WAIOPILI DITCH
SANITARY SURVEY, KAUAI

Clean Water Branch
Department of Health
2827 Waimano Home Road #225
Pearl City, Hawaii 96782
Phone: 808 586-4309

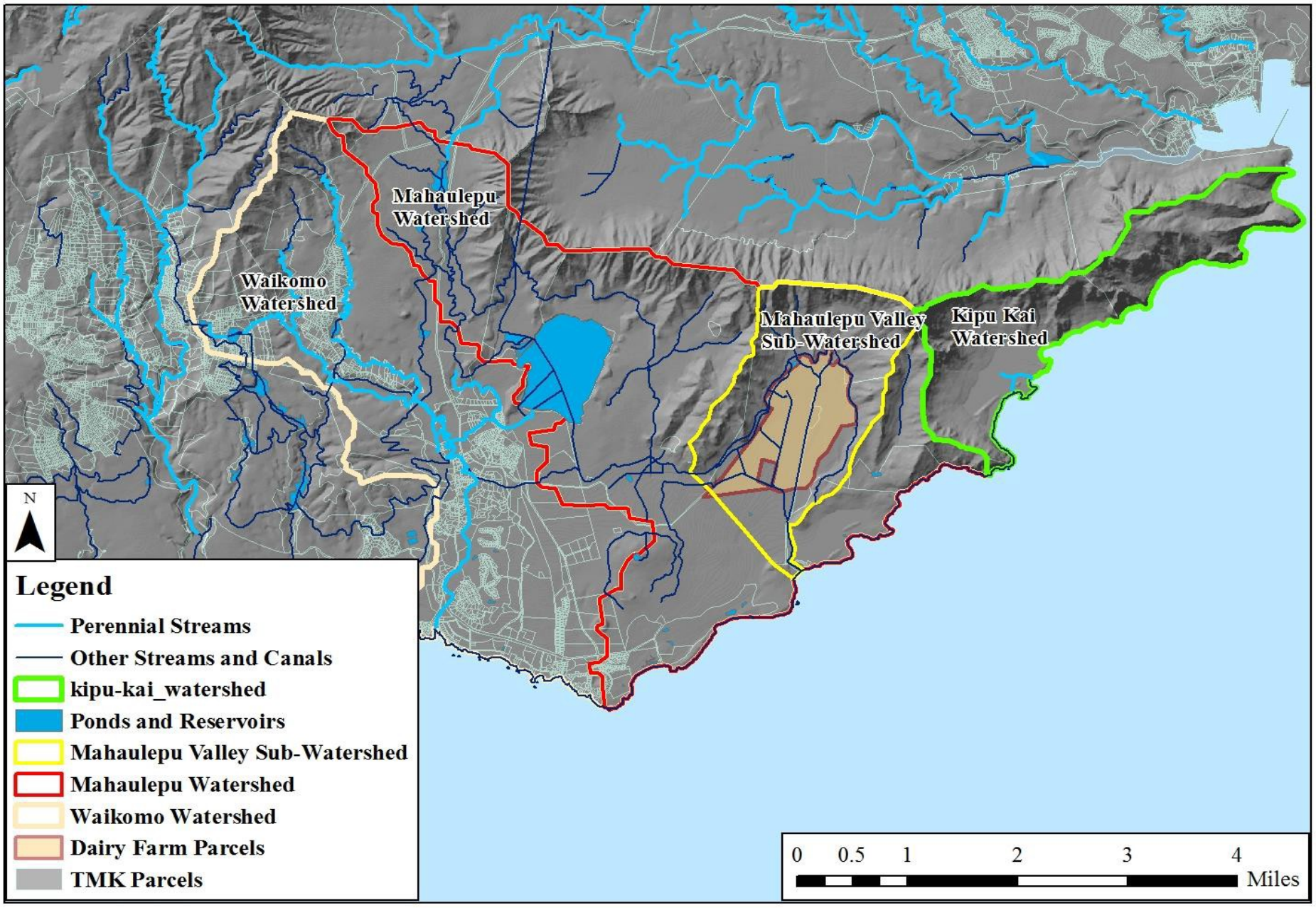
Objectives

- **Give background information on the Waiopili concerns**
- **Describe the Waiopili Sanitary Survey**
- **Present current information**
- **Provide information on the PhyloChip testing**

Background

In 2014, The Department of Health, Clean Water Branch received a complaint of high indicator bacteria (enterococci) levels in Waiopili Ditch and was asked to post warning signs along the waterway.

Mahaulepu and Adjacent Watersheds



Issues

- **DOH receives complaints of high enterococci levels in Waiopili Ditch**

- **DOH finds high enterococci levels but could not locate any human fecal source…no "smoking gun"**

- **DOH believes high enterococci levels due to natural sources**

- **DOH posts caution signs around the mouth of the Waiopili**

- **DOH initiates Sanitary Survey to investigate potential human fecal contamination of the area**

⚠ CAUTION

HIGH BACTERIA LEVELS FOUND

in ocean and stream
especially after heavy rainfall

Contact with water
may cause illness

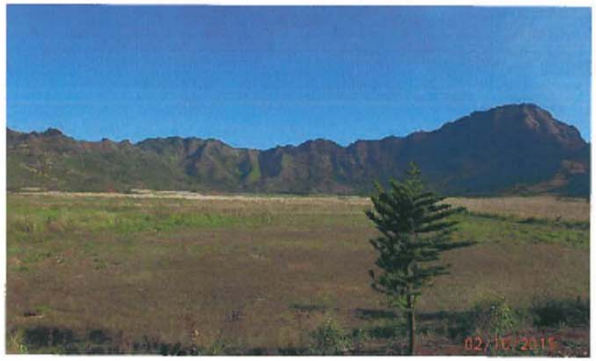
For information
health.hawaii.gov/cwb
(808) 586-4309

Hawaii State Department of Health


CAUTION

WAIOPILI DITCH
SANITARY SURVEY, KAUAI
PART I

March 2016



Prepared by Clean Water Branch,
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What is a Sanitary Survey?

