Viral Source Tracking in Karst Environments

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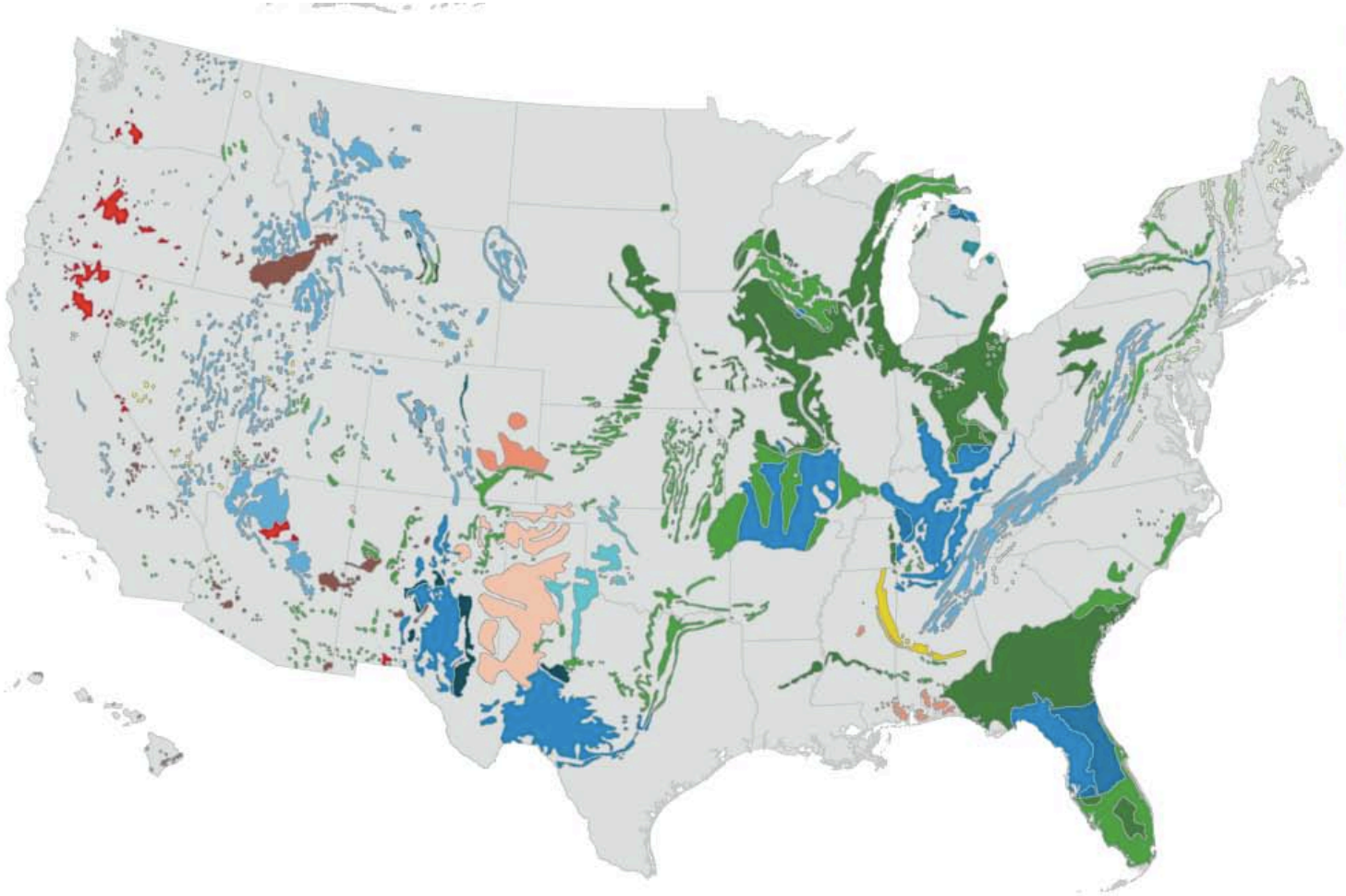
US Geological Survey



dolostone, and marble

In crystalline, highly siliceous intensely folded cabonate rock

In moderately to steeply dipping carbonate rock

In gently dipping to flat-lying carbonate rock

In gently dipping to flat-lying beds of carbonate rock beneath an overburden of noncarbonate material 10 ft (3 m) to 200 ft (60 m) thick

In moderately to steeply dipping beds of gypsum

In gently dipping to flat-lying beds of gypsum

In gently dipping to flat-lying beds of gypsum beneath and overburden of nongypsiferous material 10 ft (3 m) to 200 ft (60 m) thick

In carbonate zones in highly calcic granite

In moderately to steeply dipping beds of carbonate rock with a thin cover of glacial tilland frost-derived residual soil

FISSURES, TUBES, AND CAVES GENERALLY ABSENT; WHERE PRESENT IN SMALL ISOLATED AREAS, LESS THAN 50 FT (15 M) LONG; LESS THAN 10 FT (3 M) VERTICAL EXTENT

In crystalline, highly siliceous intensely folded carbonate rock

In moderately to steeply dipping beds of carbonate rock

In gently dipping to flat-lying beds of carbonate rock

FEATURES ANALOGOUS TO KARST

Fissures and voids present to d adepth of 250 ft (75 m) or more in areas of subsidence from piping in thick unconsolidated material

Fissures and voids present to a depth of 50 ft (15 m) in ares of subsidence from piping in thick, unconsolidated material

Fissures, tubes, and tunnels present to a depth of 250 ft (75 m) or more in lava

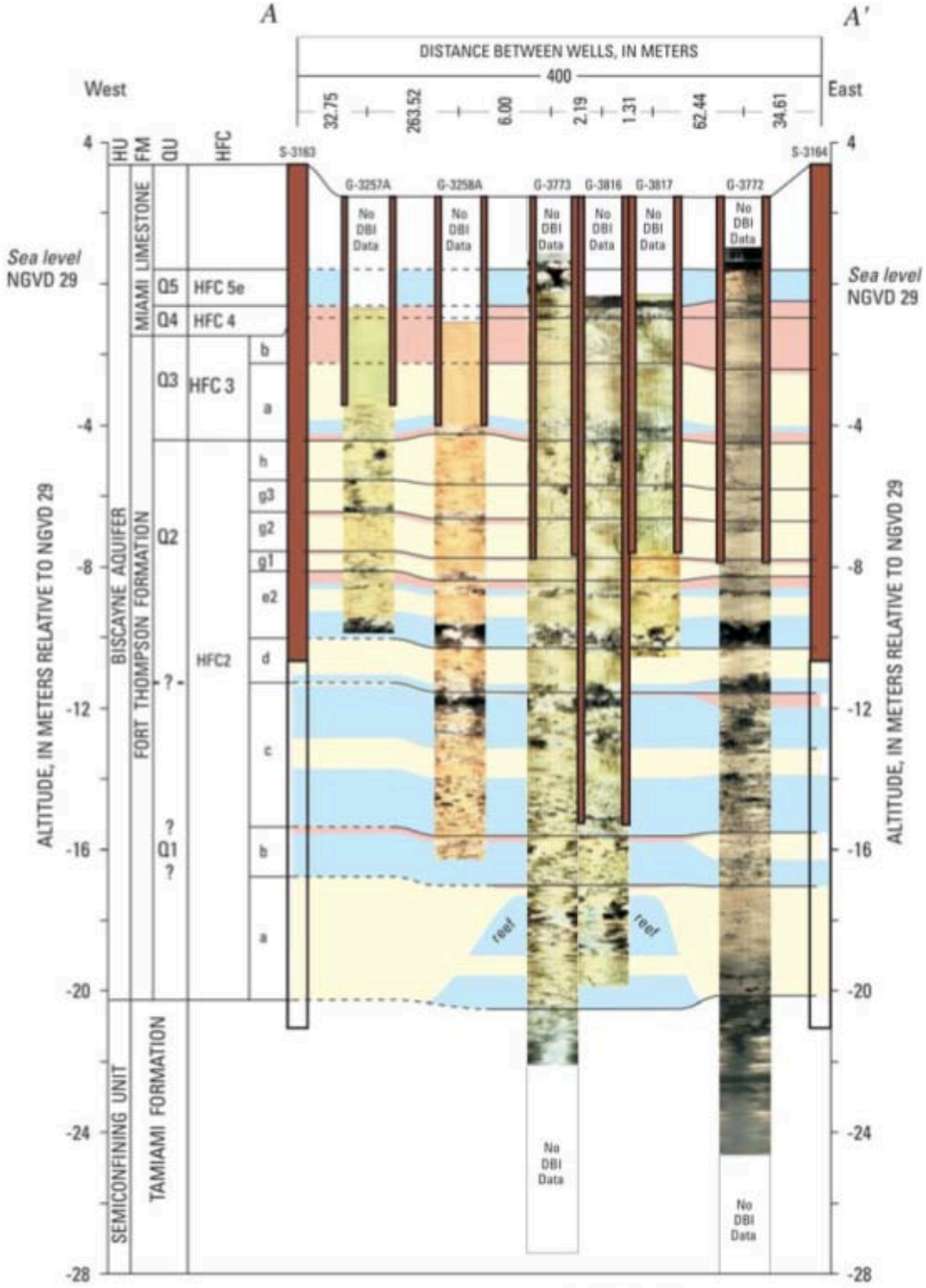
Fissures, tubes, and tunnels present to a depth of 50 ft (15 m) in lava

Abers Equal Area Projection

SCALE 1:7,500,000

Redrawn from the map by

Davies, W.E., Simpson, J.H., Ohlmacher, G.C., Kirk, W.S., and Newton, E.G., 1984, Engineering aspects of karst: U.S. Geological Survey, National Atlas, scale 1:7,5000



