeAg01	6/2/2015	7:20	310	SM9223B
MonroeAg01	6/8/2015	7:25	600	SM9223B
MonroeAg01	8/17/2015	8:38	700	SM9223B
MonroeAg02	5/27/2015	12:20	2300	SM9223B
MonroeAg02	6/2/2015	7:45	410	SM9223B
MonroeAg02	6/8/2015	7:55	5600	SM9223B
MonroeAg02	6/16/2015	9:20	4100	SM9223B
MonroeAg02	6/23/2015	8:15	5500	SM9223B
MonroeAg02	8/17/2015	9:03	500	SM9223B

Fall, 2015 Follow-up Sampling

Fall, 2015 Follow-up Sampling


Attachment F
HASKELL ST
AMSTERDAM CIR
QUEBEC CIR
CONTINENTAL DR
LIMESTONE LN
LINCOLN AV
FENWICK PL
SULKY CT
SHALIMAR PL
SONOMA ER
DARWIN ER
MARION RD
TROPICANA DR
ADAMS ST
VICTORIA AV
JACKSON ST
IRVING ST
CLEVELAND AV
DUFFERIN AV
MONROE ST
GRATION ST
ATION ST
MADISON ST
DUFFERIN AV
GRACE ST
JEFFERSON ST
SUMMIT ST
BRADLEY ST
ST LAWRENCE ST
JEFFERSON ST
HERMOSA DR
10/08/15 - 2600
10/07/15 - >2400
09/09/15 - 1800
10/07/15 - >2400
12/10/15 - 200
12/10/15 - 740
12/10/15 - ND
09/09/15 - 310
10/08/15 - 1900
12/10/15 - 4000
10/08/15 - 9300
12/10/15 - 630
09/09/15 - 980
09/09/15 - 740
09/09/15 - 6600
10/07/15 - 340 - Soil
12/10/15 - ND
09/09/15 - ND
09/09/15 - 100
12/10/15 - 6
12/10/15 - 100
12/10/15 - 840
09/09/15 - 200
10/08/15 - 4100
09/09/15 - ND
10/08/15 - ND
09/09/15 - ND
10/08/15 - ND
09/09/15 - 630
10/07/15 - >23
09/09/15 - ND
10/07/15 - 54
09/09/15 - ND
10/07/15 - 83
09/09/15 - ND
10/07/15 - 96
10/07/15 - 2
10/07/15 - >2400
10/07/15 - >2400
10/07/15 - >2400
10/07/15 - >23
E. Coli Monitoring of Agricultural Areas within the City of Riverside Greenbelt
Units for all results: MPN/100 ml
Sampling Location
Gage Canal
0
500
1,000
1,500
2,000
Feet
N