A sanitary survey is a method of investigating the sources of fecal contamination to a water body (EPA)

- Historical Information
- Land Use and Natural Setting
- Potential Contaminant Sources
- Existing Water Quality
- Water Quality Indicators

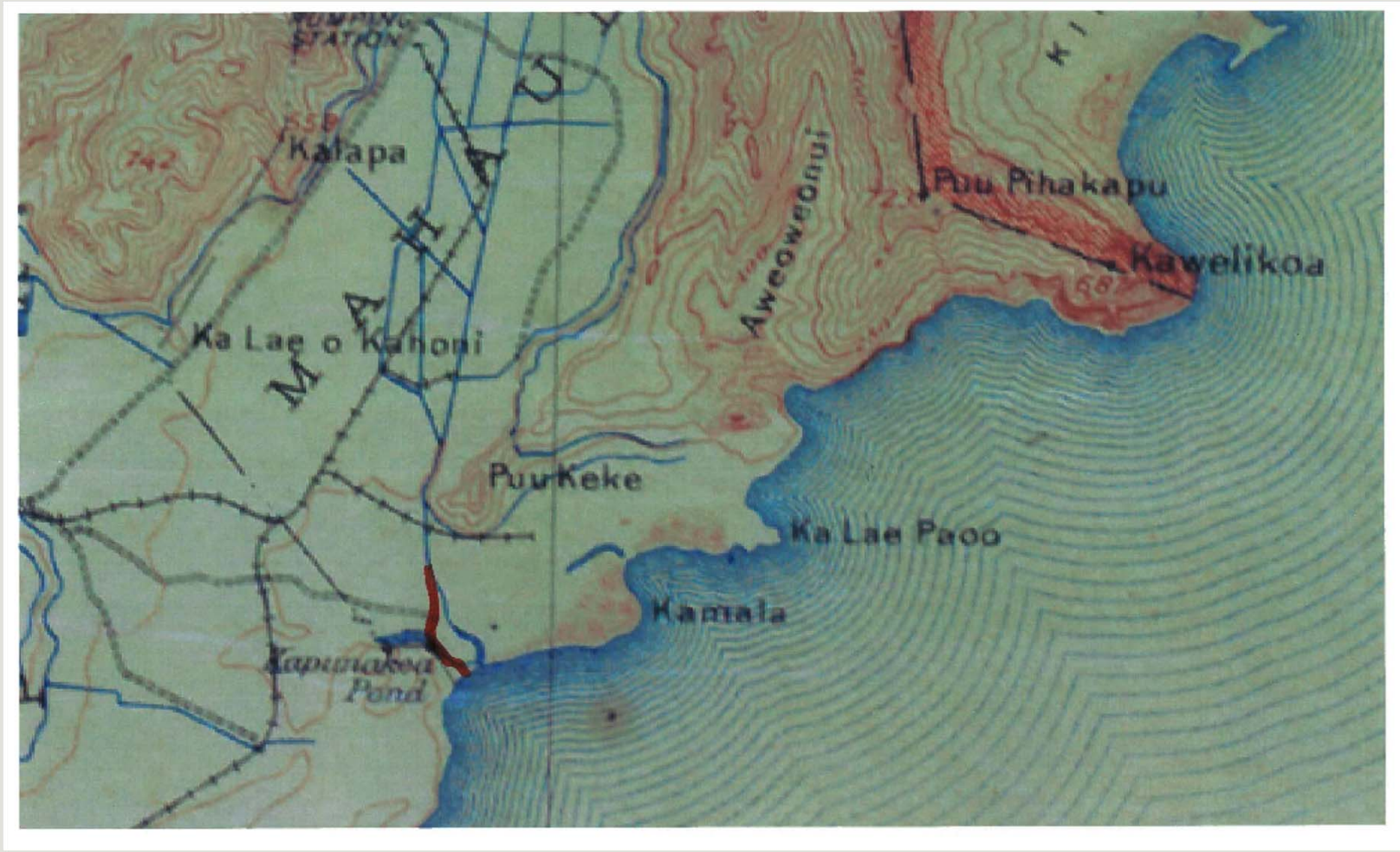
Historical Information

Note: This excerpt from an 1886 map shows the Makauwahi Cave area, including the large brackish pond (Kapunakea) that formerly existed on the site, as well as the sinkhole itself (indicated by arrow added by the author). Insert not to scale. (Courtesy of Grove Farm Museum)