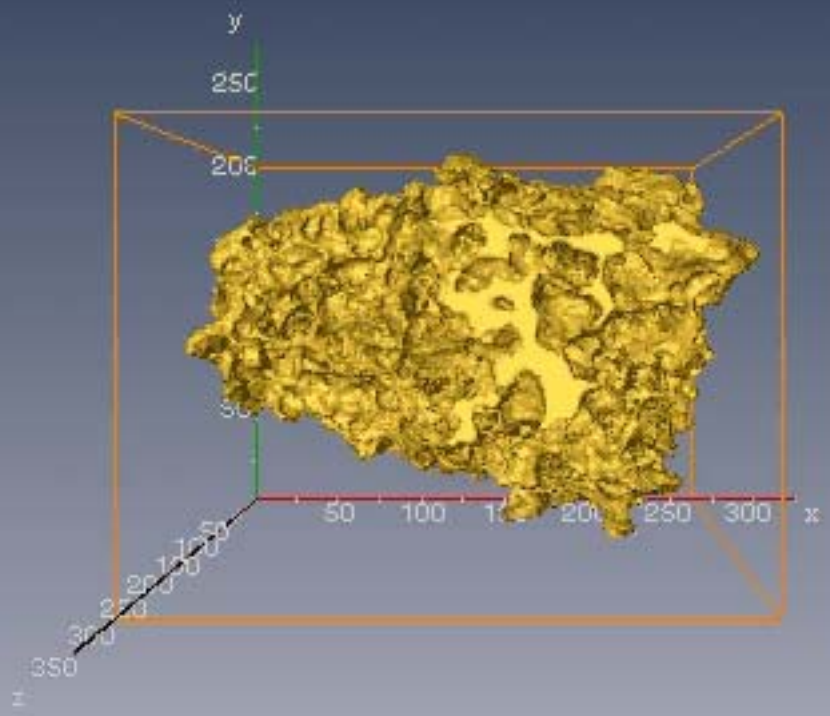
EXPLANATION

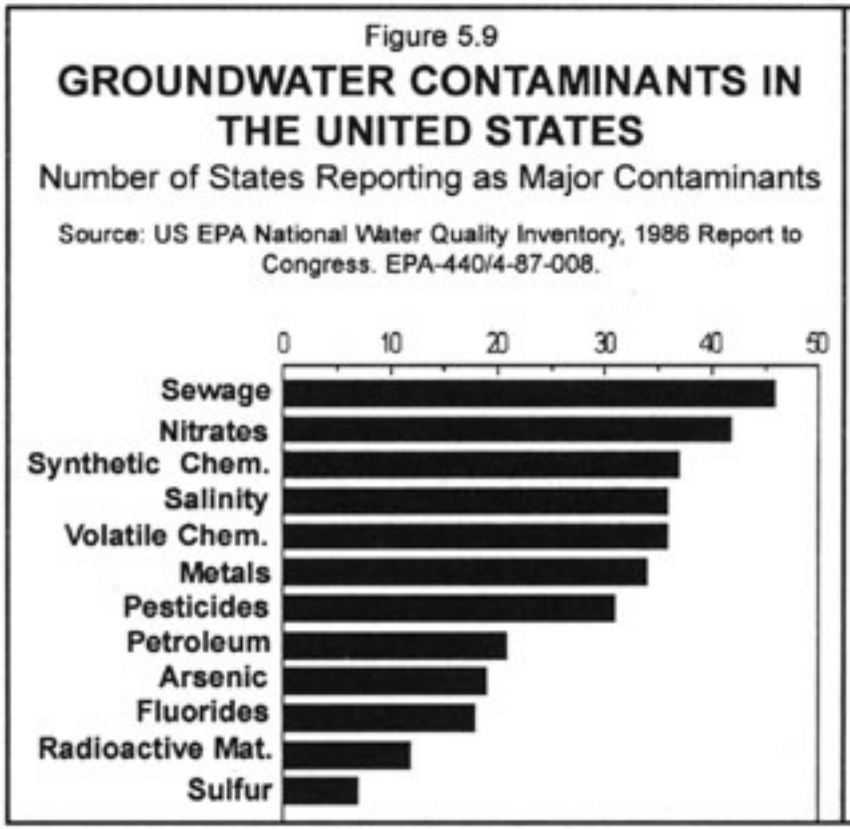
DBI DIGITAL BOREHOLE IMAGE
FM FORMATION
HFC HIGH-FREQUENCY CYCLE

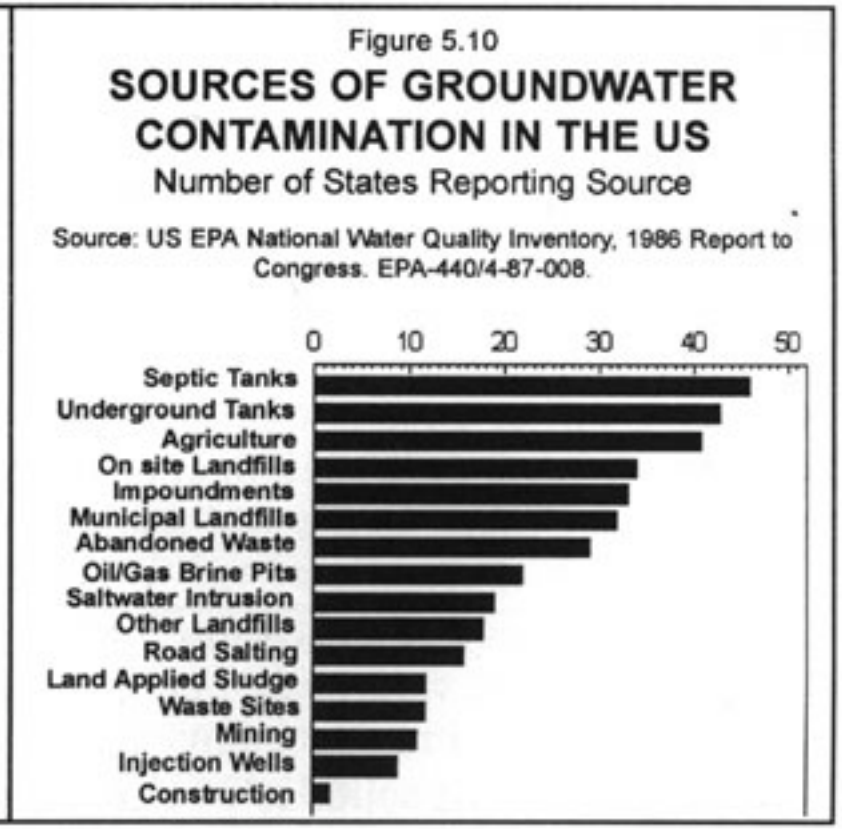
PORE CLASSES OF THE

PORE CLASS I
PORE CLASS II

Animation courtesy Kevin Cunningham, USGS

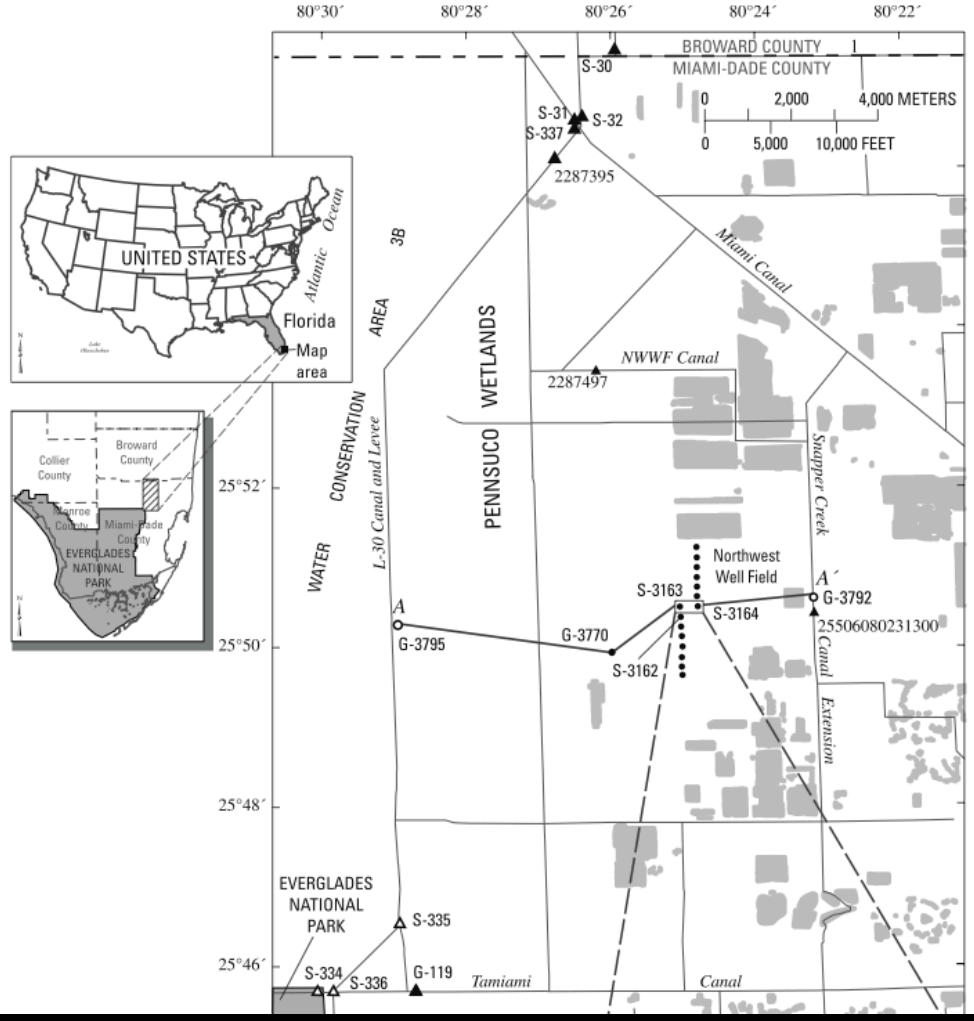






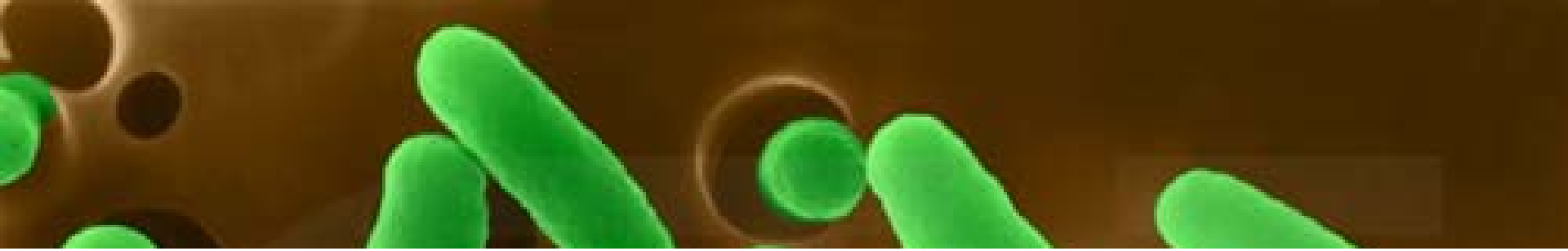
van der Leeden, F. et al. (1990). The Water Encyclopedia. Lewis Publishers Inc.: Chelsea, Michigan, 48118. (p.525).

Renkin et al. 2005. Assessing the vulnerability of a municipal well field to contamination in a karst aquifer. Env. And Eng. Geoscience. 4:319-331



'igure 2. North-facing oblique aerial photo showing a partial view of well field and active rock-mine lakes north of production well 15. Production vell 9 pump house (S-3164) is visible near the lower right corner (photo taken by Shane Ploos, U.S. Geological Survey, April 2004).

Test	Release Date	Distance to Production Well	Mean Velocity	Citation
Horizontal flow meter	4/8/96–4/9/96[1]	518–853 m (1,700–2,800 ft)	0.52–4.76 m/day (1.70–15.62 ft/day)	Krupa et al., 2001
Conservative tracers rhodamine WT and fluorescein (three traces)	1/28/98–12/17/98	257–849 m (845–1,830 ft)[2]	21.5–27.6 m/day (70.5–90.6 ft/day)	Miami–Dade Department of Environmental Resources Management, 1999
Conservative tracers rhodamine WT and fluorescein	9/10/99	926 m (3,040 ft)[3]	1.04–2.86 m/day (3.4–9.4 ft/day)	Guha et al., 2003
Conservative tracer rhodamine WT and deuterium	4/22/03	100 m (328 m)	386 m/day (1,266 ft/day)	This report



Microbiology Primer – Types/size

Bacteria ~ 0.5 to 1.5 um, genome ~ 10^7bp

Virus ~ 0.020 to 0.25 um, genome 1,700 to 360,000 bp

Satellite virus - virusoid – 375 bp circular ss RNA, use helper virus for movement/replication. Genome encodes a coat protein of its helper virus – i.e. *symbiosis*

Viroid no protein coat = naked, genome circular 240-400bp ss RNA – plant pathogens

Prion - ~ 250 aa, proteinaceous infectious particles, resistant to inactivation, causes scrapie, kuru, mad cow, etc.

