Groundwater microbiology

Marek Kirs

Water Resources Research Center

Plan

Lecture 1

- Crash course to microbiology
- Groundwater microbiology

Lecture 2

- Pathogens and groundwater quality
- Drinking water treatment

Plan

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- **Crash course to microbiology**
- Groundwater microbiology

Lecture 2

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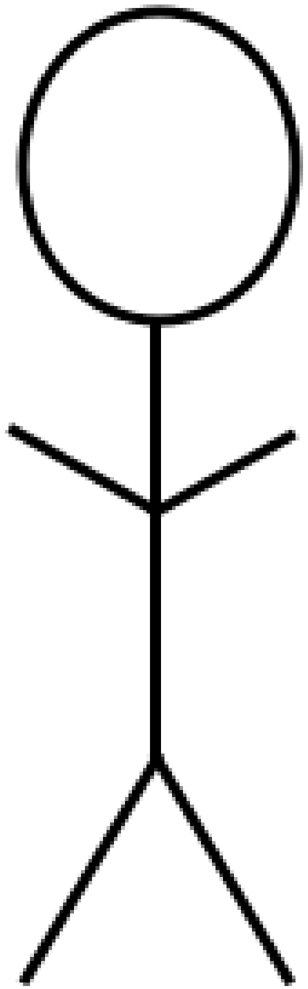
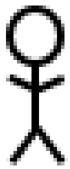


Biologist



Microbiologist

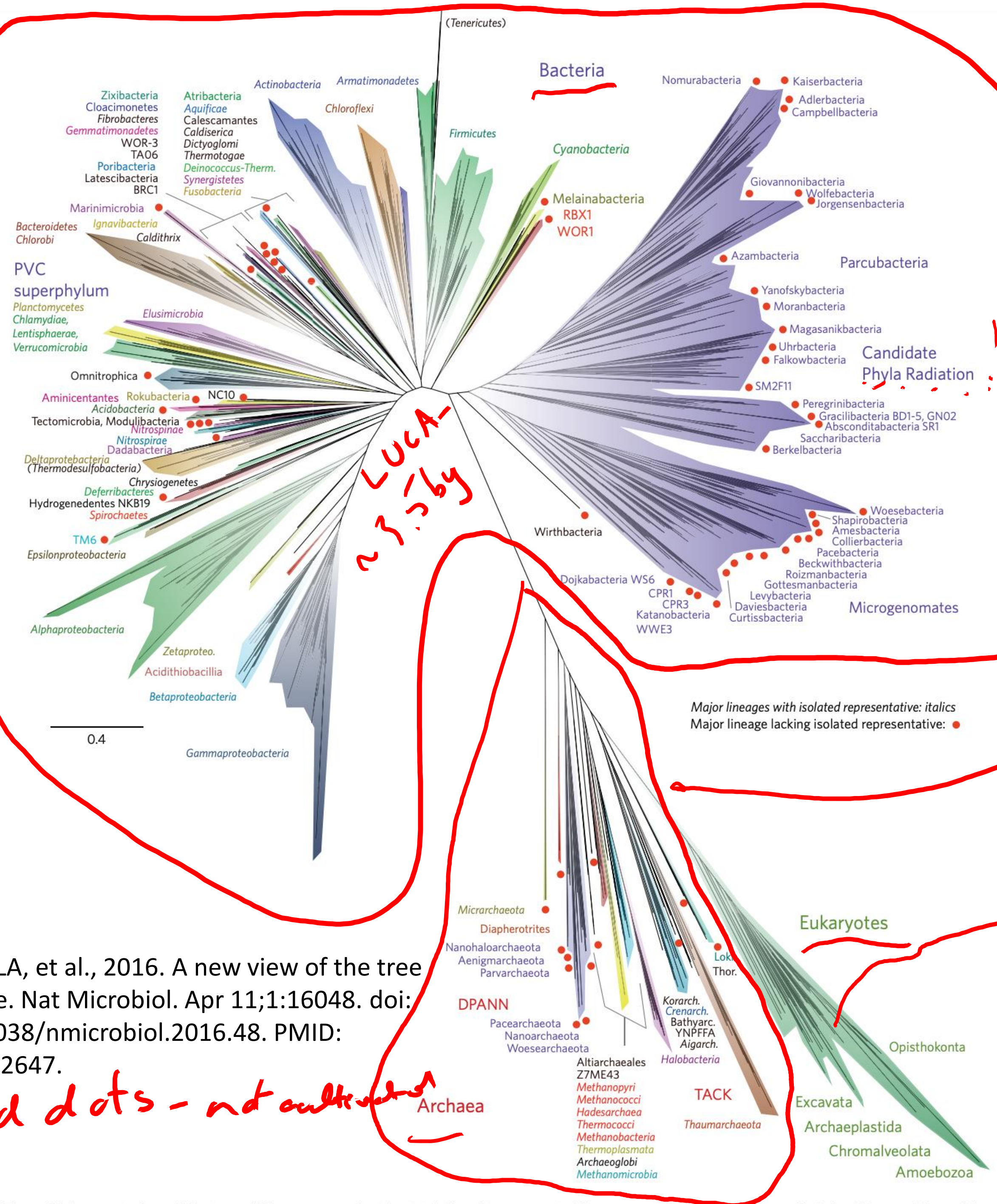


Figure 1 | A current view of the tree of life, encompassing the total diversity represented by sequenced genomes. The tree includes 92 named bacterial

Hug LA, et al., 2016. A new view of the tree of life. Nat Microbiol. Apr 11;1:16048. doi: 10.1038/nmicrobiol.2016.48. PMID: 27572647.