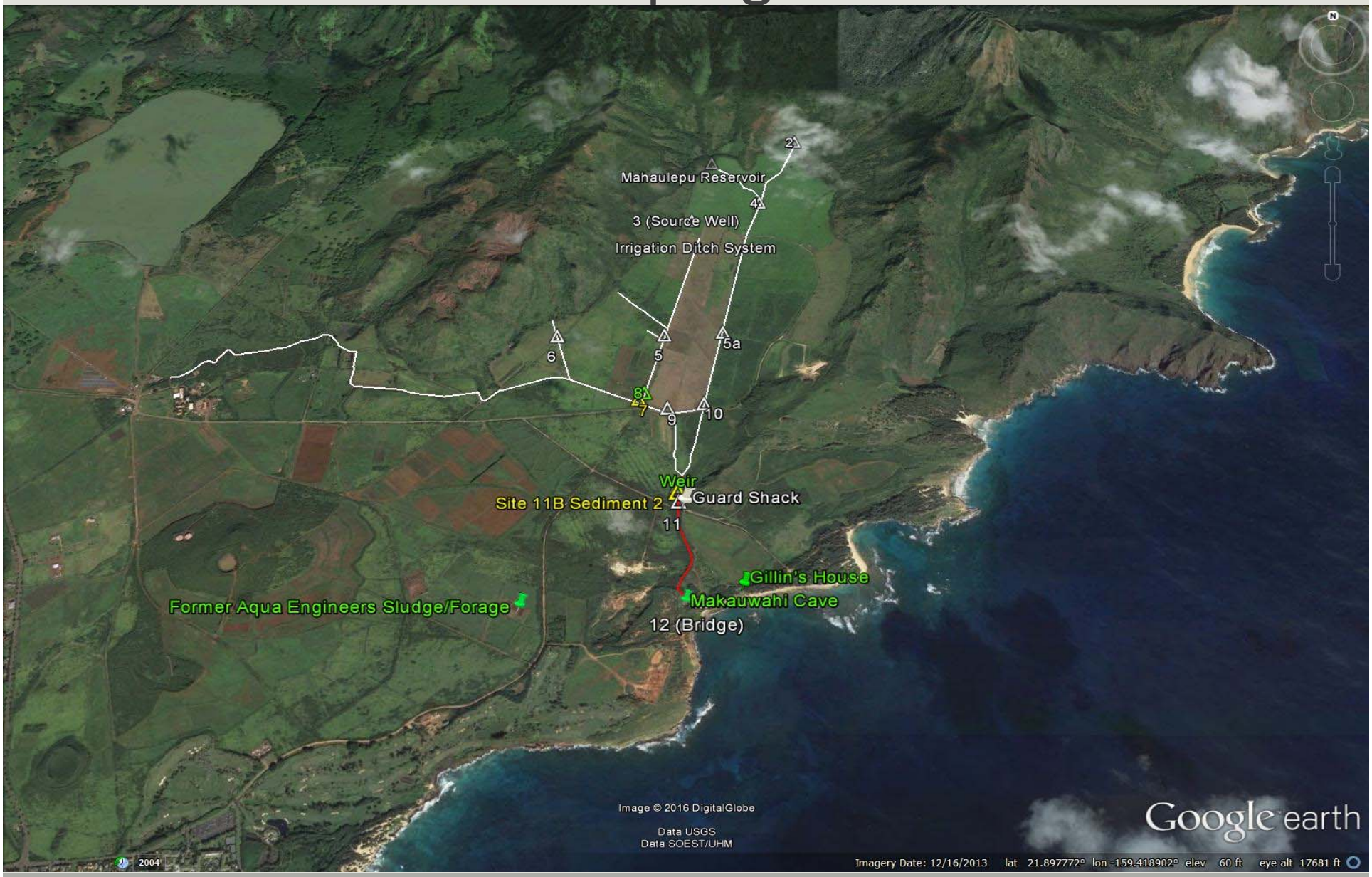
Note: Old photo, looking SW, of limestone escarpment (Keahikea Cliff) containing entrance to North Cave (on left end). Much of the hill on the right side of the picture has subsequently been quarried away. Note the lack of woody vegetation and closely grazed appearance of the landscape. Kapunakea Pond is the strip of water extending across the middle ground of the photo, up against the escarpment. Picture was probably taken between 1890–1920. (Courtesy of Bernice P. Bishop Museum).