Wastewater Microbiology

1. 13 million fecal coliforms per gram of feces
2. 10 million enterococci per gram of feces
3. 1 trillion total bacteria per gram of feces (~400-500 species - ~30% mass of feces)
4. ~140 types of enteric viruses found in humans (~1 billion per gram) and 1-10% of the population is shedding them at any given time at 1 million to 1 billion per gram of feces (Hepatitus A viruses, Noroviruses, Poliovirus, Coxsackie viruses, Echoviruses, Astroviruses, Rotaviruses, etc.)
5. Enteric virus diseases - asymptomatic, gastroenteritis, cold and flu-like symptoms, myocarditis, paralysis, etc.

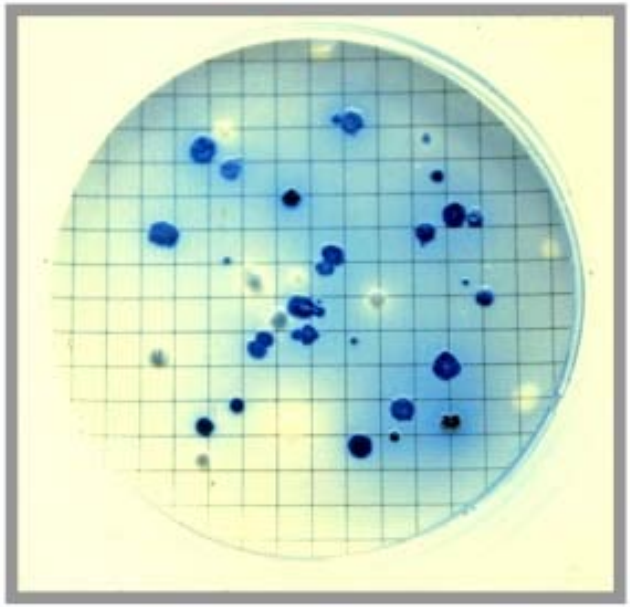
Florida Coastal Septic Tank Trivia

- Let be conservative (*really conservative as 50% of the US population reside in coastal communities*) - If 1% of the coastal counties septic tanks are coastal in nature = 137,863 septic tanks
- 2.5 people per household shedding 100g feces each.
- 1 trillion bacteria per gram
- 137863 x 2.5 x 100g x 10^{12} = 34.46 Quintillion bacteria
  - **34,465,800,000,000,000,000**
- And don't forget the viruses ~34.46 quadrillion
- ***Everyday***

Currently used indicator bacteria

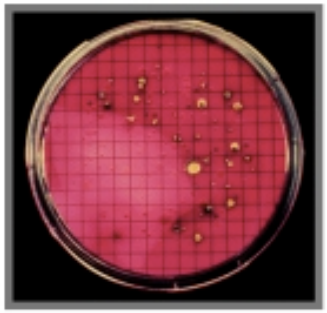
- Total coliforms (gram negative bacilli, growth at 35°C after 48 hours
  - *Escherichia* spp.
  - *Klebsiella* spp.
  - *Shigella* spp.
  - *Salmonella* spp.
  - *Yersinia* spp.
- Fecal colifroms (growth at 45°C after 48 hours)
  - Bacteria within the total coliform group, such as *E. coli*, which have adapted for growth at intestinal temperatures
- Enterococci (gram-posotive cocci, growth at 41°C after 48 hours)
  - Species of bacteria such as *Enterococcus faecalis*
- *Clostridium perfringens* (spore-forming anaerobic bacteria that grows at 45°C after 24 hours)

mFC agar



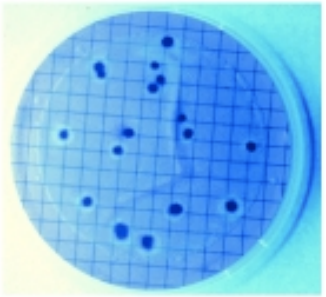
Colonies that are light to dark blue, in whole or part, are counted as fecal coliforms

mENDO



Colonies with a green metallic sheen are counted as total coliforms

NA-MUG

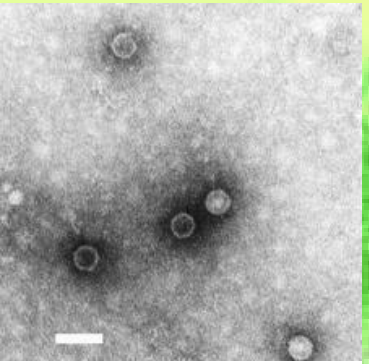


Colonies with a fluorescent halo are counted as *E. coli*

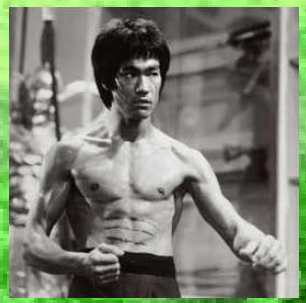
The problem with the indicator system

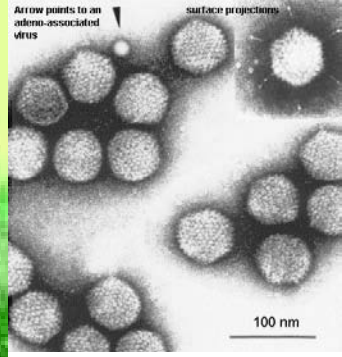
- They do not accurately reflect water quality in tropical and sub-tropical environments
  - They can survive and replicate in warm soils and shallow warm marine sediments
- They can come from animals
  - They can exchange genetic information with isolates from other animals and humans
    - This has plagued bacterial source tracking assays

Enter the Virus



Polioviruses





Astroviruses

Why use viruses?

- Host specificity
- Viruses such as Polio viruses and Hepatitis A viruses only use humans as hosts
    - i.e. if you find them in the environment you know that human wastewater is impacting that region – no question
    - there are assays specific for viruses shed from livestock and domestic animals
- This equals library independent source tracking
    - i.e. bacteria source tracking DNA libraries are extremely limited in capability
- they are shed in very high numbers (> million/gram feces).
- They readily move through karst due to their small size.
- Specific virus assays (polymerase chain reaction) are sensitive, rapid, inexpensive and give an accurate picture of the presence of host specific waste impacting ground or surface waters

Factors Influencing Virus Movement in Groundwater

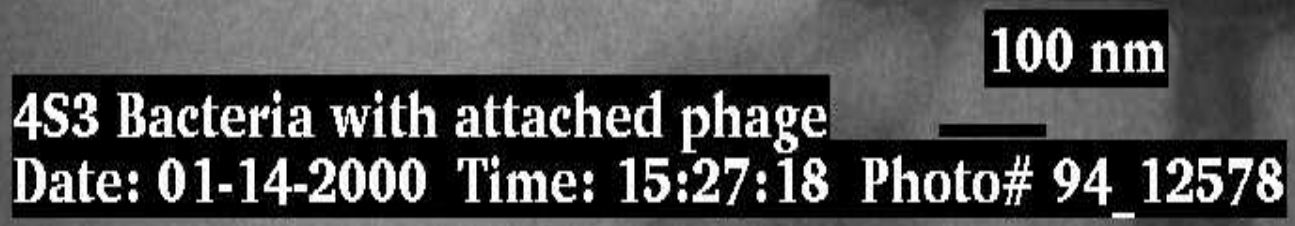
Gerba and Bitton, 1984 Microbial Pollutants: Their Survival and Transport Pattern to Groundwater. Groundwater Pollution Microbiology

- **Soil type - finer soils = increased adsorption of virus. Charged soils such as those containing iron = increased adsorption. Muck soils are poor adsorbents.**
- **pH - The lower the pH the higher the adsorption.**
- **Cations - cations lower the repulsive forces seen between soils and viruses and increase adsorption.**
- **Flow rate - increased flow equals increased movement.**
- **Saturation - increased saturation equals increased movement.**
- **Soluble organics - Humic and fulvic acid reduce virus adsorption to soils = competition**

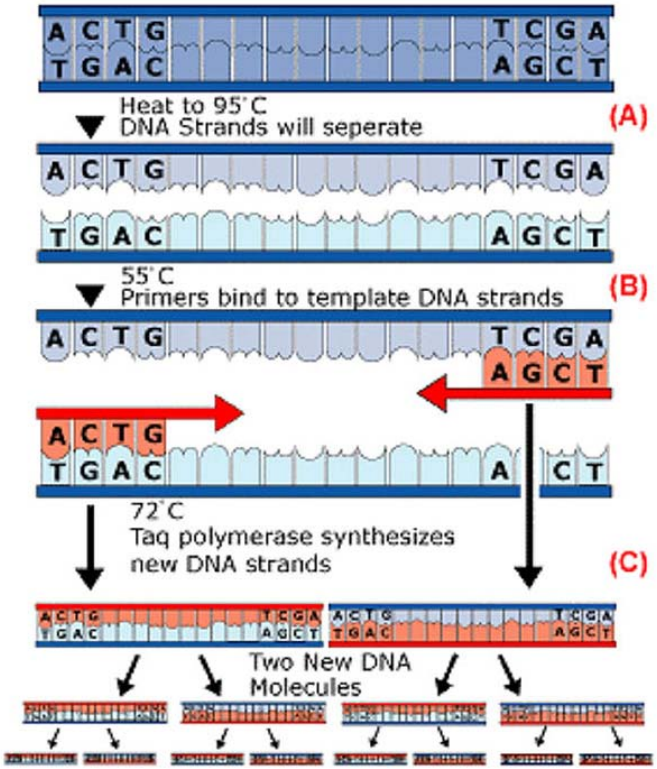
Factors Influencing Virus Survival in Groundwater

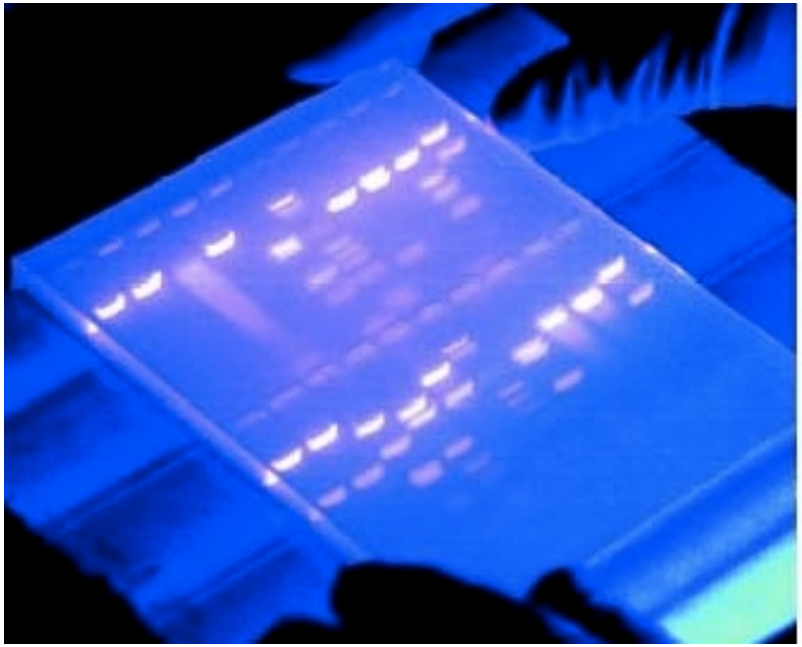
Gerba and Bitton, 1984 Microbial Pollutants: Their Survival and Transport Pattern to Groundwater. Groundwater Pollution Microbiology

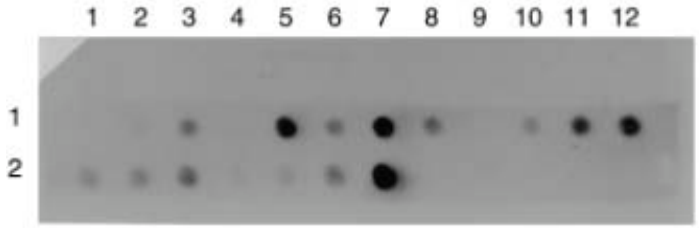
- **Temperature - lower temperatures = higher viral survival rates.**
- **Desiccation - drier soils = lower viral survival rates.**
- **Soil texture - clays and humics increase survival via water retention.**
- **pH - low pH may increase adsorption = higher survival rates.**
- **Cations - cations (calcium,magnesium) increase adsorption = higher survival rates.**



eppendorf
vapo.protect
eppendorf
Mastercycler
pro S


Heat to 95°C
DNA Strands will seperate
(A)
55°C
Primers bind to template DNA strands
(B)
72°C
Taq polymerase synthesizes
new DNA strands
(C)
Two New DNA
Molecules




1 2 3 4 5 6 7 8 9 10 11 12
1
2

StepOne™ Software v2.1

File Edit Instrument Analysis Tools Help

New Experiment Open... Save Close Send Experiment to Instrument... Download Experiment from Instrument... Export... Print Report...

