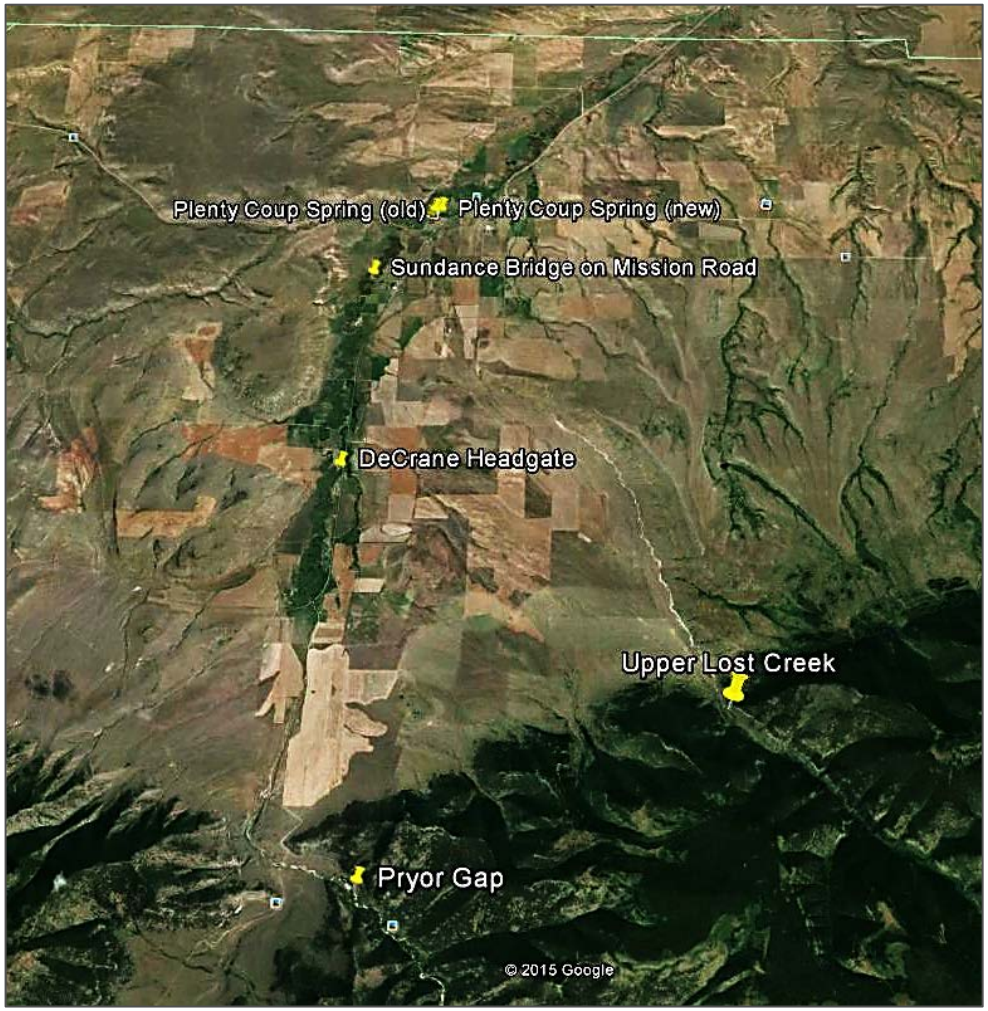
Addressing Fecal Contamination of Chief Plenty Coups Spring

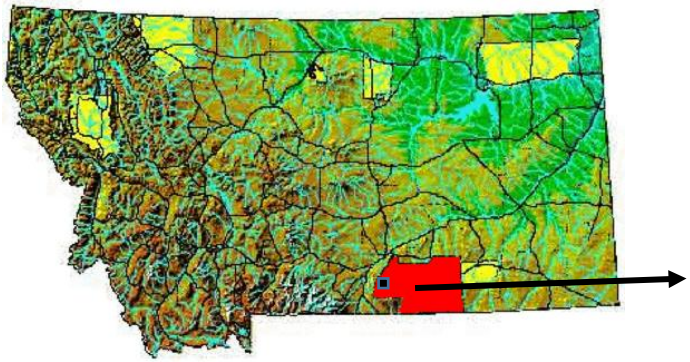
John Doyle, Mari Eggers, Sara Young
Also: Stephanie Ewing, Jane Klassen, Julie Geddes, Sue Pellegrini, Myra Lefthand, Ada Bends, Jessica Eggers, Pryor 107 Elders