1912 Topographic map of Mahaulepu

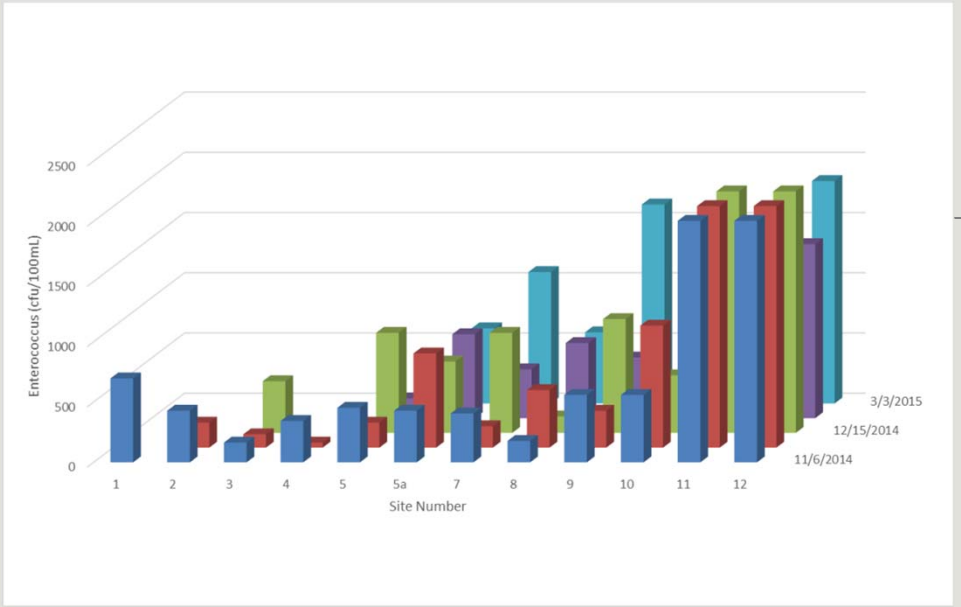
Altered Path of Waiopili

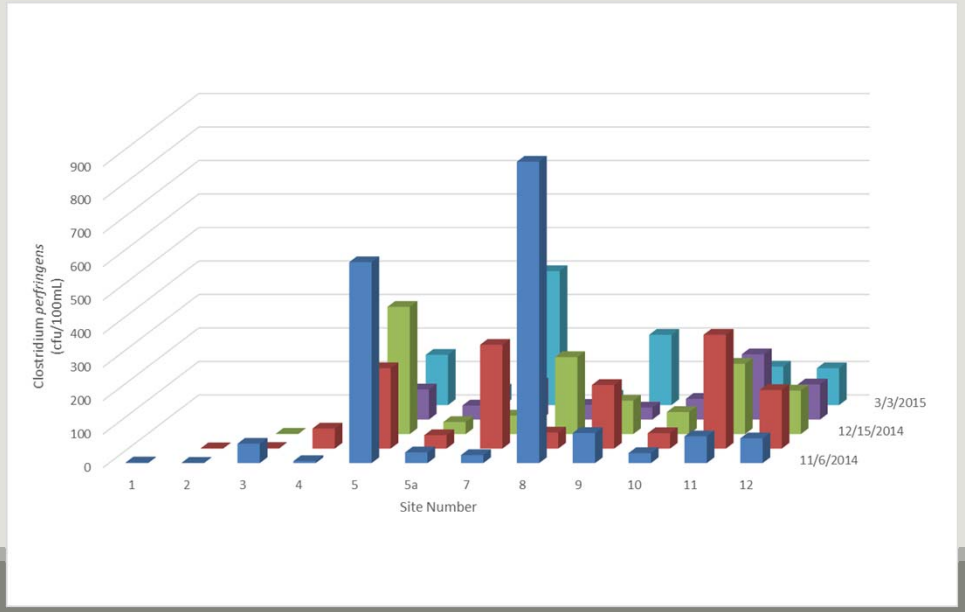


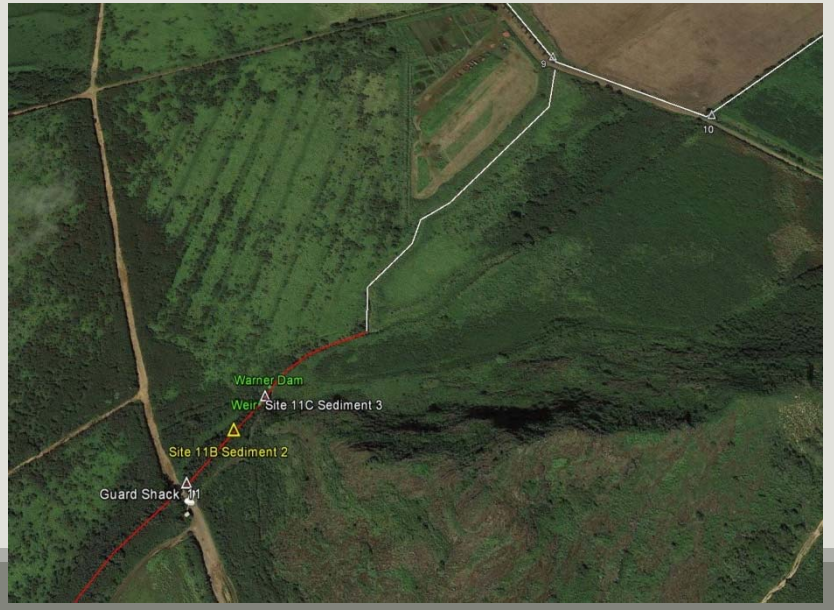
DOH Sampling Stations



DOH Water Data







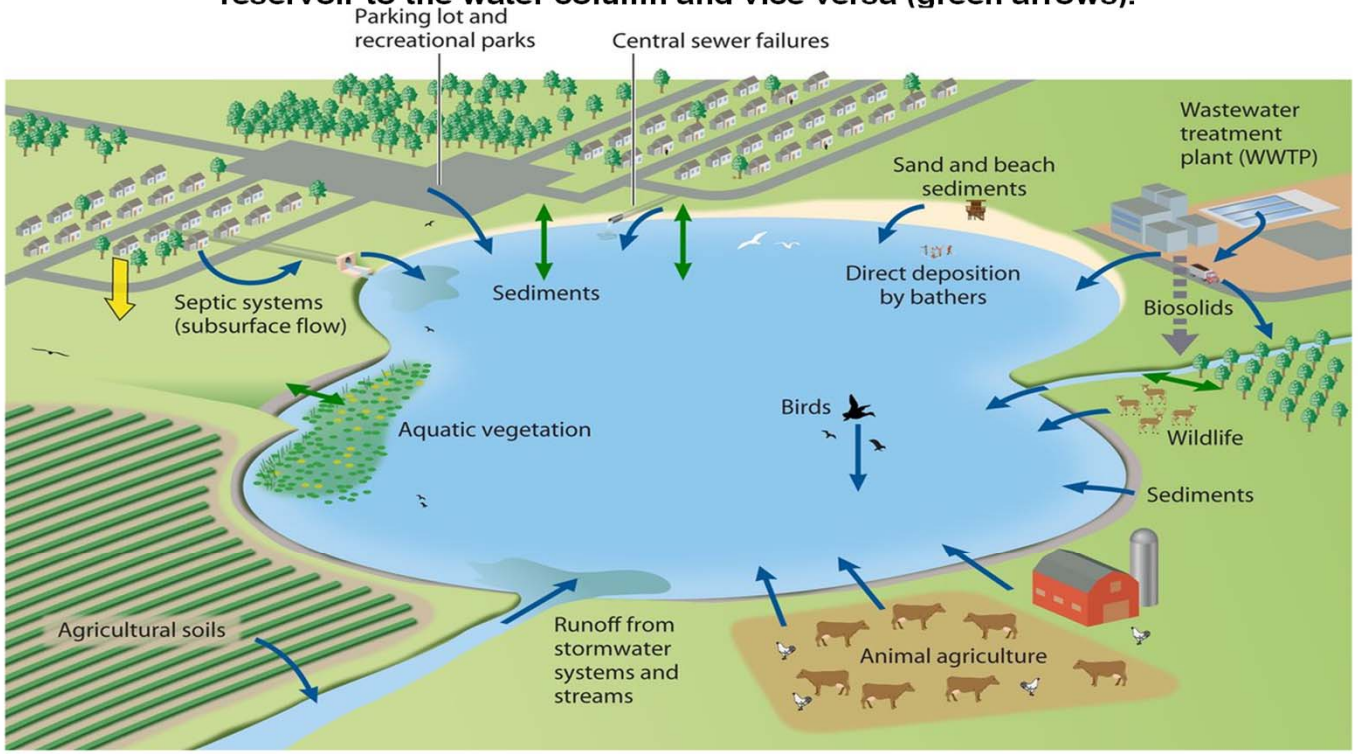
What does the data mean?

WE HAVE OBSERVED HIGH LEVELS OF ENTEROCOCCI AND C. *PERFRINGENS* BUT ARE LEFT TO SPECULATE AS TO THE SOURCE OF THE BACTERIA. WHERE IS THE

"SMOKING GUN?"

Enterococci in the Environment

Sources of enterococci in water bodies (blue arrows) as well as sinks where enterococci are immobilized (yellow arrow) and areas of flux, in which enterococci can transition from a reservoir to the water column and vice versa (green arrows).



Muruleedhara N. Byappanahalli et al. Microbiol. Mol. Biol. Rev. 2012;76:685-706

Microbiology and Molecular Biology Reviews

Species of the Genus Enterococcus in Their Known Habitats

Group	Species	Known habitats	Human pathogen
E. faecalis	E. faecalis	Human, animal (multiple), plant, insect	**Yes**
	E. haemoperoxidus	Surface water	
	E. moraviensis	Surface water	
	E. silesiacus	Drinking water	
	E. termitis	Animal (termite)	
	E. caccae	Human	
E. faecium	E. faecium	Human, animal (multiple), plant, insect	**Yes**
	E. durans	Human, animal (multiple), insect	**Yes**
	E. hirae	Animal (multiple), plant	
	E. mundtii	Soil, plant	**Yes**