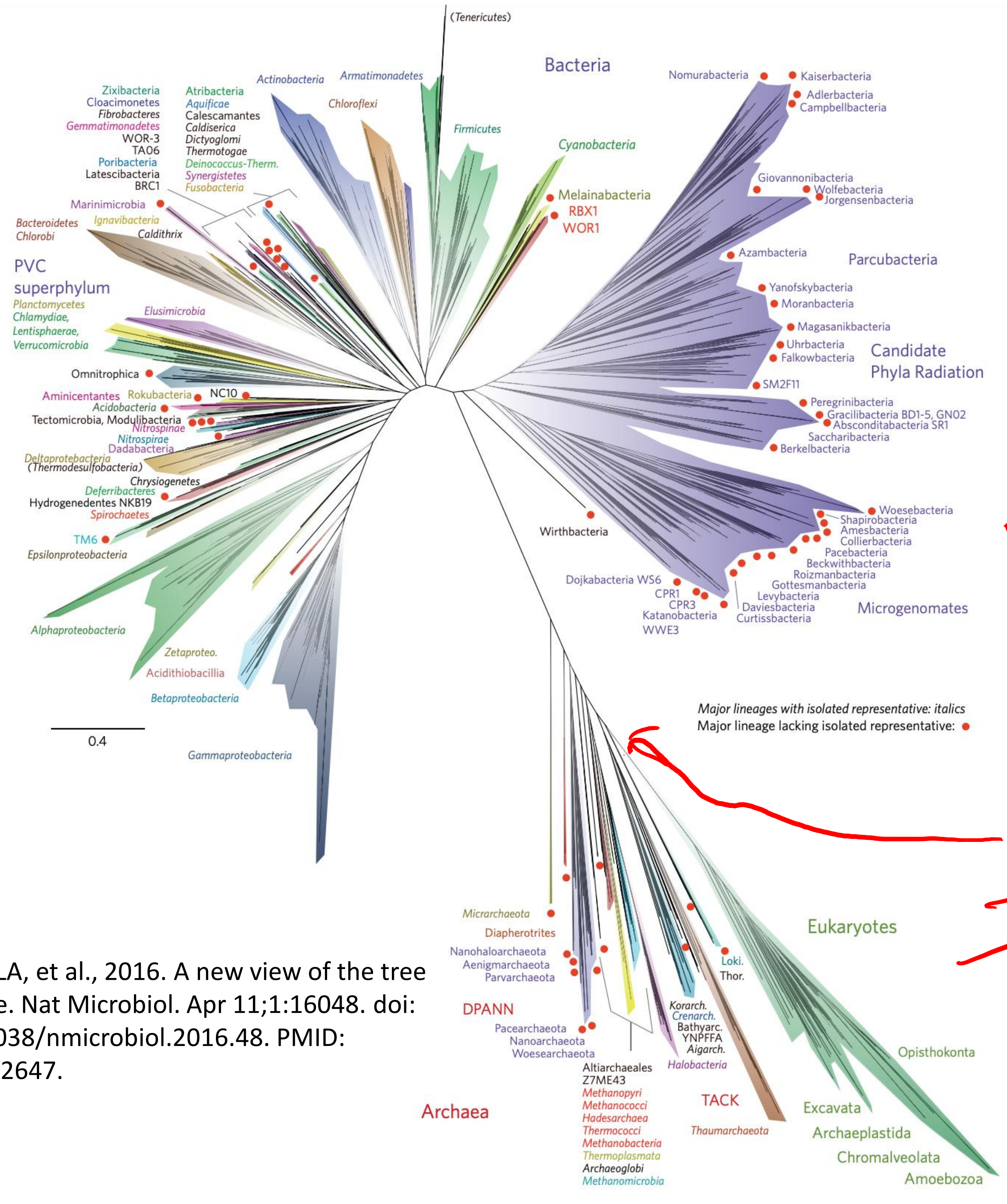
red dots - not cultured

- Prokaryotes vs Eukarotes
- Bacteria
- Archaea
  - less diverse
  - tend to be in extr. env.
  - cell walls & membr. dif.
  - no pathogens, not sensit. to antib.
- Eukaryotes
- (viruses) - In all domains

not primit. org.

- 3 or 2 domains?

Archaea
Bacteria
Eukaryotes?