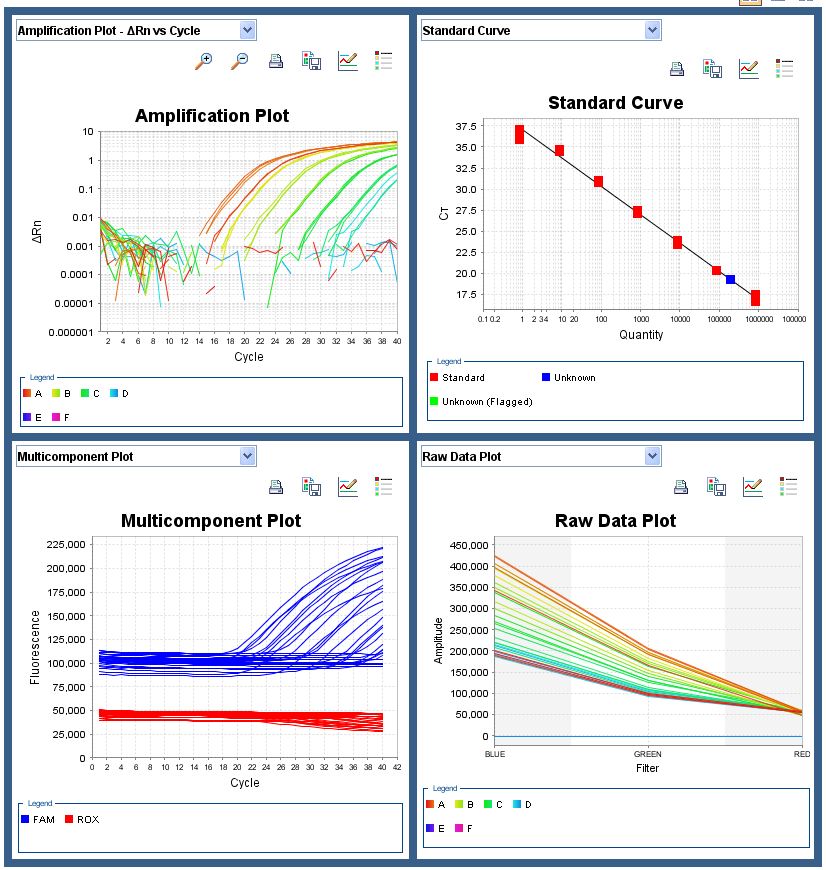
Experiment Menu

- Setup
- Run
- Analysis
  - Amplification Plot
  - Standard Curve
  - Multicomponent Plot
  - Raw Data Plot
  - QC Summary
  - Multiple Plots View

Experiment: Ypestis3aprimers Type: Standard Curve Reagents: TaqMan® Reagents

Analyze Analysis Settings

Multiple Plots View



View Plate Layout / View Well Table

Select Wells With: - Select Item - - Select Item -

Show in Wells View Legend

	1	2	3	4	5	6	7	8
A	N Target 1 Ct: Undetermin...	N Target 1 Ct: Undetermin...	N Target 1 Ct: Undetermin...	Sample 1 U Target 1 1.98E5 Ct: 19.25	Sample 1 U Target 1 1.91E5 Ct: 19.3	S Target 1 8.52E5 Ct: 16.69	S Target 1 8.52E5 Ct: 17.12	S Target 1 8.52E5 Ct: 17.49
B	S Target 1 8.52E4 Ct: 20.28	S Target 1 8.52E4 Ct: 20.29	S Target 1 8.52E4 Ct: 20.33	S Target 1 8.52E3 Ct: 23.47	S Target 1 8.52E3 Ct: 23.89	S Target 1 8.52E3 Ct: 23.92	S Target 1 852.35 Ct: 27.05	S Target 1 852.35 Ct: 27.22
C	S Target 1 852.35 Ct: 27.42	S Target 1 85.24 Ct: 30.79	S Target 1 85.24 Ct: 30.96	S Target 1 85.24 Ct: 31	S Target 1 8.52 Ct: 34.53	S Target 1 8.52 Ct: 34.71	S Target 1 8.52 Ct: 34.47	1 S Target 1 0.85 Ct: 37.1
D	1 S Target 1 0.85 Ct: 35.9	1 S Target 1 0.85 Ct: 36.99	2 S Target 1 0.09 Ct: Undetermin...	2 S Target 1 0.09 Ct: Undetermin...	2 S Target 1 0.09 Ct: Undetermin...			
E								
F								

Wells: U 2 Unknown S 24 Standard N 3 Negative Control 19 Empty

Analysis Summary: Total Wells in Plate: 48 Wells Set Up: 29 Wells Omitted Manually: 0 Wells Flagged: 6 Wells Omitted by Analysis: 0 Samples Used: 1 Targets Used: 1

Home | 5Feb10 Lake S...eroviruses.eds | Lake Seminole...ve to zero.eds | Polio Standar...rve 052610.eds | Ypestis3aprimers.eds

Disconnected

start | StepOne™ Soft... | Search Desktop | 3:02 PM

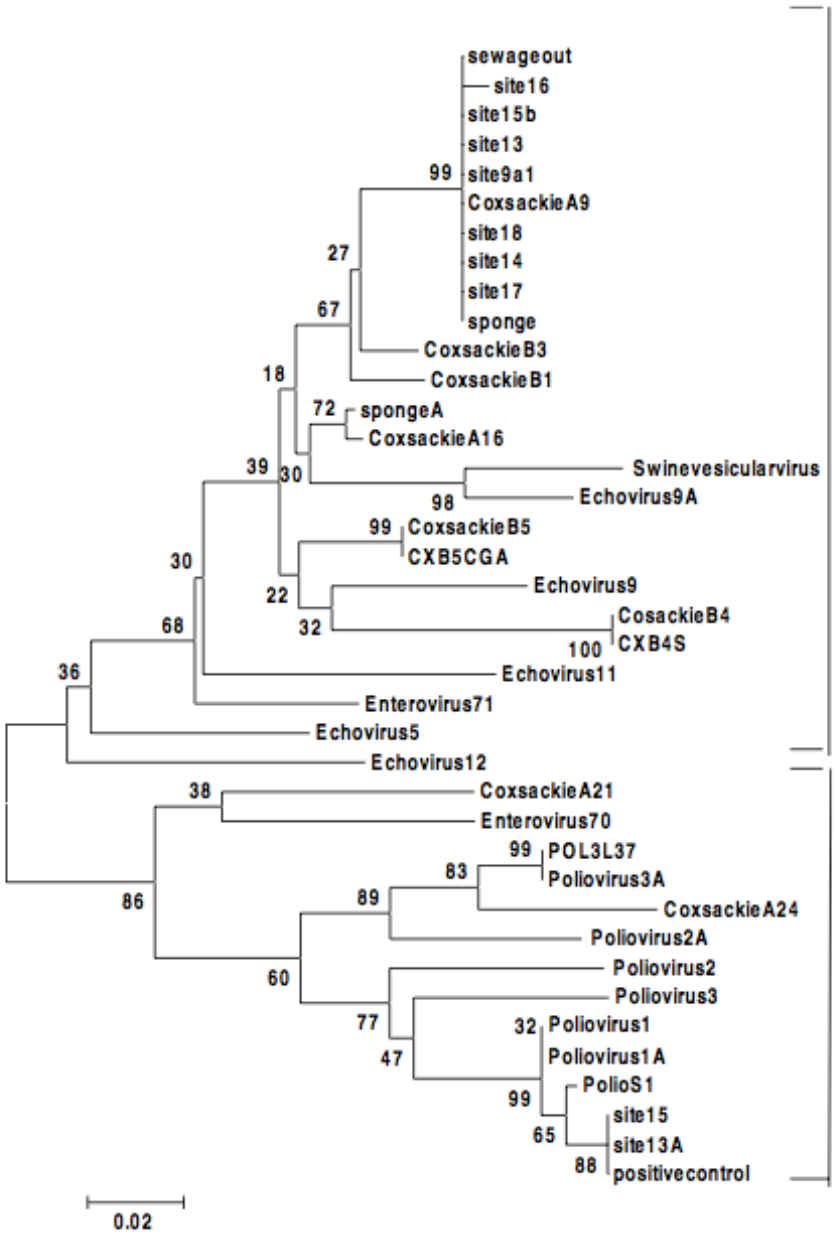
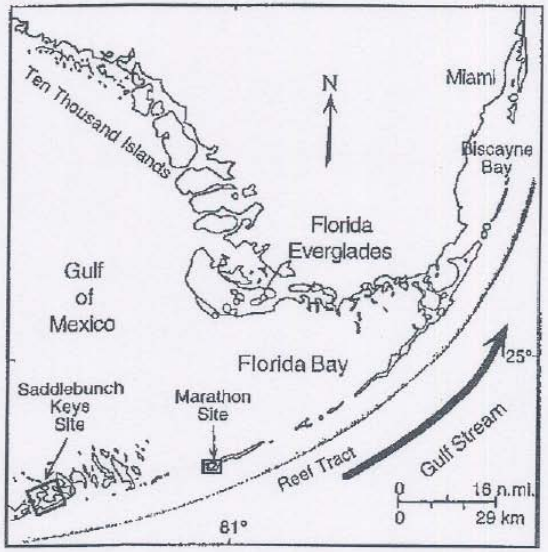
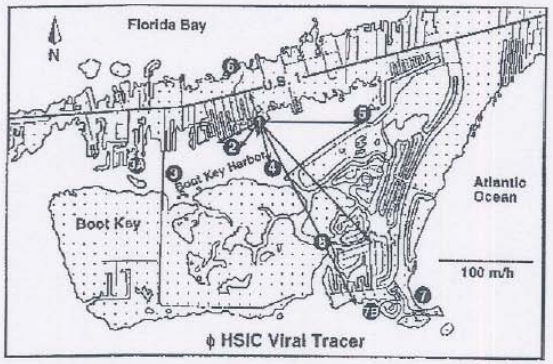


Fig. 3. A neighbor-joining tree derived from an alignment of 196 base pairs of the 5′ UTR from enteroviral standards and our specimens from the Florida Keys.