Project Advisors:
Pryor 107 Committee of Elders

Research Sites in Pryor, Crow Reservation

Seven test sites





Teaching a new generation another way of looking

Collecting samples for testing at new (2011) spring at Chief Plenty Coup State Park

Crew samples Chief Plenty Coup's Spring, while Dr. Ewing explains our project to Crow students

Lost Creek

Climate change & waterborne pathogens

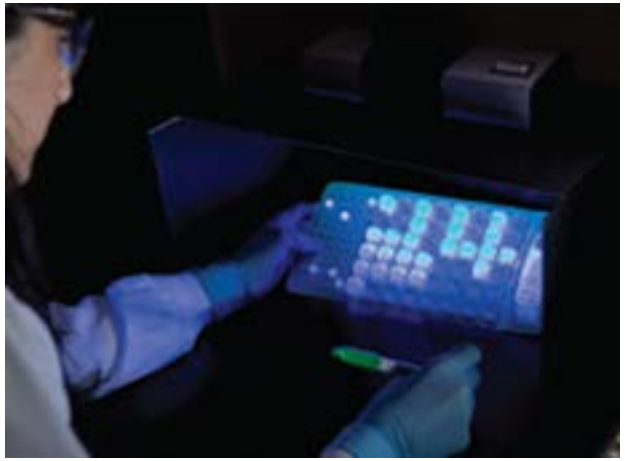
Our approach:

- Community engaged research and local environmental knowledge

  Crow Environmental Health Steering Committee and Pryor 107 Committee of Elders
- Testing water samples for coliform & *E. coli* – indicators of fecal contamination
- Isotopic analysis of water samples
- Source tracking: using DNA sequencing to compare bacterial communities
- Planned: Testing water samples for caffeine

E. coli –Indicator bacteria for fecal contamination Method for Most Probable Number/100 mL

IDEXX Quantitray 2000



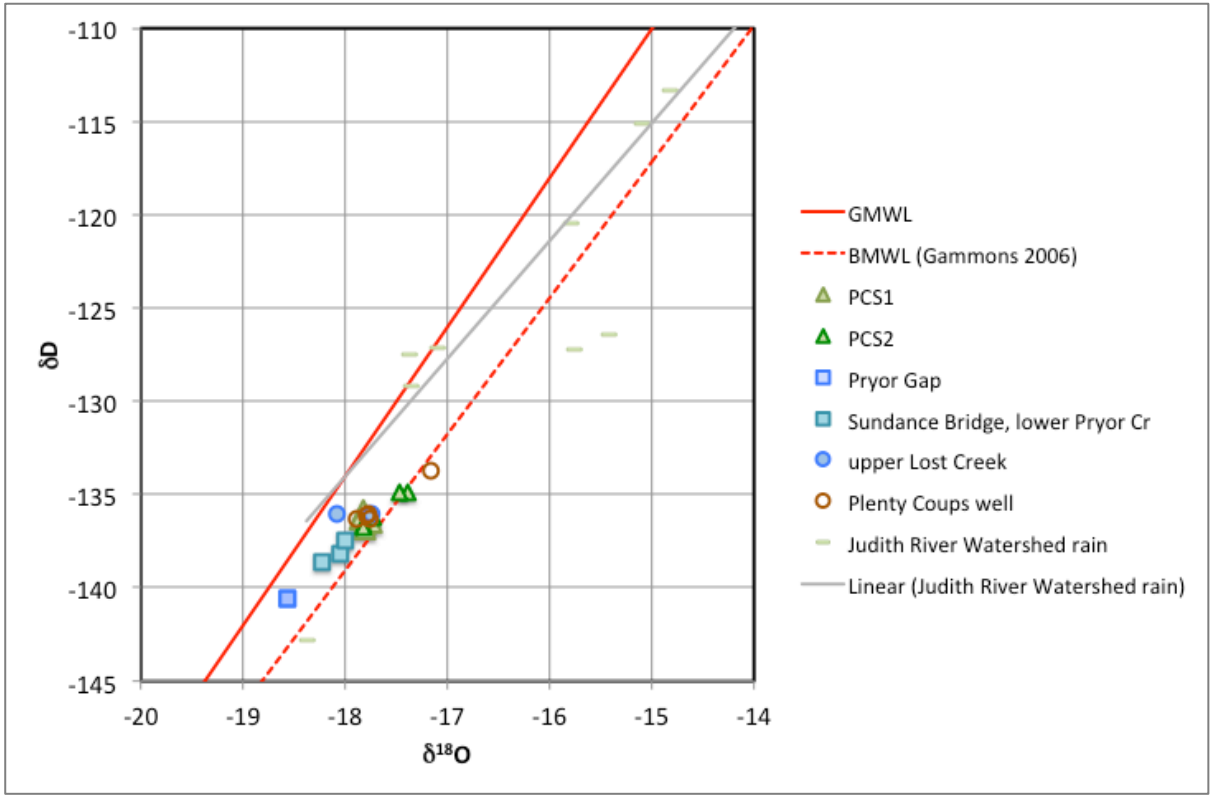
E. coli in Chief Plenty Coup springs: Primarily in summer