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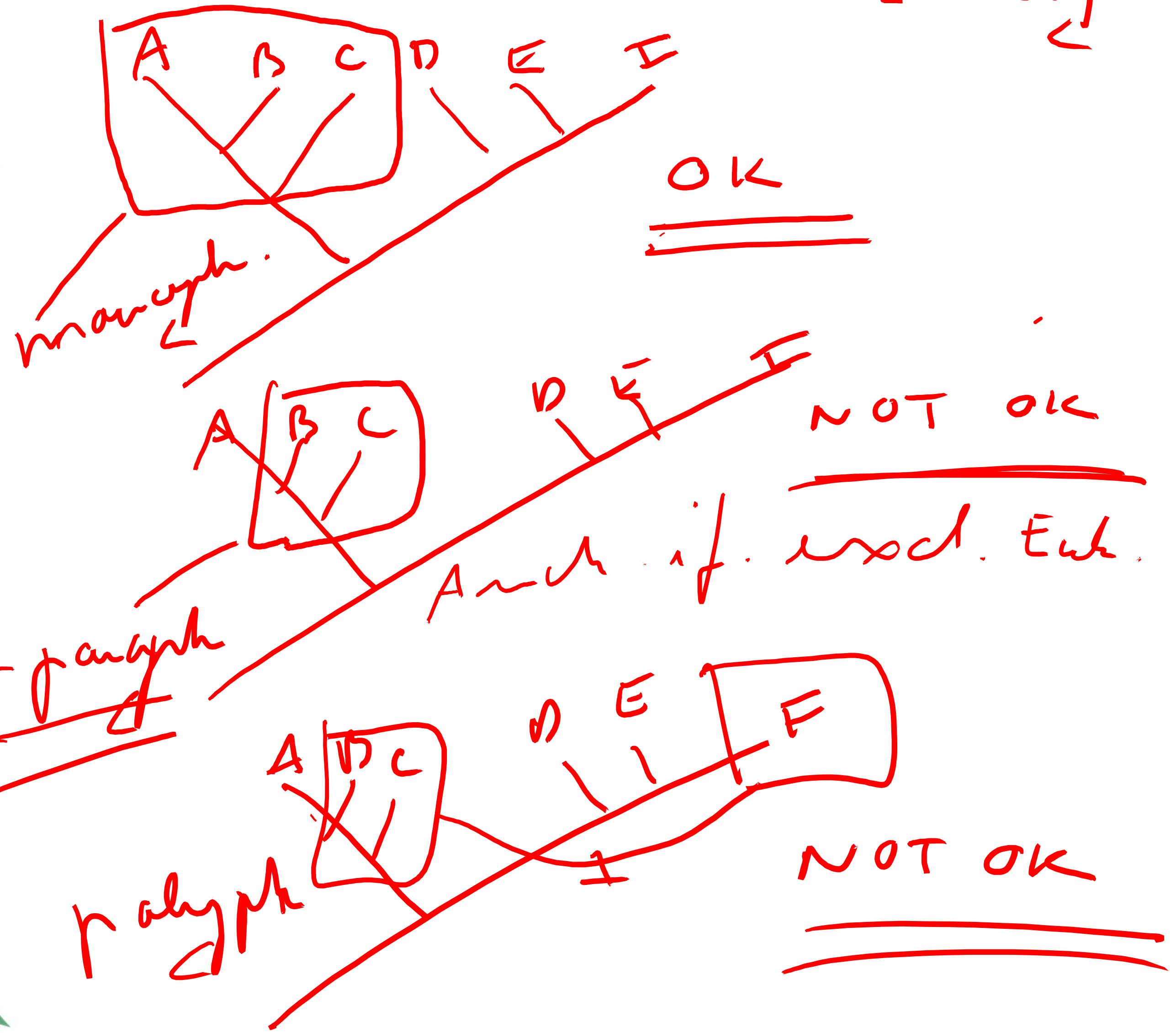
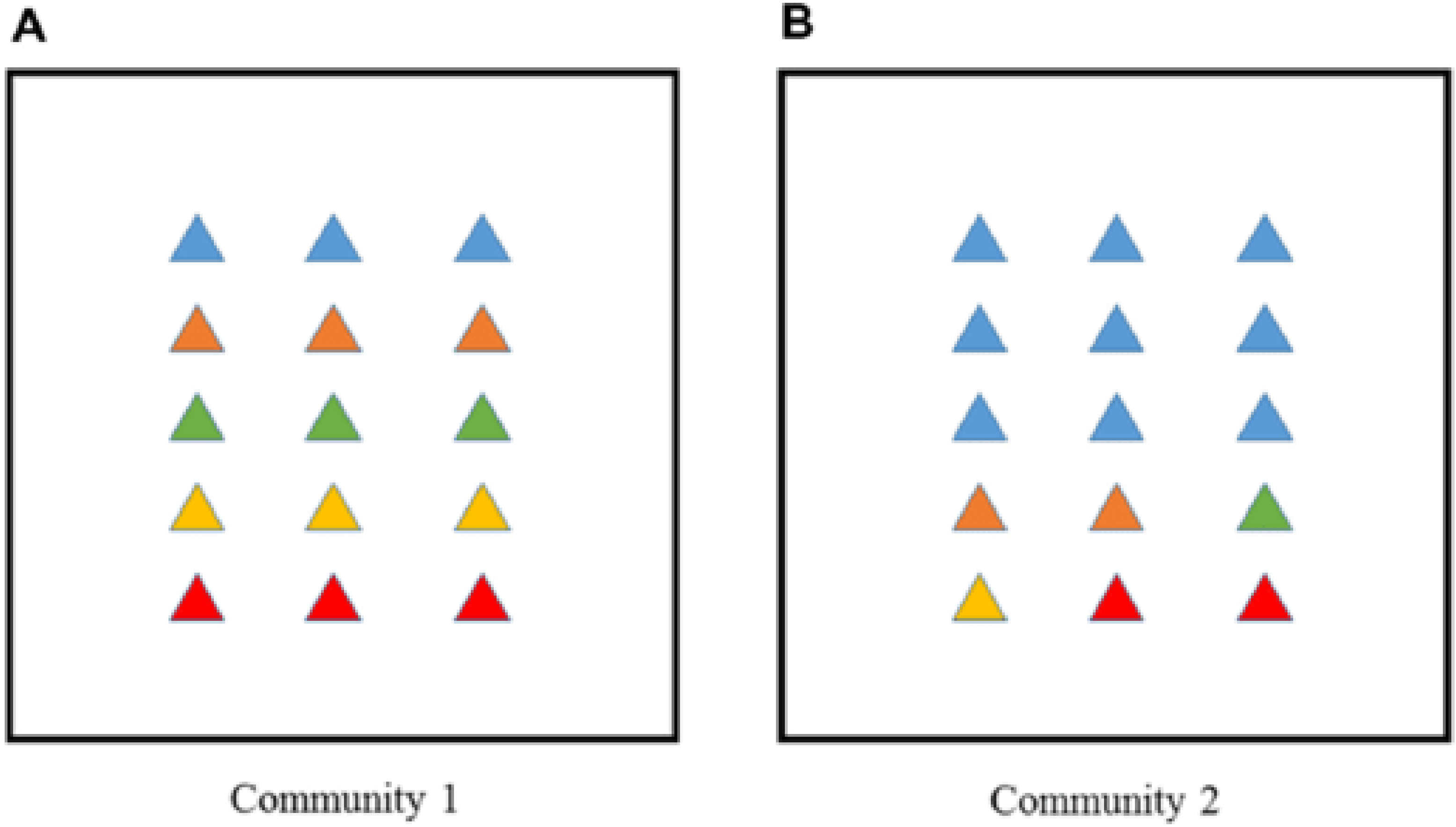