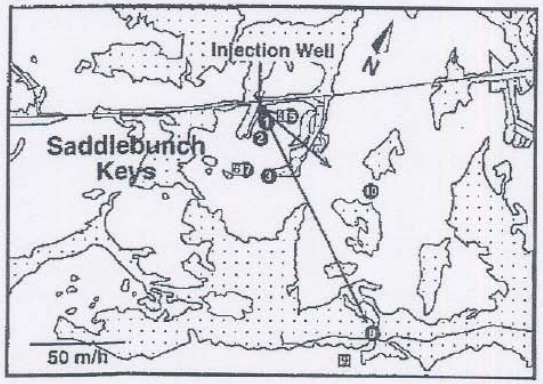
a

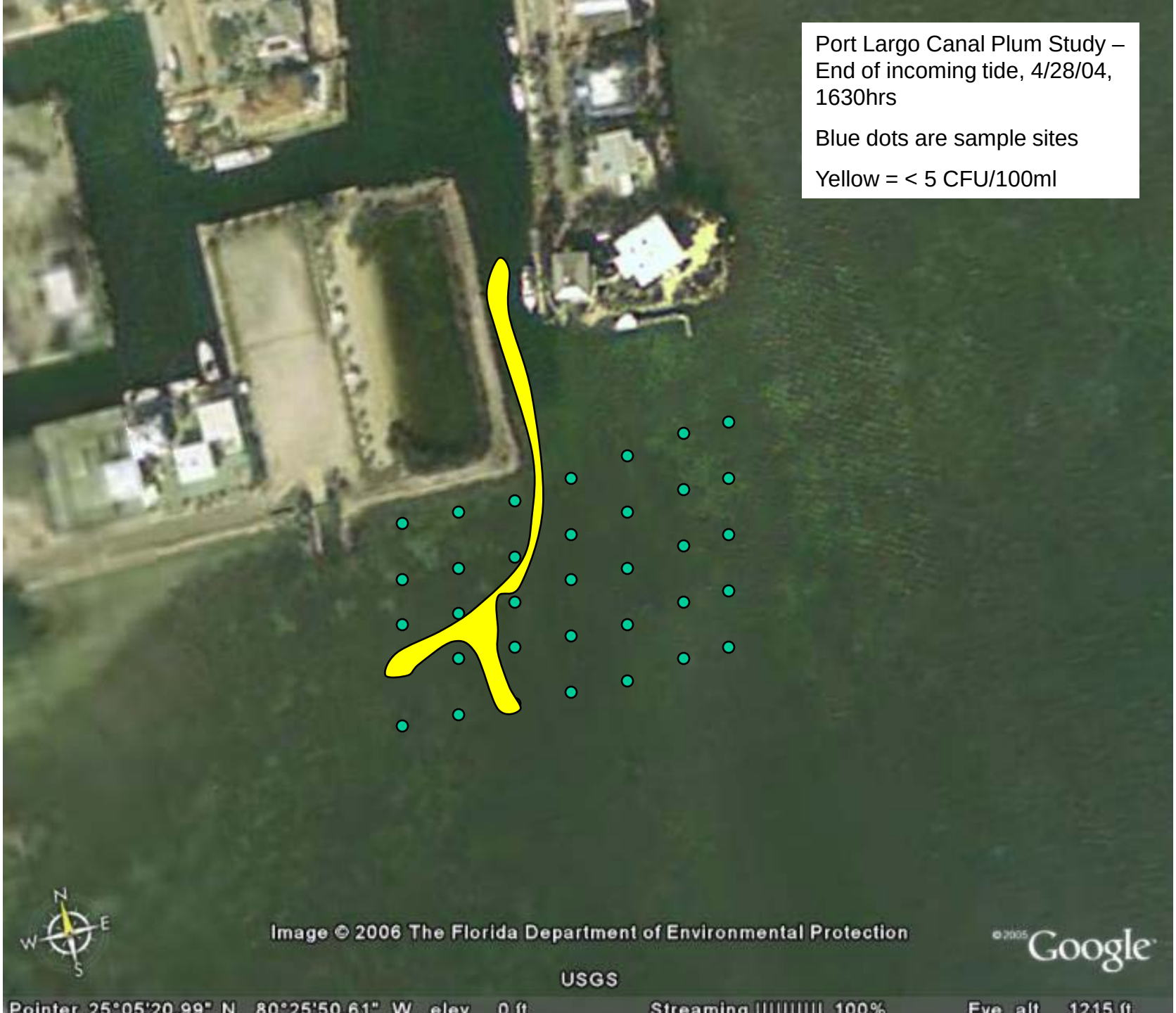

Ten Thousand Islands
N
Miami
Biscayne Bay
Florida Everglades
Gulf of Mexico
Florida Bay
25°
Saddlebunch Keys Site
Marathon Site
Reef Tract
Gulf Stream
0 16 n.mi.
0 29 km
81°

b

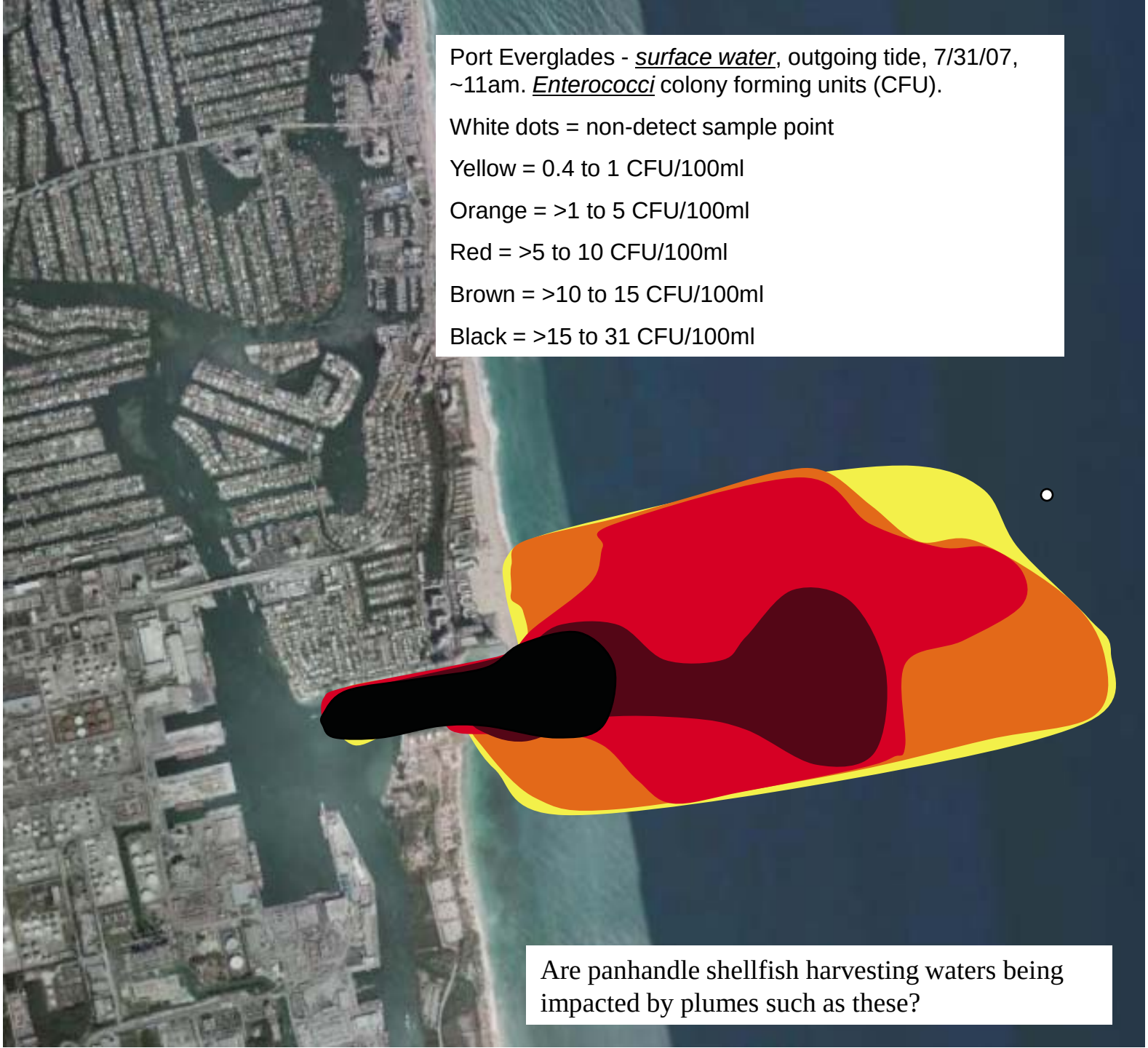

Florida Bay
N
U.S. 1
Boot Key Harbor
Atlantic Ocean
Boot Key
100 m/h
ϕ HSIC Viral Tracer

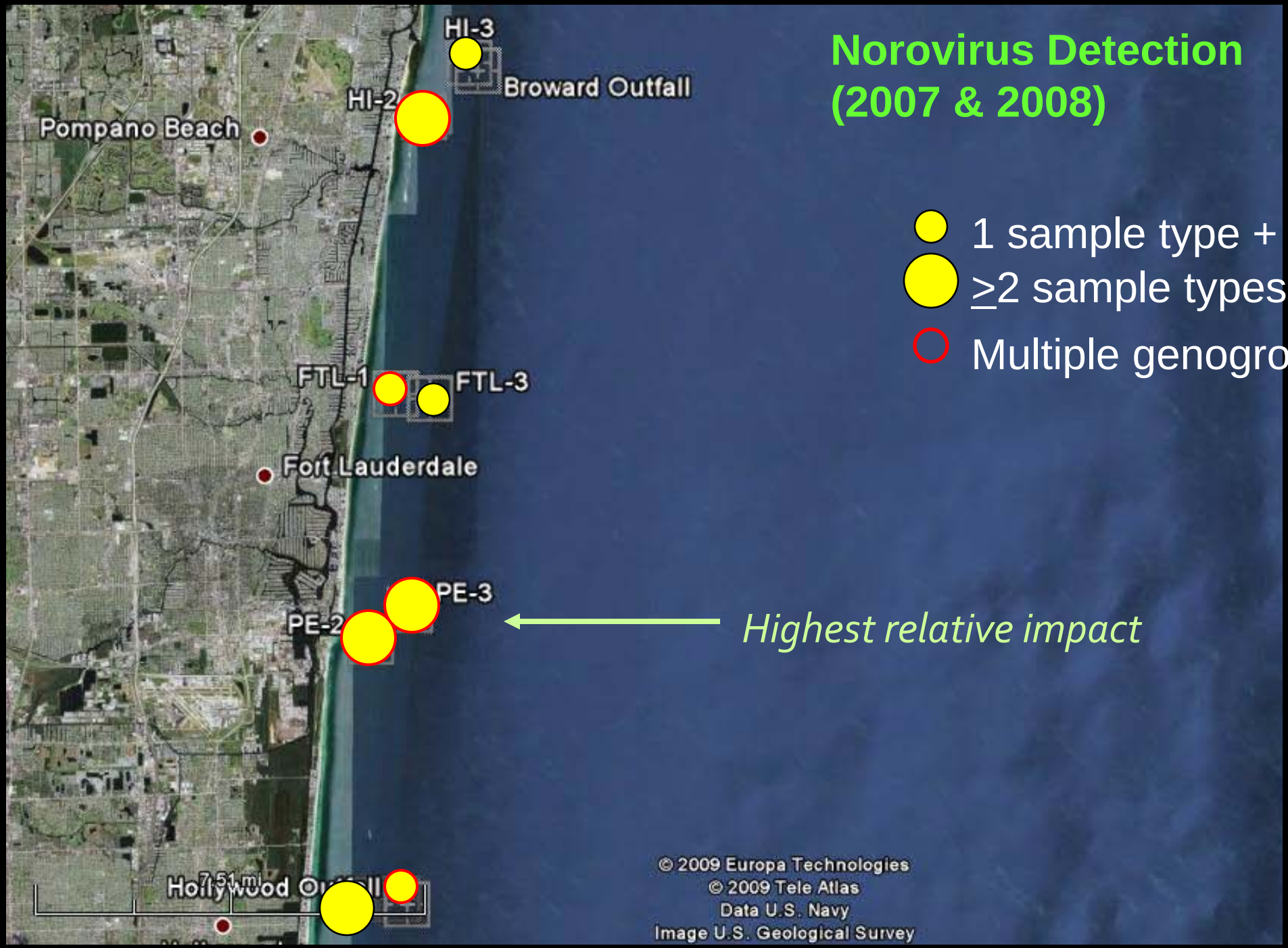
c


Injection Well
N
Saddlebunch Keys
50 m/h


Port Largo Canal Plum Study – End of incoming tide, 4/28/04, 1630hrs
Blue dots are sample sites
Yellow = < 5 CFU/100ml
N
E
S
W
Image © 2006 The Florida Department of Environmental Protection
©2005 Google
USGS
Pointer 25°05'20.99" N 80°25'50.61" W elev 0 ft
Streaming 100%
Eye alt 1215 ft