	E. villorum	Animal (hog)	
	E. canis	Animal (dog)	
	E. ratti	Animal (rat)	
	E. asini	Animal (donkey)	
	E. phoeniculicola	Animal (bird)	
	E. canintestini	Animal (dog)	
	E. thailandicus	**Human, animal (cattle)**	

From: Enterococci in the Environment. Microbiology and Molecular Biology Reviews, December 2012, Volume 76, Number 4.

Species of the Genus Enterococcus in Their Known Habitats (cont.)

E. avium	**E. avium**	**Human, animal (multiple)**	**Yes**	**67, 121, 203, 257**
	E. pseudoavium	Human		**65**
	E. malodoratus	Animal (cattle)		**67**
	E. raffinosus	Human	Yes	**65, 241**
	E. gilvus	Human		**330**
	E. pallens	Human		**330**
	E. hermanniensis	Animal (dog)		**195**
	E. devriesei	Animal (cattle)		**322**
	E. viikkiensis	Animal (broiler plant)		**269**
E. gallinarum	E. gallinarum	Human, animal (multiple), insect	Yes	**67, 70, 203**
	E. casseliflavus	Plant, soil, human, animal (multiple)	Yes	**67, 203, 239, 241**
E. cecorum	E. cecorum	Animal (chickens)		**88, 360**
	E. columbae	Animal (pigeon)		**87**
Ungrouped	E. saccharolyticus	Animal (cattle)		**203, 270**
	E. aquimarinus	Seawater		**321**
	E. sulfureus	Plant		**218**
	E. dispar	Human		**68**
	E. italicus	Animal (cattle)		**107**
	E. camelliae	**Plant**		**316**

From: Enterococci in the Environment. Microbiology and Molecular Biology Reviews, December 2012, Volume 76, Number 4.