Microbial diversity

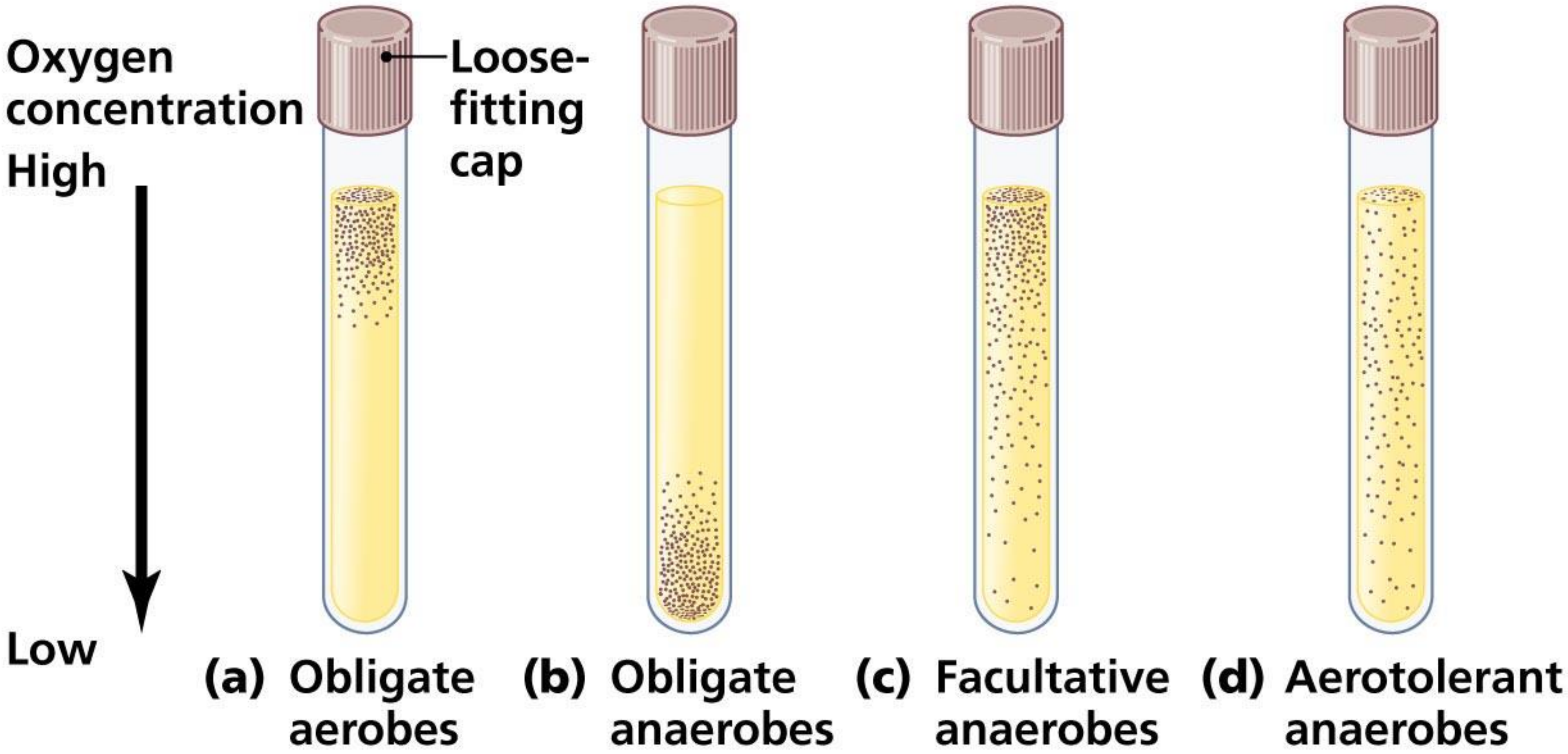
- Richness
- Evenness
- Diversity



Alfa-, beta-, and gammadiversity

Metabolic diversity

strict anaerobes
- thioglycolate tubes



Metabolic diversity

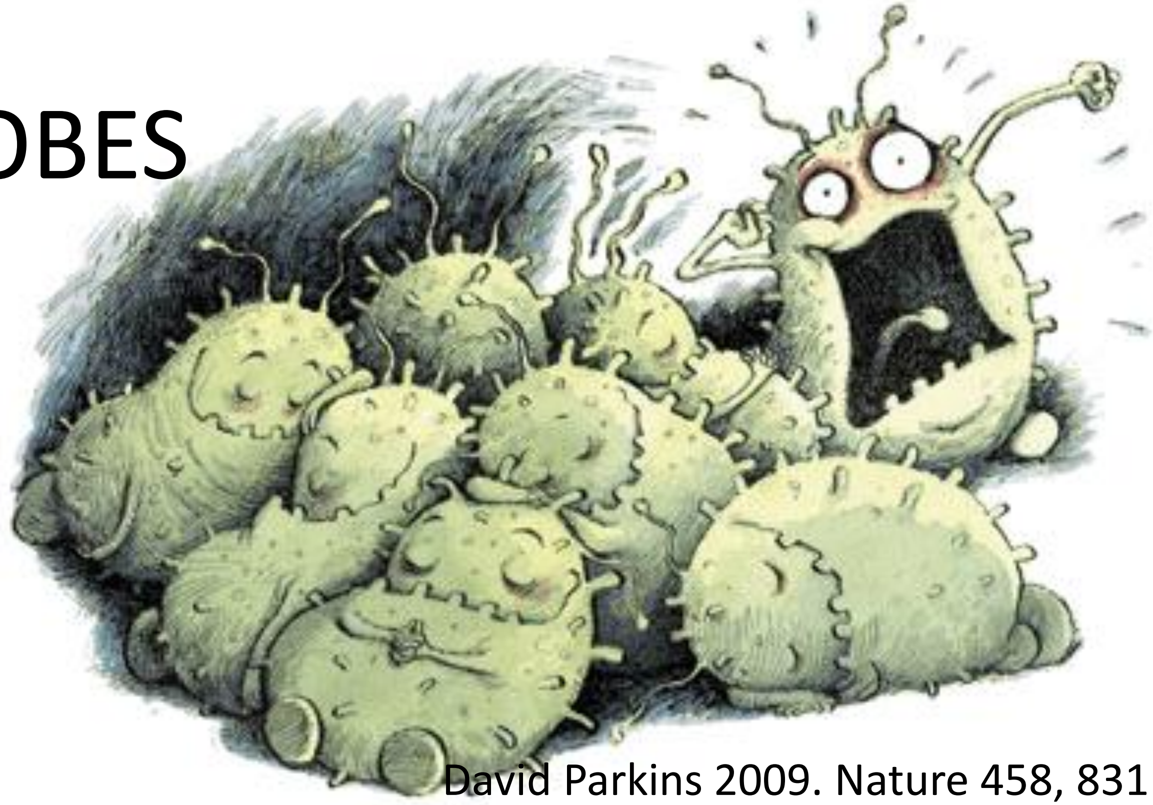
Energy source:

- Phototrophs
- Chemotrophs

Carbon source:

- Autotrophs
- Heterotrophs

MICROBES



David Parkins 2009. Nature 458, 831

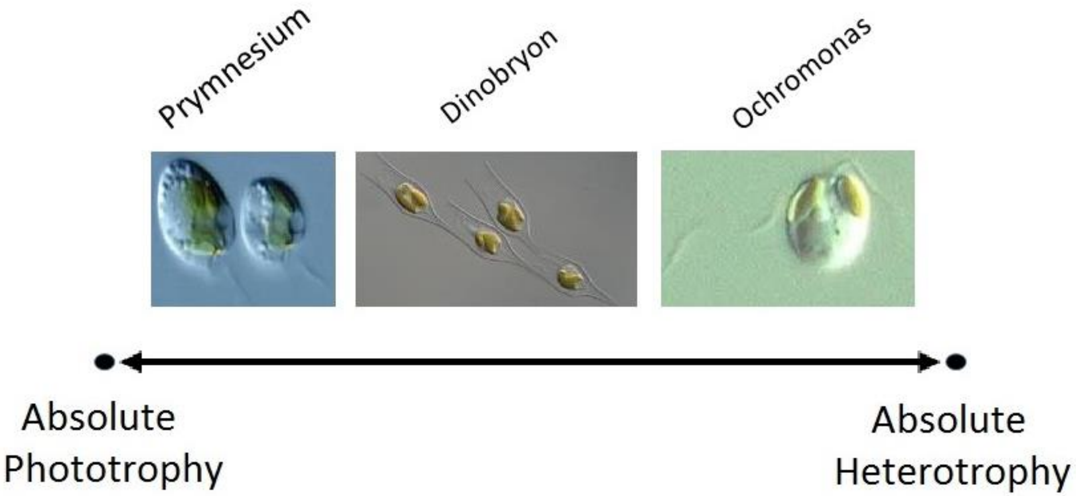
Metabolic diversity

	ENERGY source for generating ATP	CARBON sourcse	Microbial examples
Photo**autotroph**	Light (photon)	**CO_2**	Cyanobacteria
Chemo**autotroph**	*Inorganic* compounds (H_2, CO, NH_3, NO_2,H_2S, S, Fe^{2+})	**CO_2**	methanogens, halophiles, sulfur oxidizers and reducers, nitrifiers, anammox bacteria, and thermoacidophiles.
Photo**heterotroph**	Light (photon)	CO_2 and simple **organic compounds**	purple non-sulfur bacteria, green non-sulfur bacteria, heliobacteria
Chemo**heterotroph**	Preformed *organic* compounds	Preformed **organic matter**	Most bacteria, fungi and protozoa HUMANS

Mixotrophs

switch

Mainly used for protists, but bacteria to!