Port Largo Canal Plum Study – End of outgoing tide, 4/28/04, 10:25hrs
Blue dots are sample sites
Yellow = < 5 CFU/100ml
Orange = > 5 CFU/100ml
Red = ≥ 10 CFU/100ml
Brown = ≥ 20 CFU/100ml
Black = ≥ 50 CFU/100ml
N
E
S
W
Image © 2006 The Florida Department of Environmental Protection
©2005 Google
USGS
Pointer 25°05'20.99" N 80°25'50.61" W elev 0 ft
Streaming 100%
Eye alt 1215 ft


Port Everglades - *surface water*, outgoing tide, 7/31/07, ~11am. *Enterococci* colony forming units (CFU).
White dots = non-detect sample point
Yellow = 0.4 to 1 CFU/100ml
Orange = >1 to 5 CFU/100ml
Red = >5 to 10 CFU/100ml
Brown = >10 to 15 CFU/100ml
Black = >15 to 31 CFU/100ml
Are panhandle shellfish harvesting waters being impacted by plumes such as these?


Norovirus Detection
(2007 & 2008)
HI-3
Broward Outfall
HI-2
Pompano Beach
FTL-1
FTL-3
Fort Lauderdale
PE-3
PE-2
Highest relative impact
Hollywood Outfall
7.51 mi
© 2009 Europa Technologies
© 2009 Tele Atlas
Data U.S. Navy
Image U.S. Geological Survey
1 sample type +
≥2 sample types
Multiple genogro

Septic system failure rate 25%

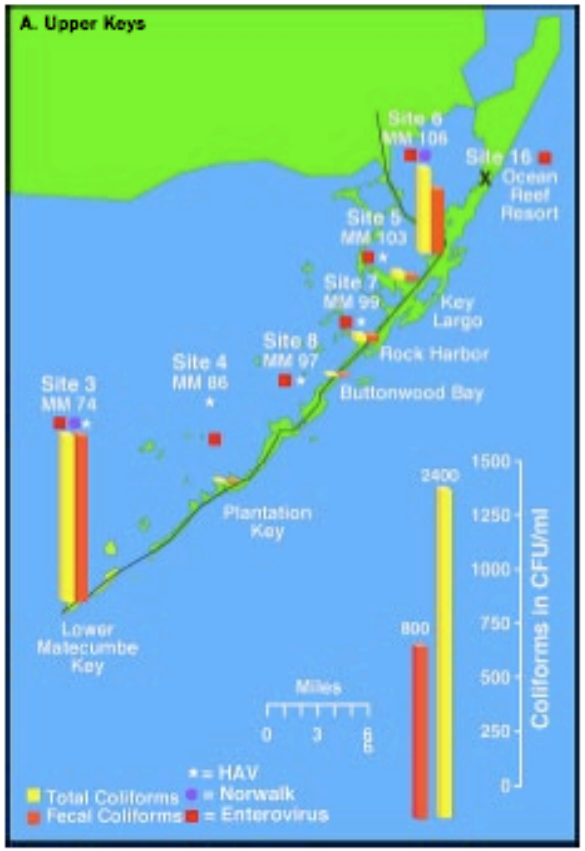
- ~ 1,000 Class VI injection wells
- ~ 11,000 cesspits
- ~36,000 septic tanks
- Every septic system made has a drain-field……..which is used each time waste material goes down a sink or toilet…….microbes, nutrients, toxic chemicals….
- System failure??????

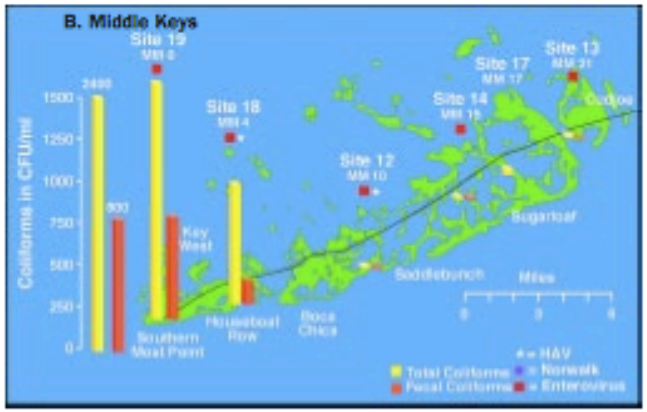
Most coastal pollution research = Central and South Florida
This example = viral source tracking in the Florida Keys

Keys Canal Study

Standard Microbial indicators
= good water quality

Pathogenic human viruses
= 95% of sites positive





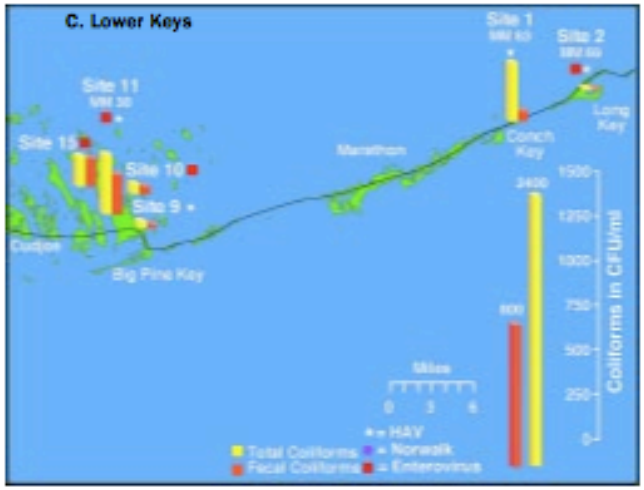


Figure 3. Occurrence of coliform indicators versus human pathogenic viruses in canals and nearshore waters of the Florida Keys. The total coliform and fecal coliform columns next to the reference concentration bar in each panel indicate the maximum single-sample limits for the respective coliform group. No coliforms were isolated at the X at site 16.

Both molecular and conventional methods were used to study the microbiological risk to swimmers at wastewater-impacted beaches in Hawaii (Colwell et al. 1996). It was found

Indicator rank data. Each site is given a value based on the number of each indicators found for each sample date.
1 = lowest number
7 = highest number
Indicator values are then summed (all dates and then FC and Ent.).
#’s to the right are The final score for each site.