Concentration of Indicator Bacteria in Animal Feces

Concentrations of Indicator Bacteria in Animal Feces*

(ranked by Enterococci)

SAMPLE	BACTERIA (CFU/gram)			
	Fecal Coliform	*Escherichia coli*	Enterococci	*C. perfringens*
Chicken	1.62×10^8	1.62×10^8	3.81×10^7	<67
Pig	5.73×10^7	5.80×10^7	9.33×10^6	1.73×10^5
Rat	1.08×10^7	1.03×10^7	6.33×10^6	<67
Quail	5.40×10^7	3.40×10^7	5.80×10^6	<67
Pigeon	1.87×10^7	1.71×10^7	5.00×10^6	<67
Rabbit	1.55×10^7	1.57×10^7	3.23×10^6	<67
Mice	2.03×10^6	3.83×10^6	1.99×10^6	<67
Sheep	3.47×10^5	2.80×10^5	1.67×10^6	4.07×10^4
Duck	7.60×10^5	1.60×10^6	1.40×10^6	2.90×10^5
Cat	1.32×10^6	1.46×10^6	3.57×10^5	5.50×10^4
Monkey	1.45×10^7	2.35×10^7	1.35×10^5	<67
Dog	6.47×10^4	7.13×10^4	5.33×10^4	1.47×10^4
Cow	1.87×10^6	1.87×10^6	4.27×10^4	2.67×10^2
Guinea Pig	8.00×10^3	1.17×10^3	1.57×10^3	<67

*Fujioka, R.S., and Byappanahalli, M.N. 1996. Assessing the Applicability of USEPA Recreational Water Quality Standards to Hawaii and Other Tropical Islands. Water Resources Research Center, University of Hawaii, WRRC Project Completion Report: WRRC 96-01.

Enterolert Test

- EPA approved method for Ambient waters, Ground water and Wastewater
- 24-hour detection of enterococci
- Detects species:

  faecalis, faceum, avium, gallinarium, casselifavis and durans.

Other Possible Enterococci Sources

- Leaking Sewer Lines, breaks in line, manhole overflow, and/or overflow at WWTP/pump stations
- **Malicious pumper truck dumping**
- **Sludge/forage crop operation**
- Cesspools
- **Contaminated groundwater**
  - **-Karst topography**

qPCR Molecular Testing

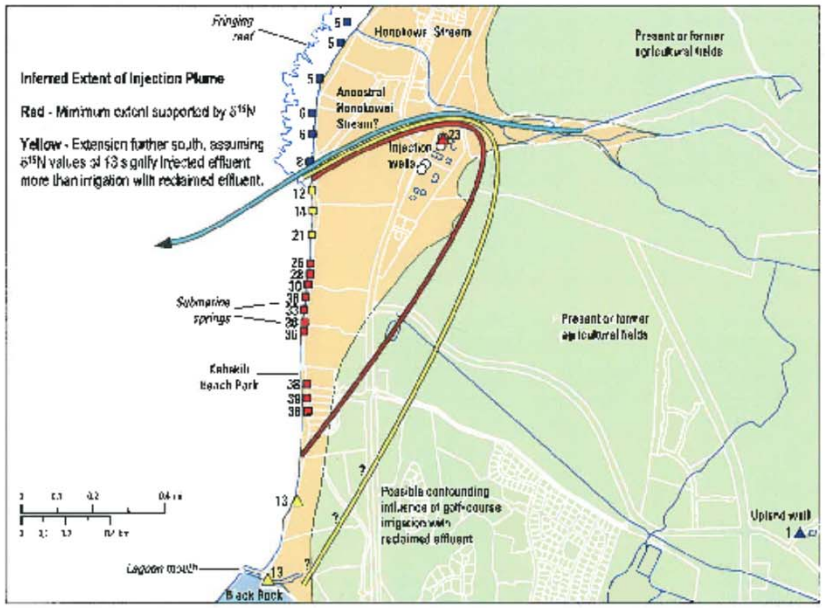
Quantitative Polymerase Chain Reaction (qPCR) is a somewhat rapid molecular method to test recreational waters for human markers. qPCR measures all DNA, including the DNA from live cells, viable but not culturable cells, dead cells, and the DNA that is found outside of a cell and in the environment (free DNA).

qPCR samples were collected on two occasions (7/23/2014 and 5/26/2015) and tested using the markers Entero 1A and HF183. Entero 1A marker were found at all sites sampled, while HF 183 was found at only one site. However, Entero 1A and HF 183 are not human specific. In Hawaii, dogs have high concentrations of HF183 and also mongoose and chickens carry the human markers. So more work is needed with qPCR to supplement current cultivation methods.

USGS
science for a changing world

Prepared in Cooperation with the Hawaii State Department of Health, Clean Water Branch

A Multitracer Approach to Detecting Wastewater Plumes from Municipal Injection Wells in Nearshore Marine Waters at Kihei and Lahaina, Maui, Hawaii



Scientific Investigations Report 2009–5253

U.S. Department of the Interior
U.S. Geological Survey

USGS Pharmaceutical Sampling

USGS/DOH collected samples at 4 sites in the Mahaulepu watershed (Sites 10, 11, 12, and in the Makauwahi Cave) as well as Poipu watershed (Waikomo stream and two coastal springs) during October of 2015.