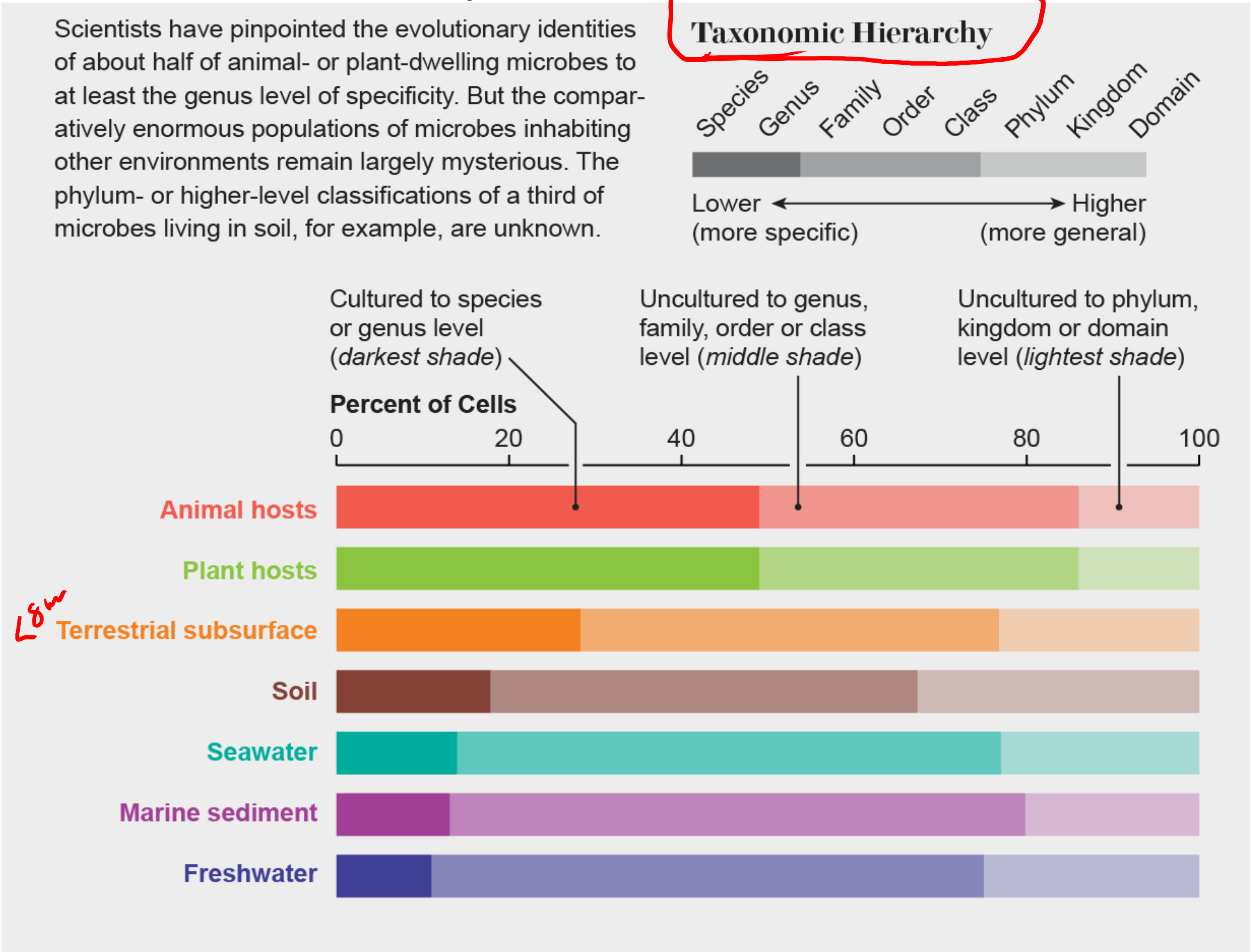
↑ uptake org. c. & CO_2

Most abundant bacteria in oligotrophic marine waters are mixotrophic

Huge impact on nutrient cycles! Climate Change! Fisheries!

Microbial world: Huge knowledge gaps

Perhaps around trillion microbial species



E. coli

E. coli

binomial

Escherichia coli

Enterobacteriales coli

seq. similarity

97% typ

OTUs vs species

ASVs

cult. needed to name sp.

~31,000 full genomes

"univ." primers

not univ.

named tow. cultiv.

Source: "Phylogenetically Novel Uncultured Microbial Cells Dominate Earth Microbiomes," by Karen G. Lloyd et al., in mSystems, Vol. 3, No. 5; September/October 2018

Unique traits of microbes

- Small size
- Ubiquitous distribution through Earth's habitats
- High specific surface areas
- Potentially high rate of metabolic activity
- Potentially rapid growth rate
- Physiologically responsive
- Unrivaled nutritional diversity
- Unrivaled enzymatic diversity

Ecological consequences of microbes

- Geochemical cycling of elements
- Detoxification of organic and inorganic pollutants
- Release of essential limiting nutrients from the biomass
- Maintaining the chemical composition of soil, sediment, water, and atmosphere required by other forms of life
- Major impact on environmental quality, agriculture, and climate

Microbial diversity is the key to human survival

Environmental Microbiology: Core Concepts

- Primary directive of microbial life: survive, maintenance, generate energy, grow and replicate
- No method is perfect
- Several independent studies (papers)
- Every form (trait) has its function