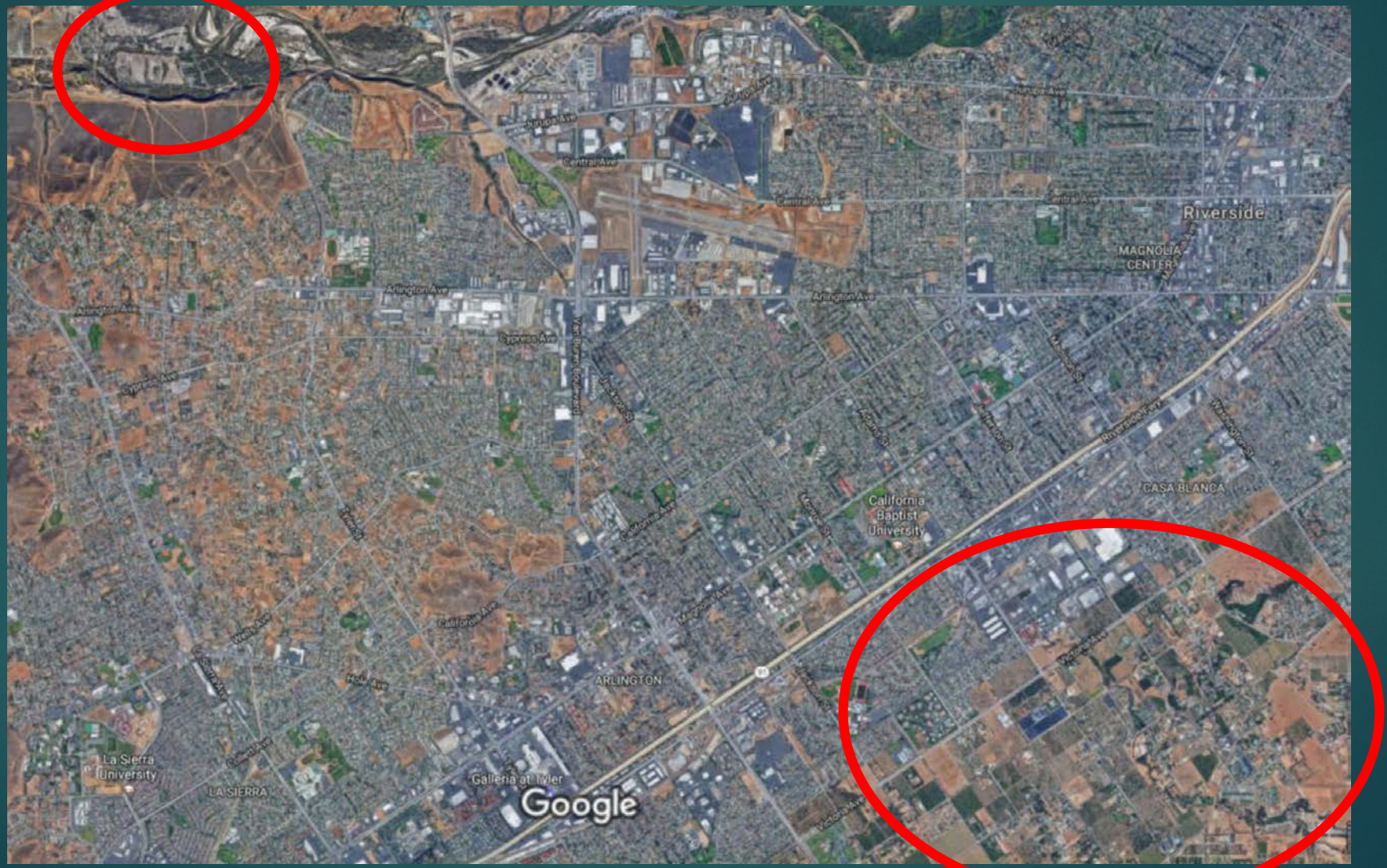
Next Steps:

- Bacteroides analysis to exclude human sources
- Sanitary survey if human signals are detected
- DNA testing for non-human sources (birds, dogs, critters)
- Recon-level fate and transport study (dry weather conditions)
- Preliminary alternatives analysis for BMPs

Task Force Initiative

- Establish a rigorous approach for natural source exceptions
- Develop approach for distinguishing ag vs. urban sources
- Standardize procedure for addressing bacteria inputs to the MS4 unrelated to Urban Runoff
- Potential opportunity for a large-scale regional BMP solution by re-routing dry weather runoff through the Hidden Valley Wetlands

Hidden Valley Wetlands



Funding:

Approved FY 2016-17 Budget: Middle Santa Ana River Pathogen TMDL Task Force — **March 14, 2016**

Summary: TMDL Task Force Implementation Schedule and Budget				
Summary Expenses	Task Force Budget 2016-17	SAW Bacteria Monit Budget 2016-17	Tier 2 Source Eval 2016-17	Total Budget 2016-17
TASK FORCE: Administration (assumes quarterly meetings)	$ 30,000			$ 30,000
Grant Preparation	$ 20,000			$ 20,000
TASK FORCE: TMDL Compliance Expert	$ 25,000			$ 25,000
TMDL Implementation: Task 3 - Watershed-wide Monitoring Program	$ 140,000	$ 149,175		$ 289,175
TMDL Implementation: Tasks 4 & 5 - Source Evaluation / Management	$ -		$ -	$ -
Estimated Total Annual TMDL Implementation Budget	$ 215,000	$ 149,175	$ -	$ 364,175
Applied Task Force Carryover Funds:	$ 100,000			$ 100,000
Estimated Total Stakeholder Cash Contribution	$ 115,000	$ 149,175	$ -	$ 264,175
Contingency Reserve (estimated):	$ 50,000	$ -	$ -	$ 50,000