Following USGS Protocol for Sampling and Analysis

- 109 Pharmaceutical Compounds Schedule 2440
- 69 Waste-Indicator Compounds Schedule 4433
- Nutrients: NH3, NO3, NO2, Total N, Total P, Orthophosphate
- Isotopes of H and O (useful for determining source of water)
- Isotopes of N and O (useful for detecting waste water)
- Suite of Samples Similar to Previous Oahu and Maui Sampling

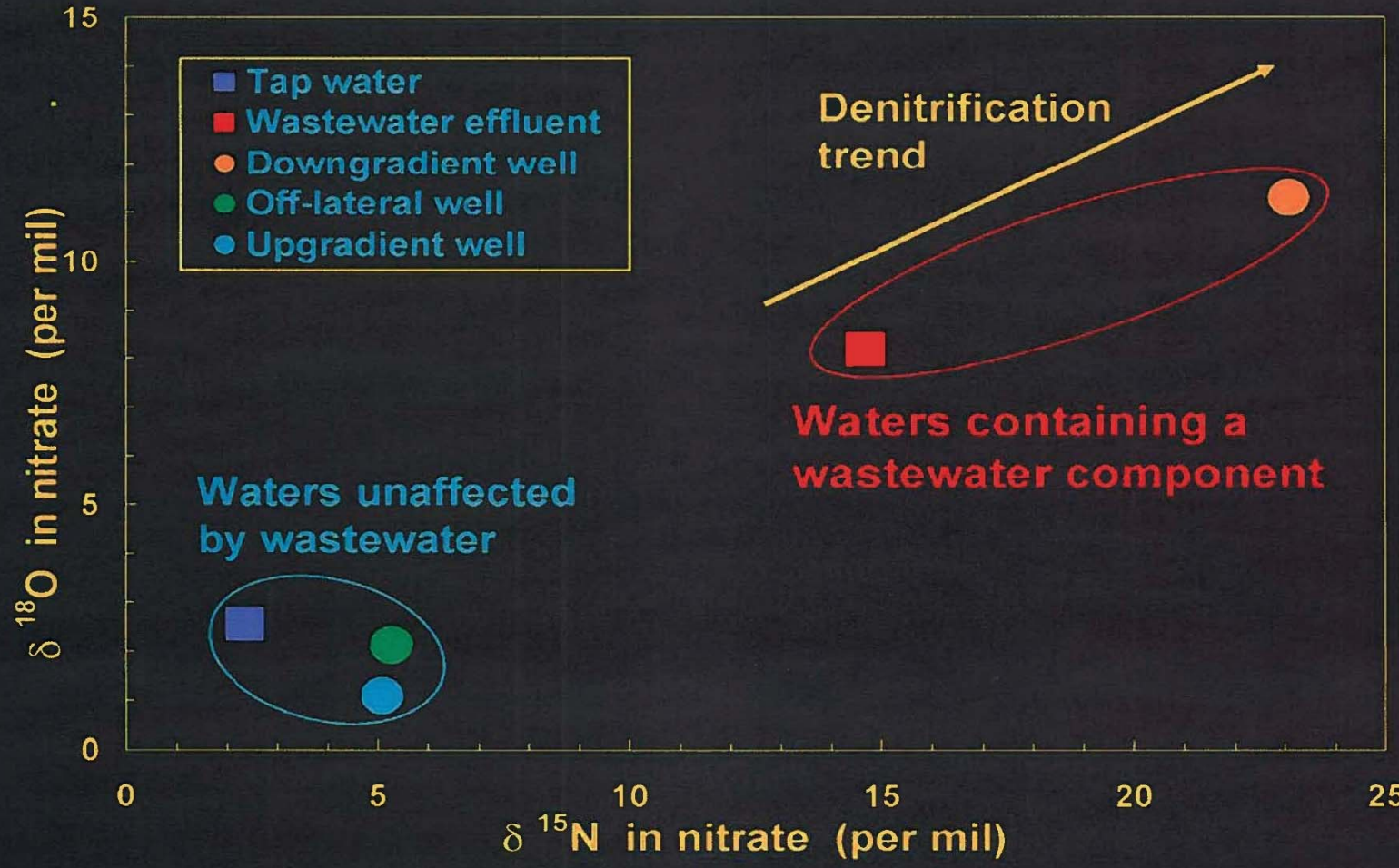
maceutical Indicators

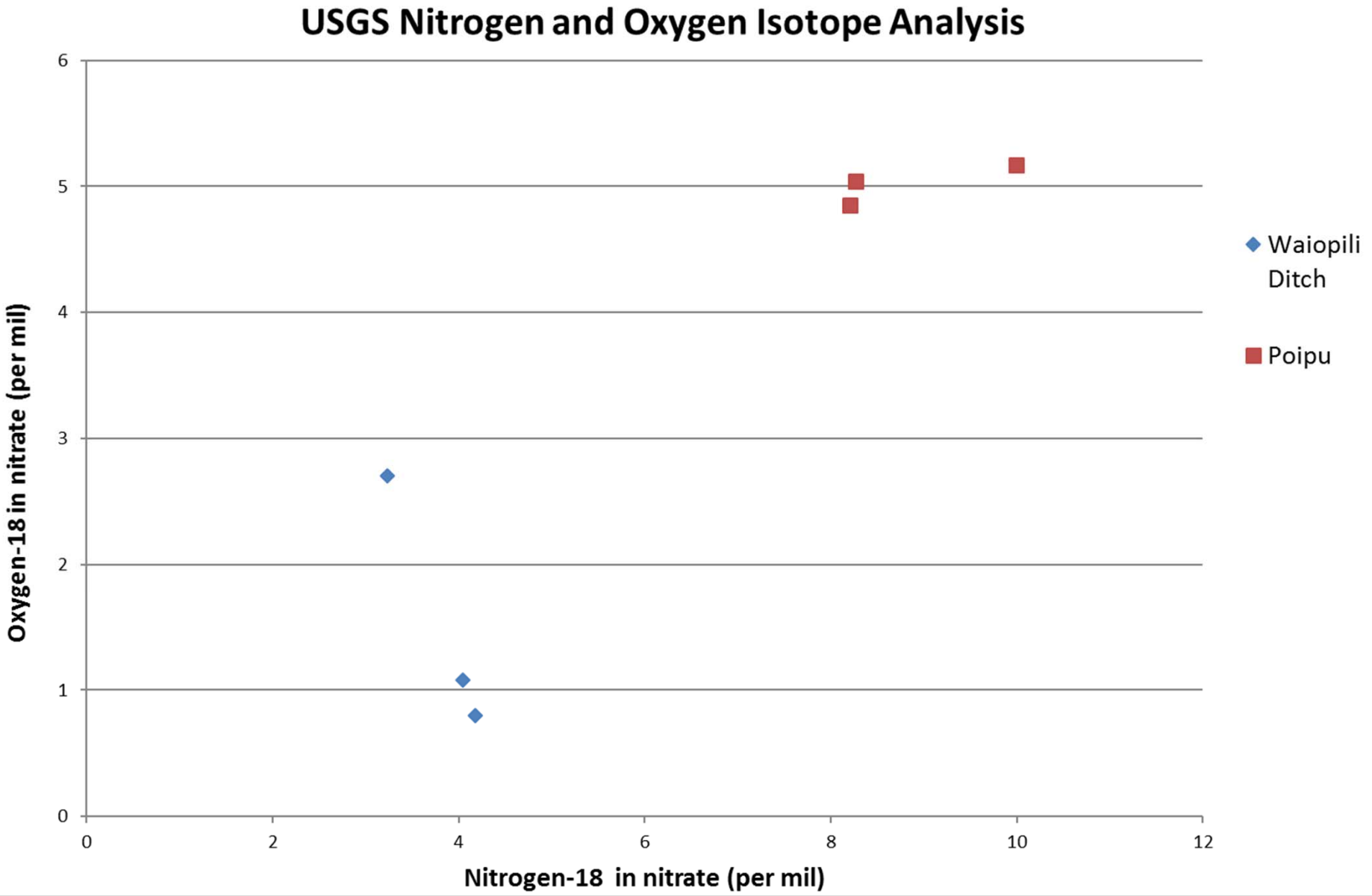
Vahiawa, Oahu		Kealakehe, Kona		Kihei, Maui				Lahaina, Maui				Waiopili, Kauai			Poipu, Kauai	
TP ent	Kaukonahua Ditch Effluent[1]	WWTP Effluent	Monitoring Well	WWRF Effluent	Monitoring Well	K1 Submarine Spring	K2 Submarine Spring	WWRF Effluent	L1 Submarine Spring	L2 Submarine Spring	South Seep	Site 10	Site 11	Site 12	Seep 3	Seep 4
-	+	+	+	ND	ND	ND	+	+	+	+	+	ND	ND	ND	+	ND
-	+	+	+	+	+	+	+	+	+	+	+	ND	ND	ND	ND	ND
D	ND	--	--	+	ND	+	--	+	+	--	+	ND	ND	ND	+	+

Schofield WWTP

d

Here's how ^{15}N worked out at Kihei




USGS Nitrogen and Oxygen Isotope Analysis
Oxygen-18 in nitrate (per mil)
Nitrogen-18 in nitrate (per mil)
0
1
2
3
4
5
6
0
2
4
6
8
10
12
Waiopili Ditch
Poipu

Weight of Evidence

Sanitary Survey

- No sewer lines in Mahaulepu Valley Watershed