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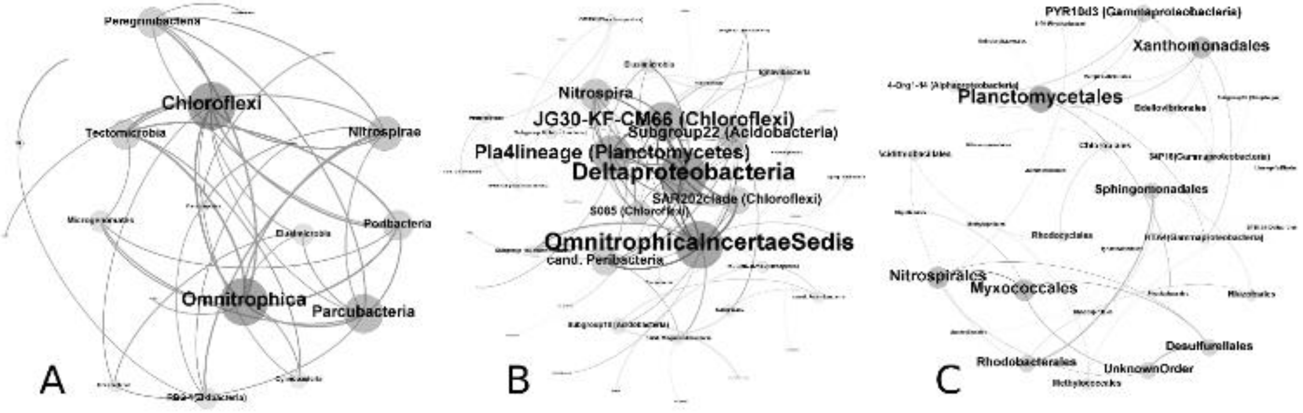
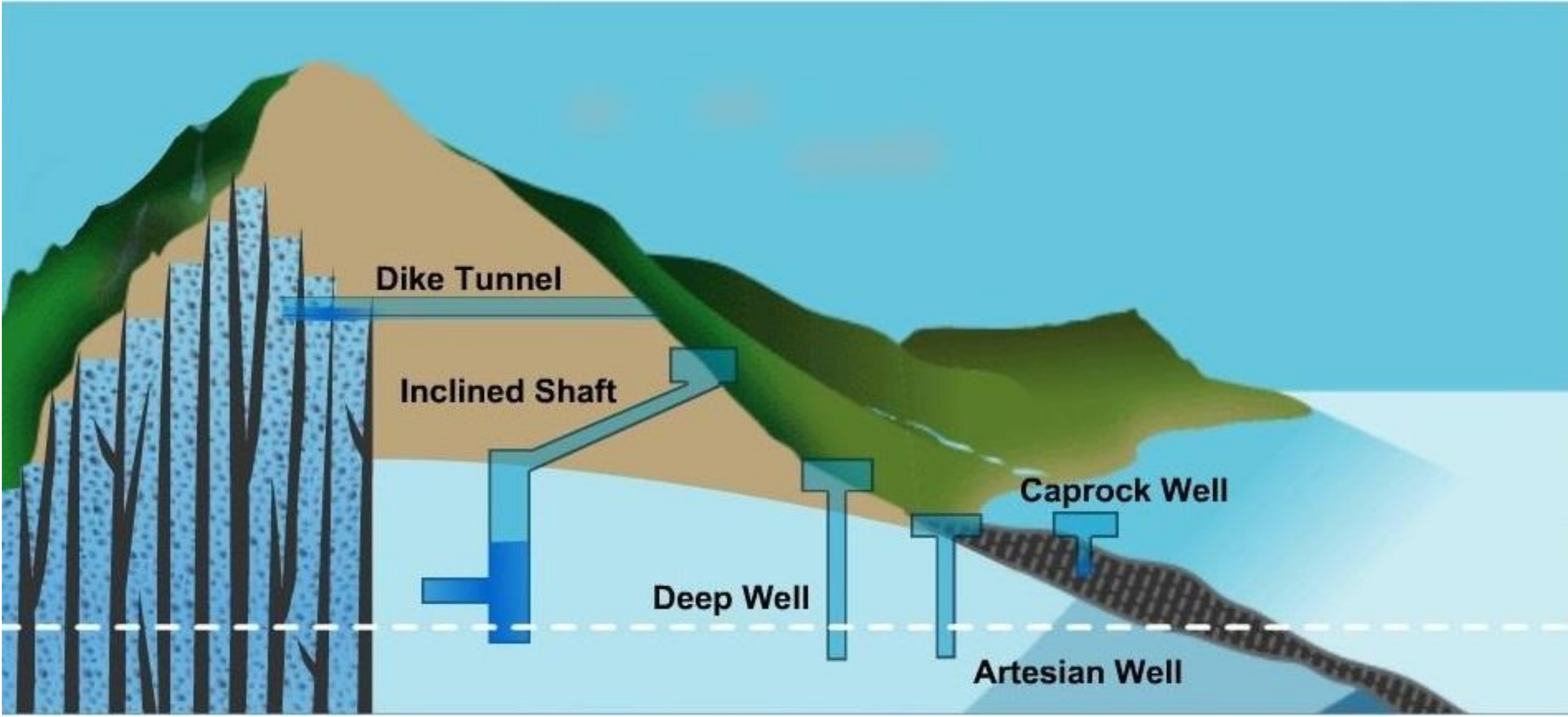
Microbes in (Hawaii) Groundwater



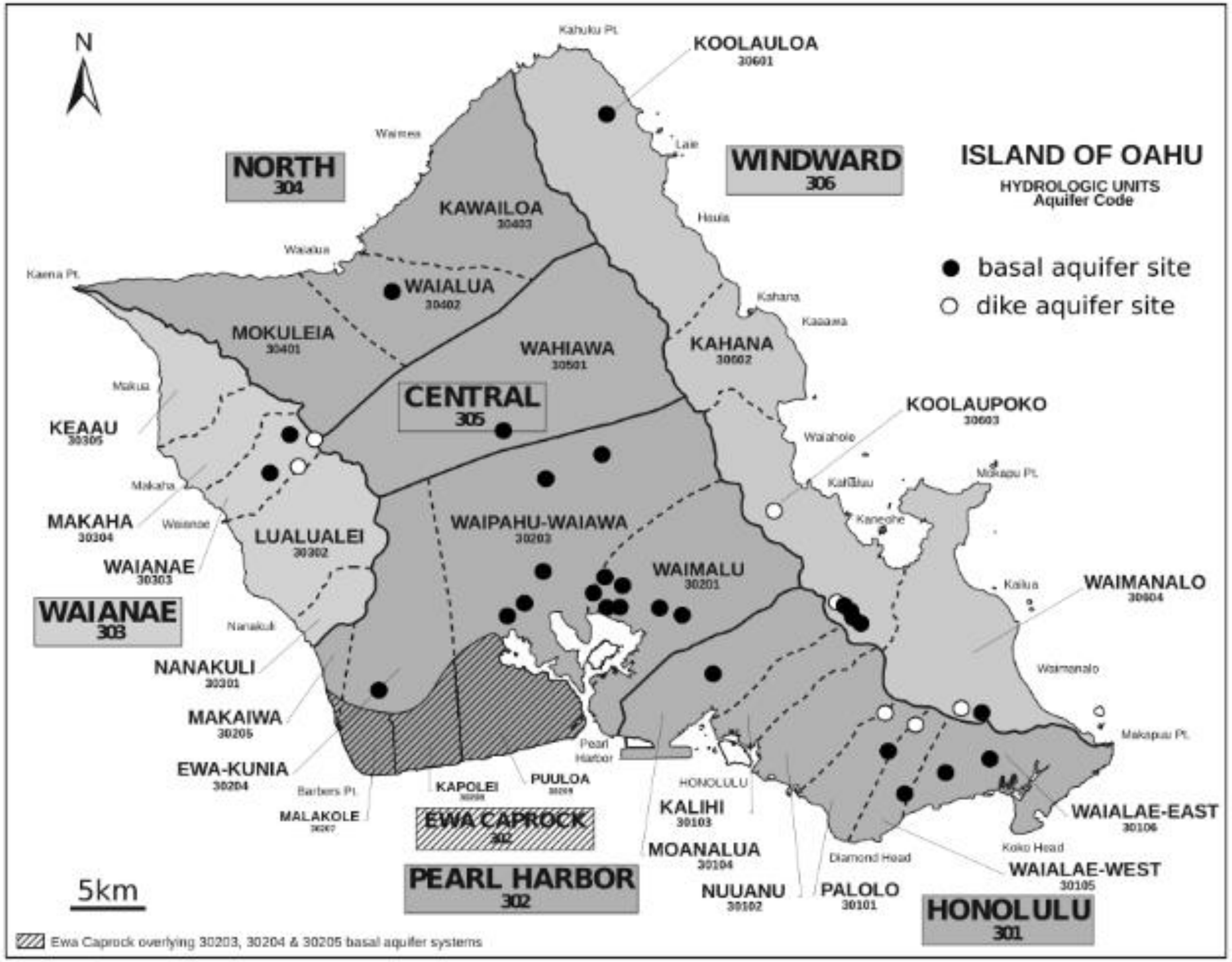
Why microbes in GW matter?