Plenty Coup Spring and Pryor Creek Data 2014

		1/24/2014		2/19/2014		3/26/2014		4/24/2014		5/13/2014		6/18/2014		7/17/2014		8/5/2014		8/28/2014		10/14/2014	
Site		coliform	E. coli	coliform	E. coli	coliform	E. coli	coliform	E. coli	coliform	E. coli	coliform	E. coli	coliform	E. coli	coliform	E. coli	coliform	E. coli	coliform	E. coli
PC1 = Plenty Coup Spring 1	E Labs	49.6	<1	67.7	<1	51.2	<1	present	<1	present	<1	38.4	1	34.1	<1	44.1	<1	17.5	<1		
	Doyle			193.5	<1	14.6	1	517.2?				483.3	7.2			61.2	n/a	24.3	3.1	4.1	1.0
PC2 = Plenty Coup Spring 2	E Labs	228.2	<1	16	<1	12.2	<1	present	<1	present	<1	290.9	<1	1986.3	3.1	1203.3	<1	727.0	5.2		
	Doyle			35.9	<1	9.8	1	721.5?				204.6	165					<1	<1	57.3	2.0
Pryor Creek at Sun-dance Bridge (SDB)	E Labs											1986.3	191.8	>2419.6	125	>2419.6	261.3	770.1	83.6	SDB	
	Doyle					290.9	201	307.6	160.7			250.4	250.4			>2419.6	325.5	214.3	48.8	177.0	35.0
	Doyle					235.9	172	4.1	2									248.1	45.7	SDB Pipe	
	Doyle					435.2	276													17.5	<1
Pryor Creek at Pryor Gap (PGP)	E Labs													307.6	3.1			648.8	66.3		
	Doyle							183.5	135.4									7.5	6.3		
	Doyle							5.2	5.2									85.7	58.3		