- No cesspools impacting Waiopili Ditch
- No evidence of human fecal contamination of Waiopili Ditch
- Healthy population of feral pig, Nene Goose and other water birds
- Heavy canopy over Waiopili Ditch promotes high enterococci levels
- High levels of enterococci in sediment of Waiopili Ditch where not sun exposed

USGS Multi-tracer Testing

- Persistent wastewater pharmaceuticals did not show at all 3 Waiopili sites
- Delta N-15 and Delta O-18 dual isotope approach did not show waste water influence at all Waiopili sites

UC Berkeley PhyloChip

- TBD

Mahaulepu and Waikomo Watersheds Phylochip Source Tracking Study, Hawaii (Contract is under preparation)

The Andersen lab at UC Berkeley will use PhyloChip microarray analysis to provide more conclusive identification of sources of enterococci and Clostridium *perfringens* in Mahaulepu and Waikomo watersheds.

The PhyloChip microarray test can fingerprint any animal or environmental source with a single test (Dubinsky et al. 2012).

Dubinsky, E.A.,Esmaili, L.,Hulls, J.R., Cao, Griffith, J.F., and Andersen, G.L. (2012) Application of Phylogenetic Microarray Analysis to Discriminate Sources of Fecal Pollution. *Environmental Science & Technology* **46** (8), 4340-4347

Discussion