- No meaningful alternative for island communities
- Changing climate and anthropogenic microbial contaminants: health risk and ecosystem functioning
- GW microbial communities are important in subsurface biogeochemical cycling and biodegradation
- We know nothing about microbes in HI GW, except indicators
- Detect change
- Important when making management decisions

https://www.boardofwatersupply.com/water-resources/the-water-cycle



The study


N
ISLAND OF OAHU
HYDROLOGIC UNITS
Aquifer Code
basal aquifer site
dike aquifer site
NORTH
304
WINDWARD
306
CENTRAL
305
WAIANAE
303
PEARL HARBOR
302
HONOLULU
301
EWA CAPROCK
302
KOOLAULOA
30601
KAWAILOA
30403
WAIALUA
30402
MOKULEIA
30401
WAHIAWA
30501
KAHANA
30602
KOOLAUPOKO
30603
KEAAU
30305
MAKAHA
30304
WAIANAE
30303
LUALUALEI
30302
WAIPAHU-WAIAWA
30203
WAIMALU
30201
WAIMANALO
30604
NANAKULI
30301
MAKAIWA
30205
EWA-KUNIA
30204
MALAKOLE
KAPOLEI
PUULOA
KALIHI
30103
MOANALUA
30104
NUUANU
30102
PALOLO
30101
WAIALAE-EAST
30106
WAIALAE-WEST
30105
Kahuku Pt.
Waimea
Laie
Hauula
Waialua
Kaena Pt.
Kahana
Kaaawa
Makua
Waiahole
Kahaluu
Mokapu Pt.
Makaha
Waianae
Kaneohe
Kailua
Nanakuli
Waimanalo
Makapuu Pt.
Pearl Harbor
HONOLULU
Barbers Pt.
Koko Head
Diamond Head
5km
Ewa Caprock overlying 30203, 30204 & 30205 basal aquifer systems