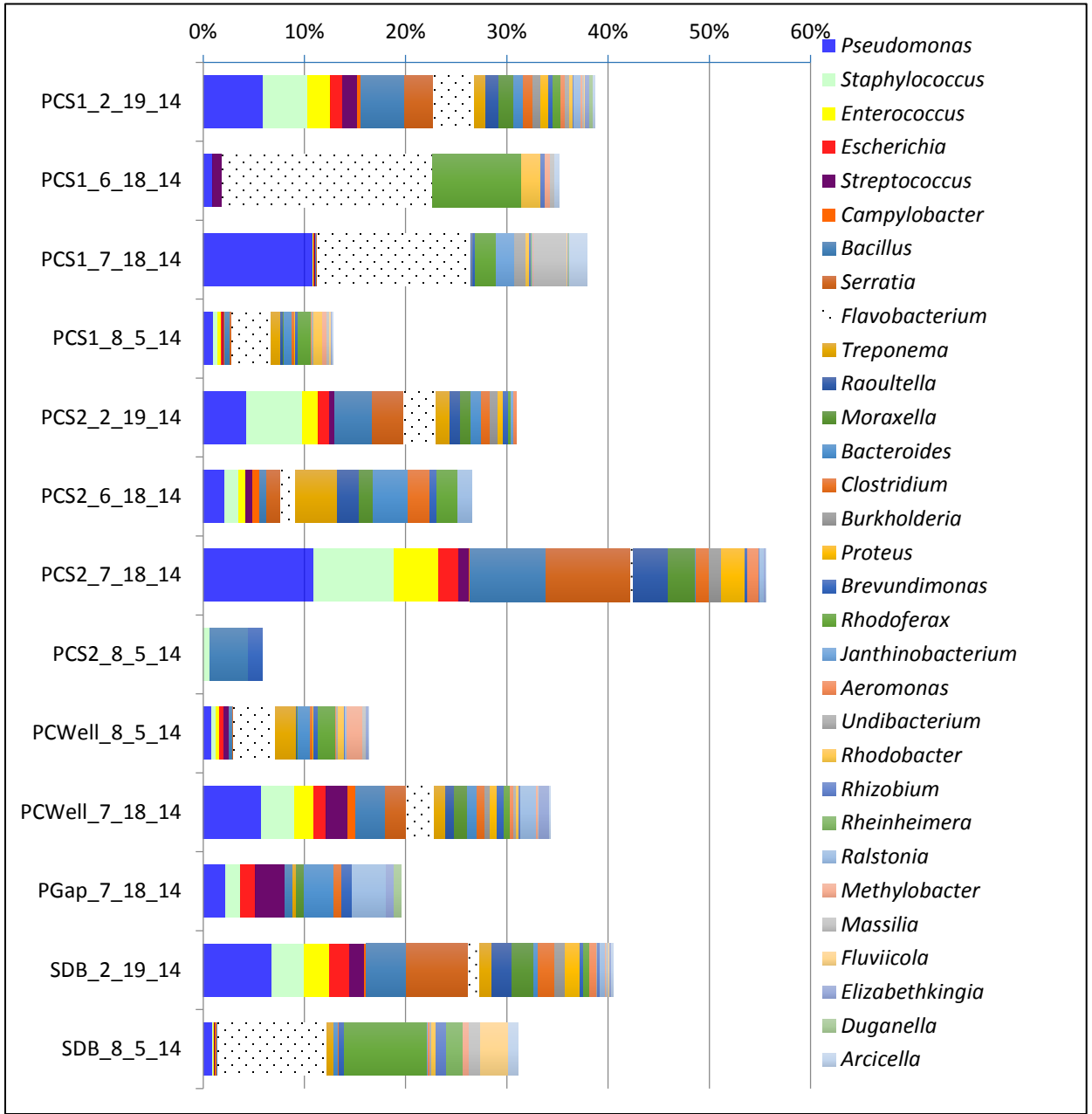
Isotope hydrology

Collaborators: Stephanie Ewing, Jane Klassen



The dual isotope plot ($d^{18}O$ vs. dD) below shows how the relationship of values in the samples are arrayed relative to the Global Meteoric Water Line (solid red line); rainfall samples from Butte, Montana (red dotted line; Gammons et al., 2006); and rainfall samples from the Judith River Watershed in central Montana (grey line fit to dashes; Ewing, Sigler et al., unpublished data).

DNA extraction, amplification & sequencing



Collaborators:

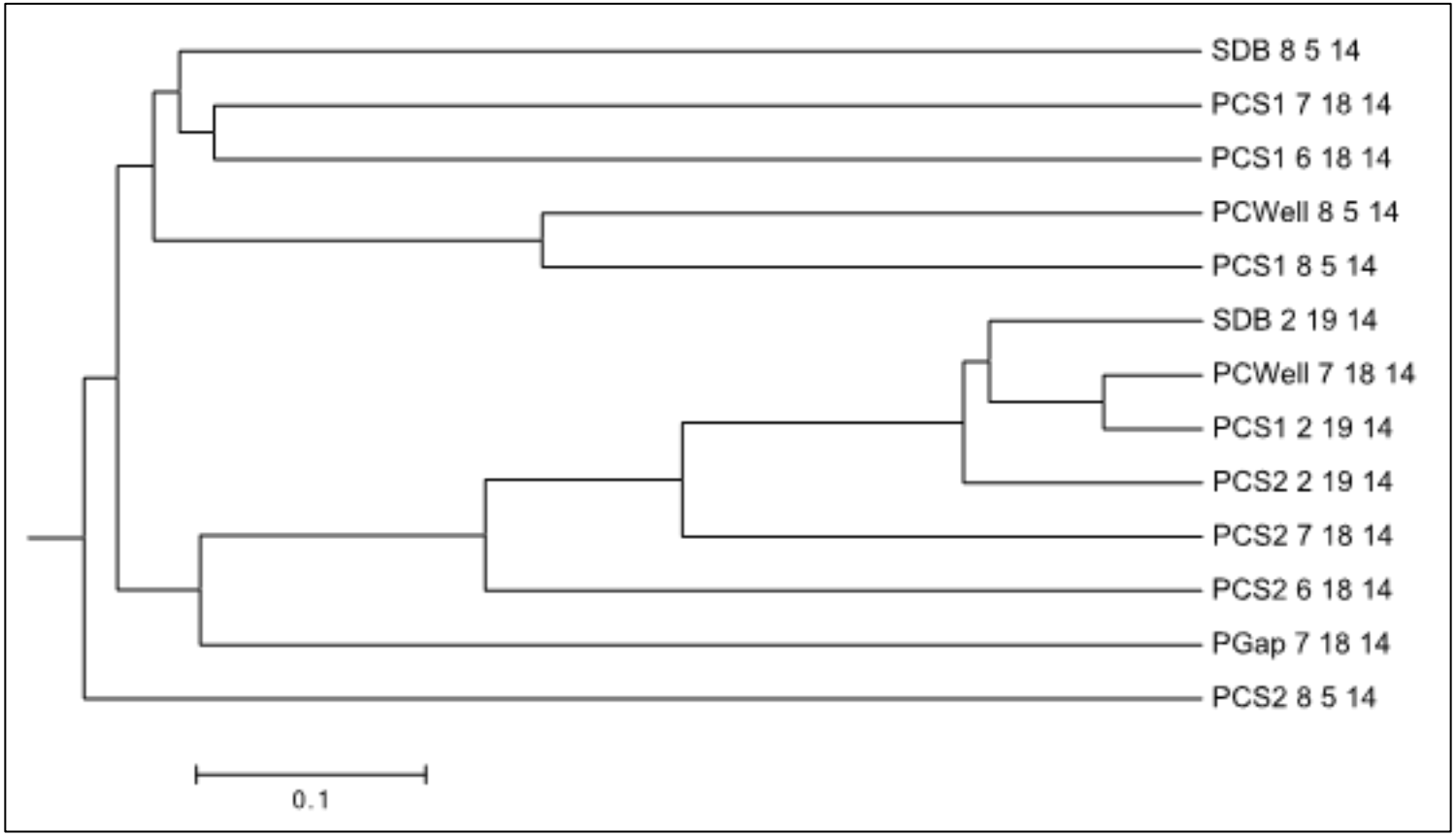
Suzanne Ishaq Pellegrini, Julie Geddes, Carl Yeoman, student Jessica Eggers

Percent abundance of selected genera, by sample

How can sequencing data be used?

Percent abundance of potentially pathogenic species, by sample													
Genera	PCS1_2_19_14	PCS1_6_18_14	PCS1_7_18_14	PCS1_8_5_14	PCS2_2_19_14	PCS2_6_18_14	PCS2_7_18_14	PCS2_8_5_14	PCWell_7_18_14	PCWell_8_5_14	PGap_7_18_14	SDB_2_19_14	SDB_8_5_14
Campylobacter	0	0	0	0	0	0	0	0	0	0	0	0	0
Citrobacter	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter	0	0	0	0	0	0	0	0	0	0	0	0	0
Escherichia	1.2	0	0.1	0.2	1.1	0	2	0	1.2	0.4	1.5	2	0
Hafnia	0	0	0	0	0	0	0	0	0	0	0	0	0
Klebsiella	0	0	0	0	0.3	0	0.1	0	0	0	0	0	0
Pseudomonas	5.9	0.9	10.8	1	4.2	2.1	10.9	0	5.7	0.8	2.2	6.8	0.9
Shigella	0	0	0	0	0	0	0	0	0	0	0	0	0
Staphylococcus	4.3	0	0	0.4	5.6	1.4	8	0.7	3.3	0.4	1.5	3.2	0.1
Streptobacillus	0.03	0	0	0	0	0.7	0.03	0	0.06	0	0	0	0

Comparing sites to each other by their samples: UPGMA dendogram



http://genomes.urv.cat/UPGMA/

Thank you

Collaborators

Crow Environmental Health Steering Committee: Myra Lefthand, Sara Young, Larry Kindness, Ada Bends, Connie Howe, Robin Stewart (and us)

Montana State University Bozeman: Anne Camper, Stephanie Ewing, Jane Klassen, Julie Geddes, Suzanne Ishaq Pellegrini, Carl Yeoman, Jessica Eggers

Pryor 107 Committee of Elders

Chief Plenty Coup State Park staff, Pryor, MT

Little Big Horn College, Crow Agency, MT

Apsaalooke Water & Wastewater Authority

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Questions?