5

3

4

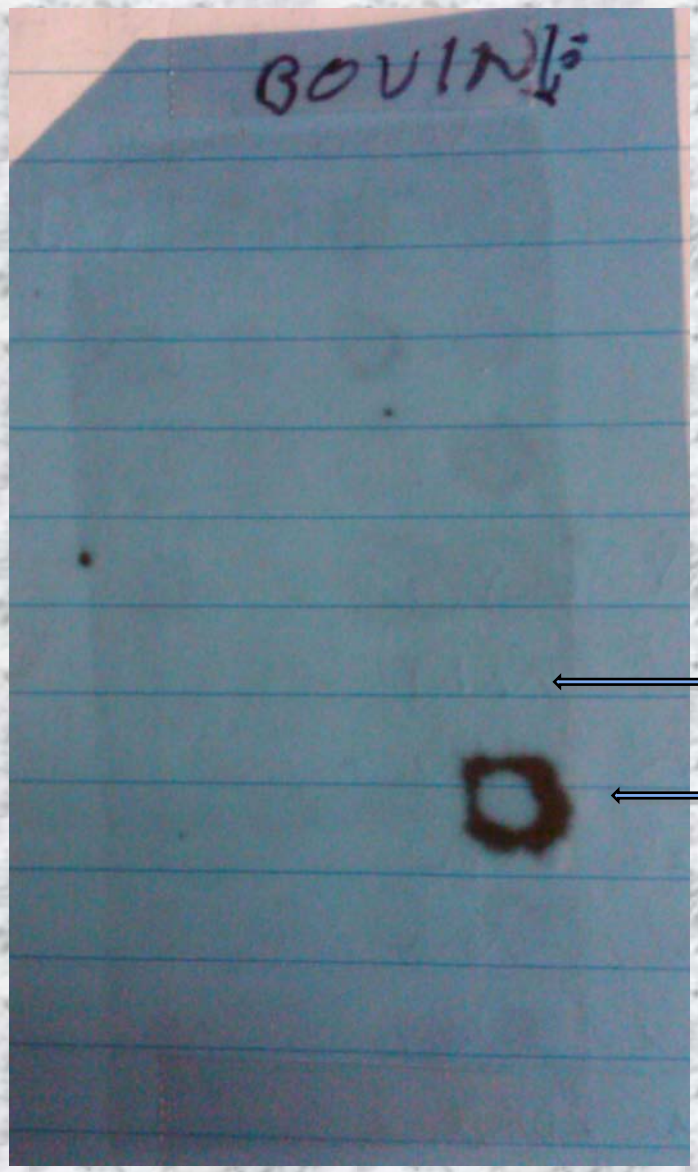
6

7

2

1

Bovine enteroviruses were not detected in any of the samples



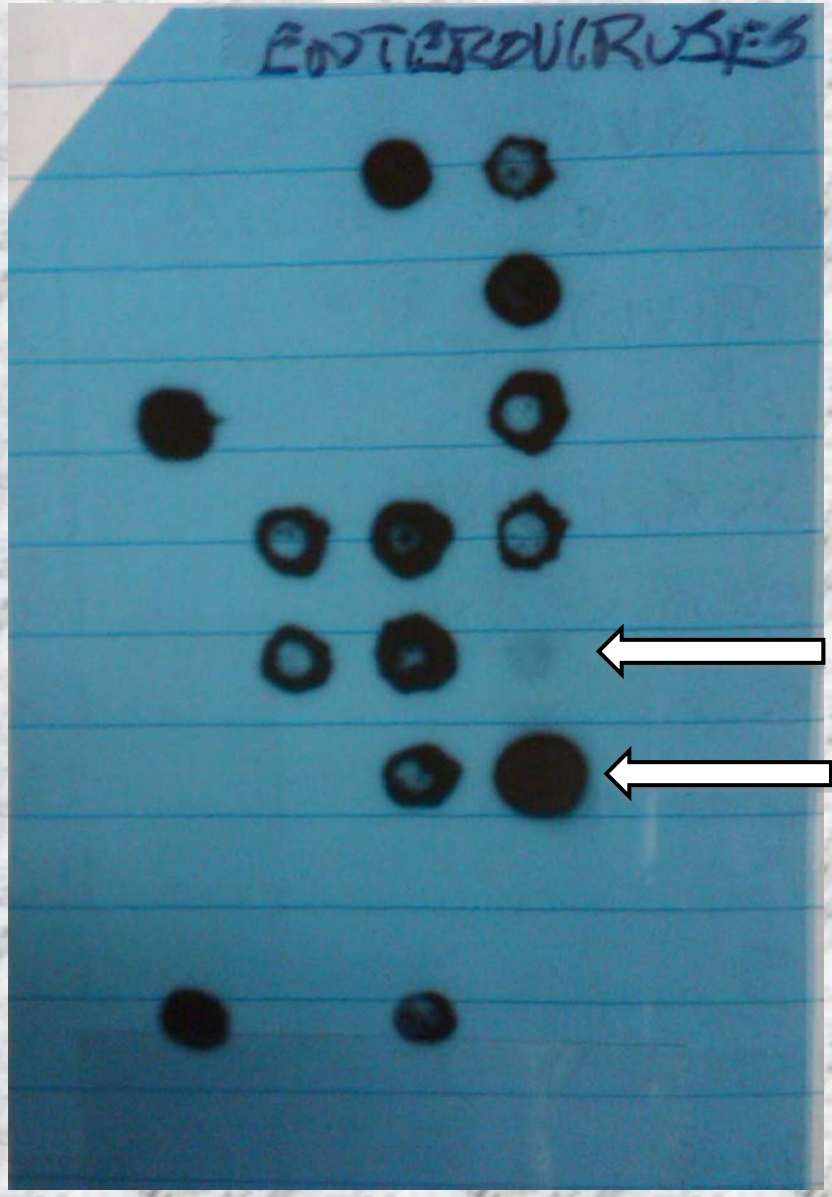
Negative control

Positive control

RT-PCR for enteroviruses
13 of 48 samples positive via dot blot ~27%.

The image to the right is of the dot-blot of the last 4 sample sets

The primer set used in this assay will detect all human enteroviruses (Polio, Coxsackie, Echo) and swine vesicular virus (a virus that evolved from a human Coxsackie B virus)



Negative control

Positive control

15%

0%

50%

15%

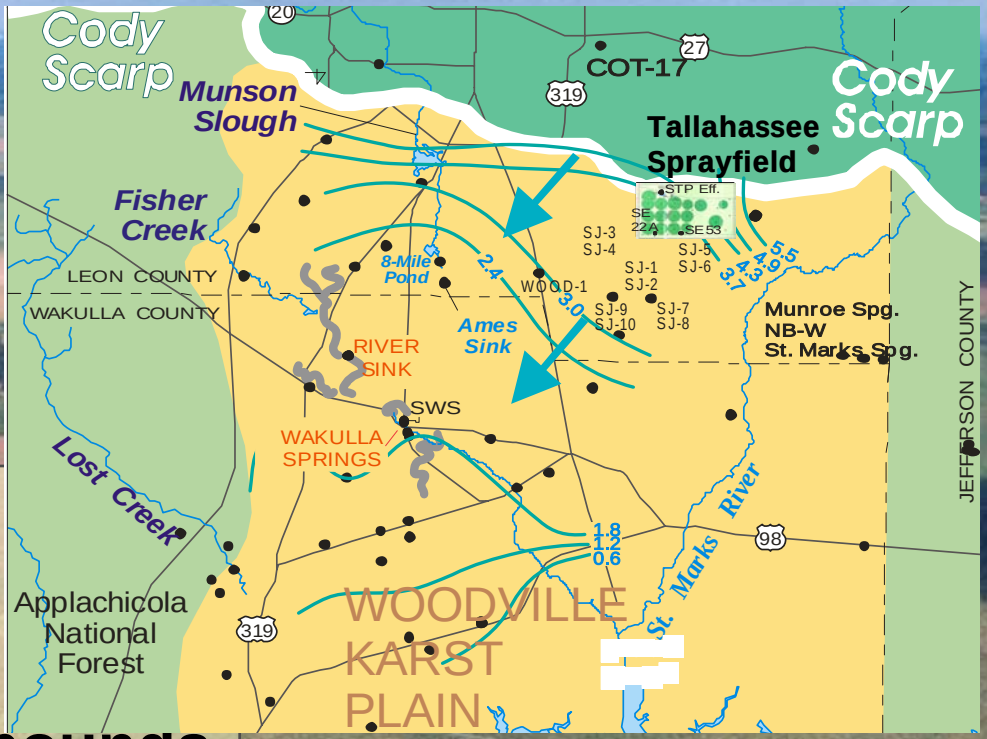
33%

50%

33%

Water-chemistry sampling

Samples were collected from 22 sites and analyzed for more than 260 chemical constituents by USGS and COT laboratories



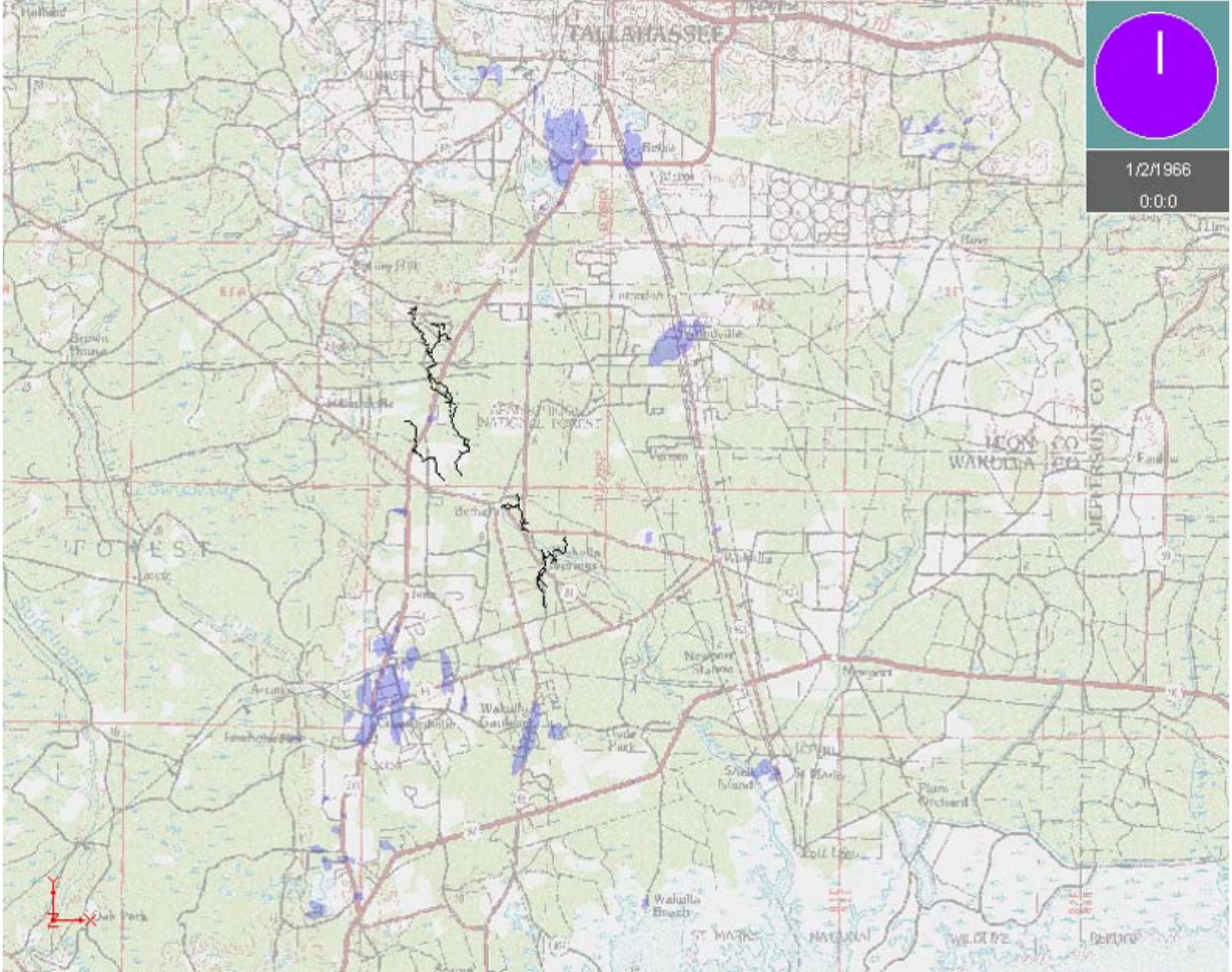
- ✓ **Nutrients (N,C,P)**
- ✓ **Major ions**
- ✓ **Stable isotopes**
- ✓ **Organic wastewater compounds**
- ✓ **Pharmaceutical compounds**
- ✓ **Age-dating tracers**
- ✓ **Volatile organic compounds**
- ✓ **Pesticides**
- ✓ **Trace elements**
- ✓ **Enteroviruses and indicators**

Site	Fecal coliforms	*E.coli*	Enterococci	*Clostridium perfringens*	MS2 phage	PRD1 phage	Enterovirus RT-PCR/Cell Culture/Volume
St. Joe well 1	0.2	0.2	0.0	0.0	0.0	40.0	-/-/6L
St. Joe well 2	0.2	0.2	0.0	0.0	0.0	0.0	-/-/6L
St. Joe well 3	0.0	0.0	0.0	0.0	0.0	0.0	-/-/6L
St. Joe well 4	0.0	0.0	0.0	0.0	0.0	0.0	-/-/6L
St. Joe well 5	0.5	0.5	0.0	0.0	0.0	0.0	-/-/6L
St. Joe well 6	0.3	0.3	0.0	0.0	0.0	0.0	-/-/6L
St. Joe well 7	0.0	0.0	0.0	0.1	Na	0.0	**+**/na/6L
St. Joe well 8	0.0	0.0	0.0	0.0	Na	0.0	-/na/0.2L
St. Joe well 9	0.0	0.0	0.0	0.0	Na	0.0	-/na/6L
St. Joe well 10	0.0	0.0	0.0	0.0	Na	0.0	-/na/0.3L
Well 22a	0.0	0.0	0.0	0.0	0.0	0.0	-/-/6L
Well 53	0.0	0.0	0.0	0.0	0.0	0.0	-/-/6L
City well 17	0.2	0.2	0.0	0.0	Na	na	-/-/6L
Woodville well 1	0.0	0.0	0.0	0.0	Na	0.0	-/na/6L
Natural Bridge well	0.0	0.0	0.0	0.0	0.0	0.0	**+**/-/4L
Wakulla Tunnel B	0.0	0.0	0.0	6.3	Na	0.0	-/na/6L
Spray-field effluent	32.5	12.5	6.5	0.0	60.0	40.0	-/-/0.25L
Munroe Spring	7.9	2.1	5.4	0.0	0.0	20.0	-/-/4L
St. Marks Spring	14.0	3.3	2.2	0.0	20.0	0.0	-/-/3.5L
Sally Ward Spring	0.0	0.0	0.4	0.0	Na	0.0	-/na/6L
Wakulla Spring	2.4	2.0	0.0	0.0	Na	na	-/-/6L
Wakulla Spring	0.0	0.0	0.0	1.6	Na	0.0	-/na/6L

Groundwater samples

Surfacewater sample

Spring vent/boil samples

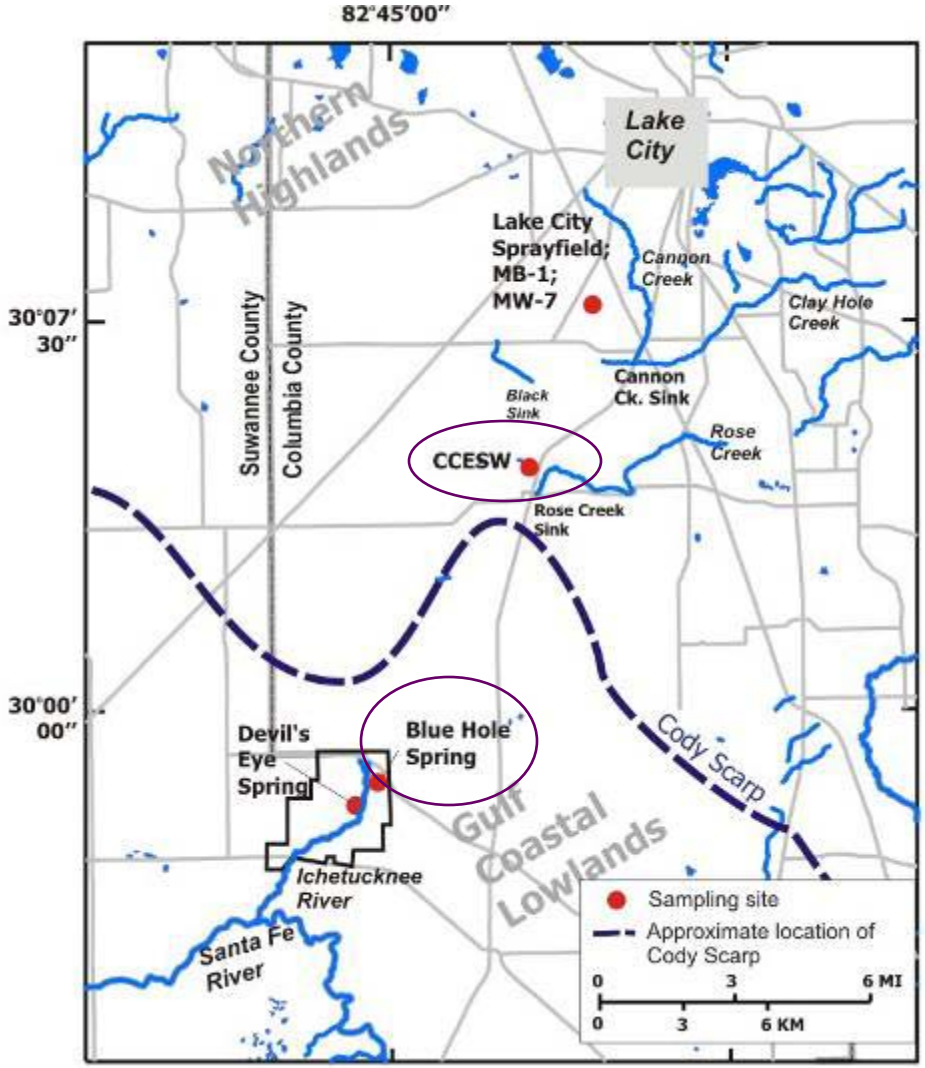

1/2/1966
0:0:0
TALLAHASSEE
Bethel
Wakulla
Newport
Wakulla Beach
WAKULLA CO
JEFFERSON CO
NATIONAL FOREST

- 70-75% removal of total N from sprayfield effluent to ground-water monitoring wells at southern boundary

- An additional 75-80% reduction in chloride and nitrate-N concentrations due to dilution after ground water leaves sprayfield boundary and flows toward Wakulla Springs

- Sprayfield farm operation is highly effective in removing pharmaceutical and organic wastewater compounds from the treated effluent thereby preventing them from entering the Floridan aquifer and associated ecosystems.

Microbiological Indicators elevated during higher flow conditions



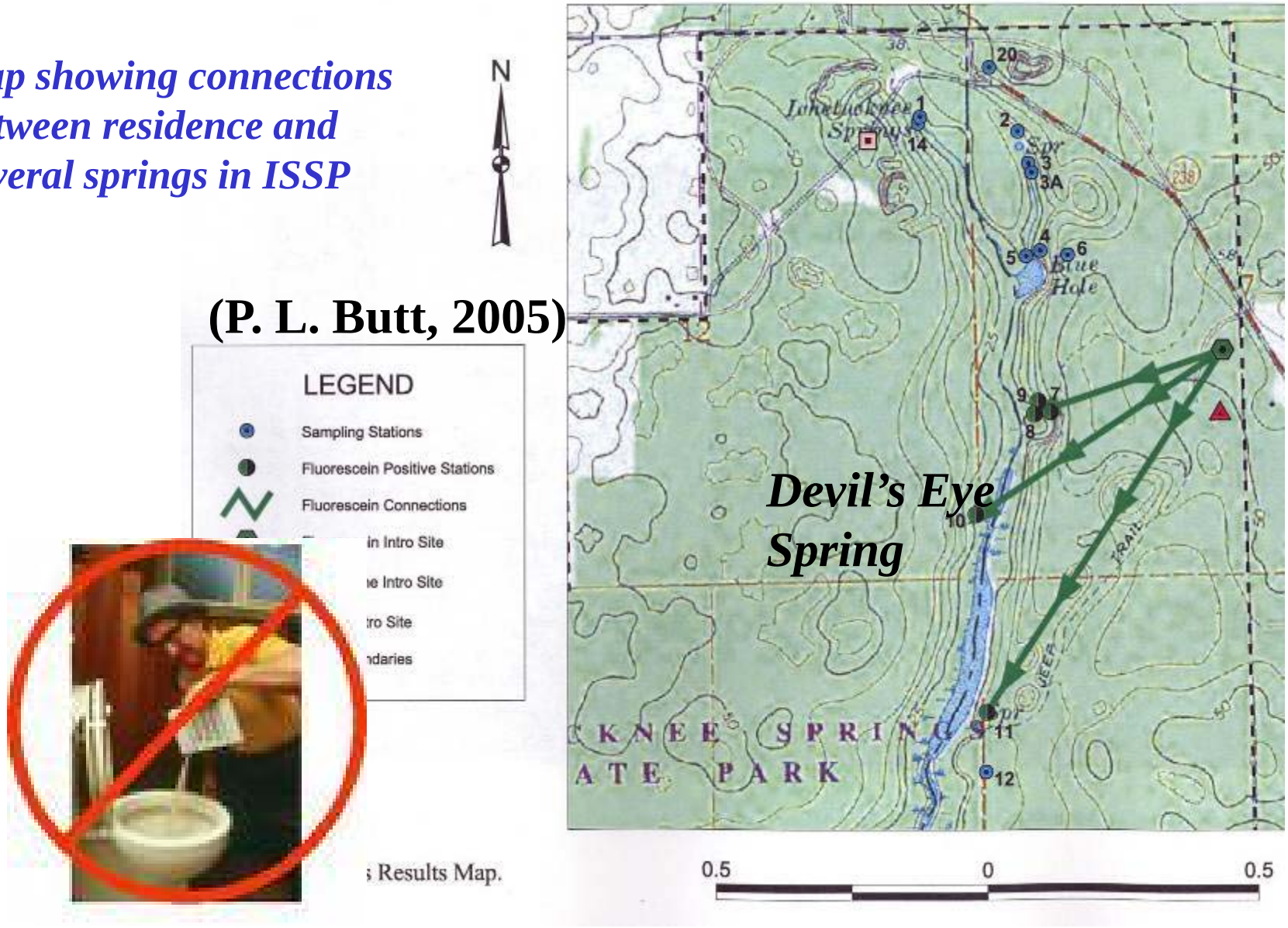
Site name	Fecal Coliforms	*Escherichia coli*	Enterococci	*Clostridium perfringens*	Enteroviruses
Sprayfield Effluent Reservoir	10.5	0	0	75	–
	67	26	0	11.5	–
MB-1	0.2	0.2	0	0	–
	0	0	0	0	–
MW-7	0.2	0.2	0	0	–
	0	0	0	0	–
CCESW	0	0	0	0	+
	0.4	0	0	0	–
Blue Hole Spring	8.1	0	15.4	0.4	+
	1.7	1	0.2	0.2	–
Devil's Eye Spring	0	0	0	0	–
	3.8	1	0.1	0.1	–

May 2005*; *October 2005

USGS

Map showing connections Between residence and Several springs in ISSP

(P. L. Butt, 2005)



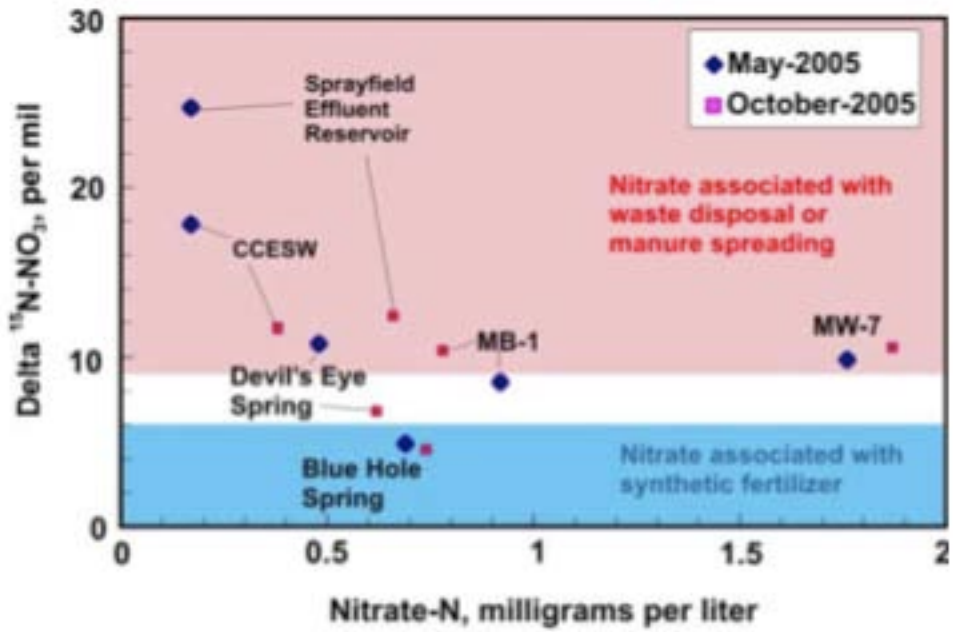


Fig. 7 Plot of $\delta^{15}N–NO_3$ and $NO_3–N$ concentrations in water from springs, wells, and sprayfield effluent reservoir

Mystery Spring

Average spring vent bacteria count/ml = 5,870
Average spring vent virus count/ml = 29,900

Conclusions

- The combination of viral source tracking and chemical analyses has successfully been used to study microbial and pollutant transport, and nutrient loading from septic systems, injection wells, and spray-fields in karst environments.
- Viral quantitative polymerase chain reaction (qPCR) is a sensitive and specific assay that can be used for source tracking of fecal-oral microorganisms in the full range of different aquatic environments (surface and ground waters, marine to fresh waters).
- These viral qPCR assays are host specific and tailored to study the influence of individual organisms.
- Any questions?

- Feline calicivirus – ~50% of cats are believed to carry this RNA virus.
- Canine norovirus – 40 to 50% of dogs carry antibodies to this virus.