BOARD OF WATER SUPPLY

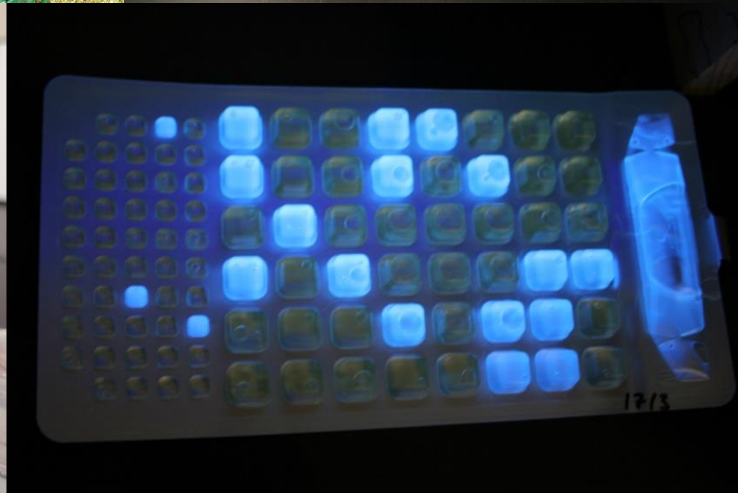
Methods

- Collection
- Filtration

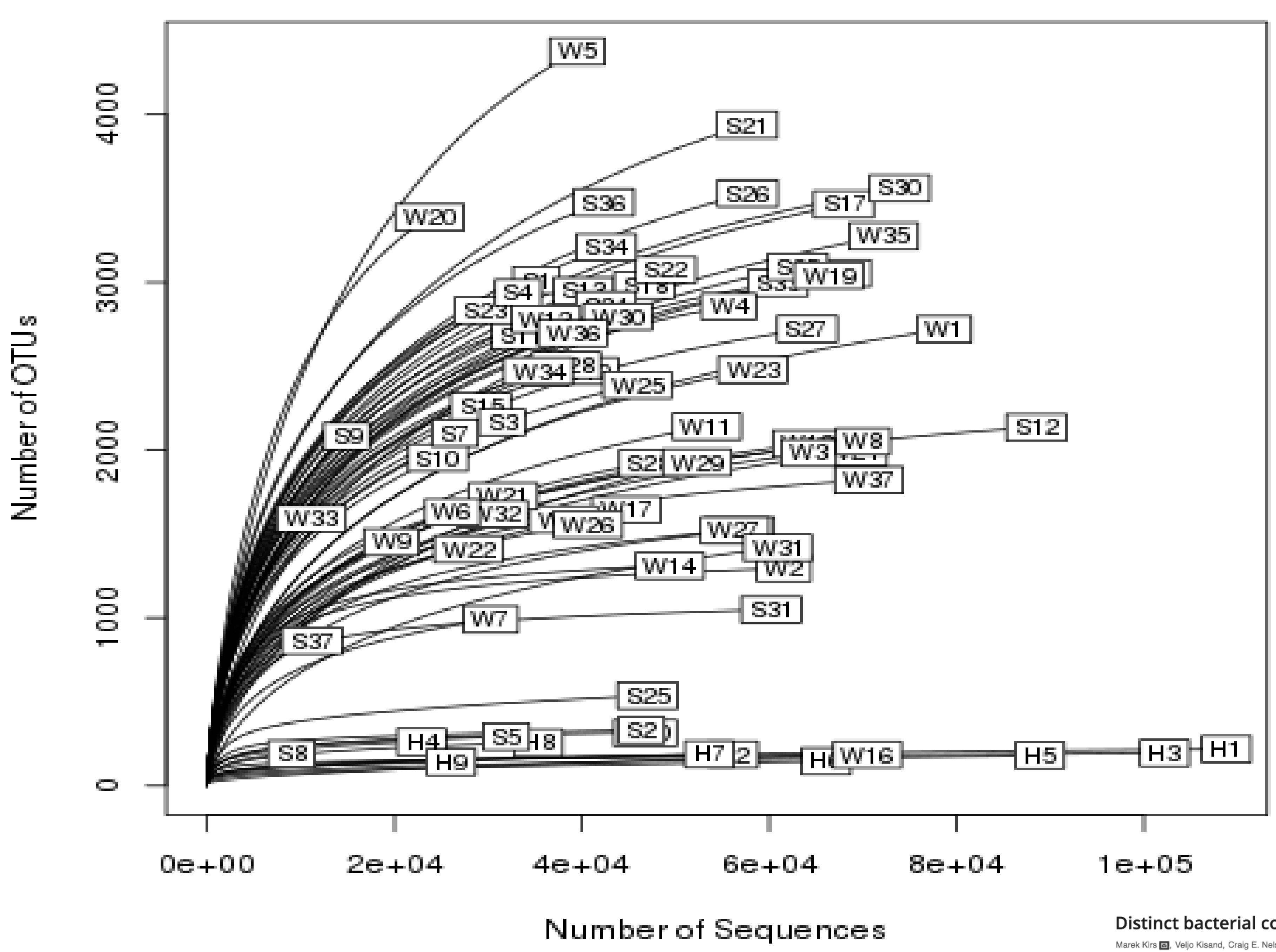
Cultivation

- DNA extraction
- Library preparation
- Sequencing
- Bioinformatics pipeline and analyses



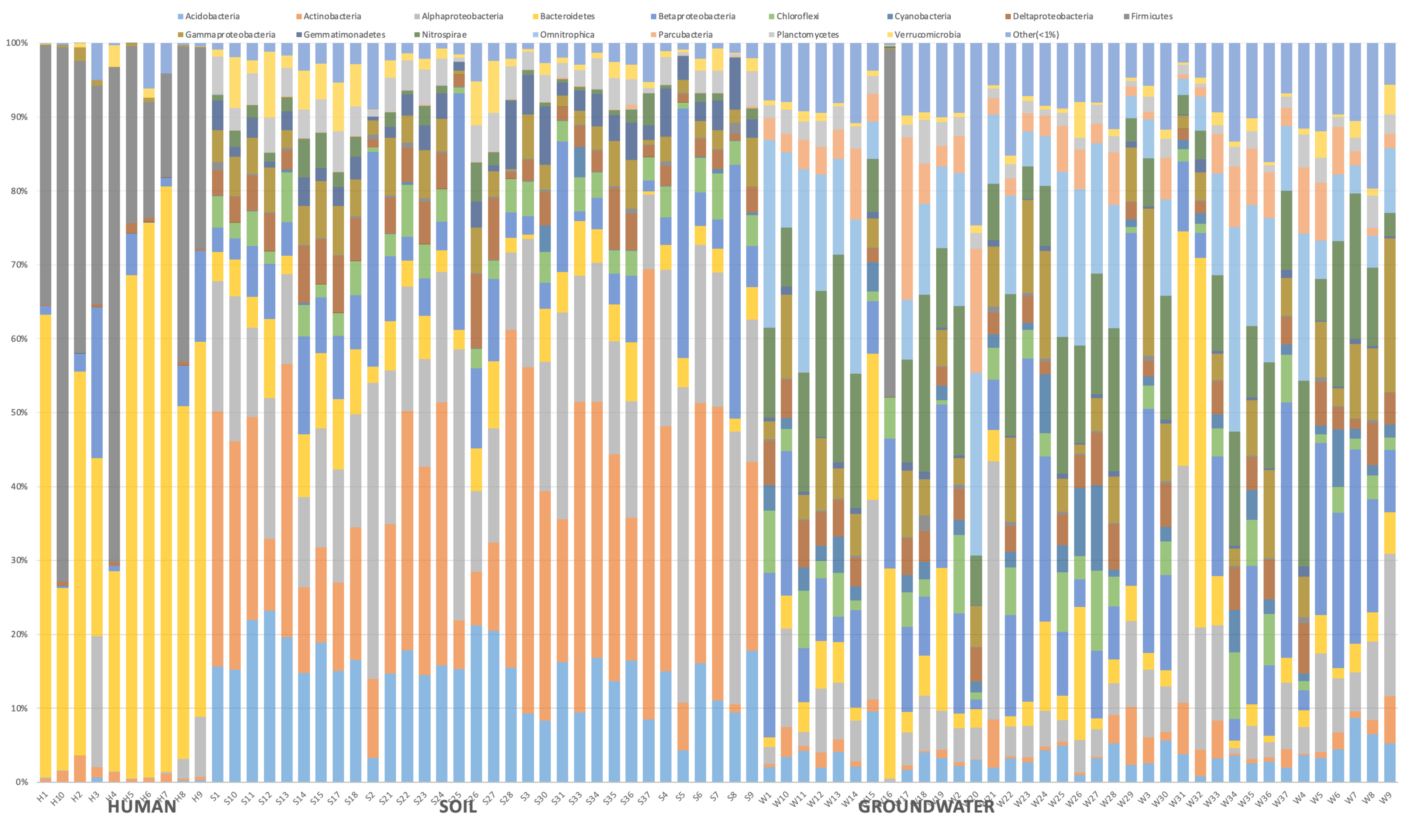
1. Quality control and filtering (Trimmomatic)
2. (PE assembly and) Clustering (Vsearch)
3. Identification (SINA aligner)
4. Statistical analyses (R: vegan and other packages)



Distinct bacterial communities in tropical island aquifers

Marek Kirs, Veljo Kisand, Craig E. Nelson, Tineill Dudoit, Philip S. Moravcik

Published: April 30, 2020 • https://doi.org/10.1371/journal.pone.0232265


Acidobacteria
Actinobacteria
Alphaproteobacteria
Bacteroidetes
Betaproteobacteria
Chloroflexi
Cyanobacteria
Deltaproteobacteria
Firmicutes
Gammaproteobacteria
Gemmatimonadetes
Nitrospirae
Omnitrophica
Parcubacteria
Planctomycetes
Verrucomicrobia
Other(<1%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
H1 H10 H2 H3 H4 H5 H6 H7 H8 H9
S1 S10 S11 S12 S13 S14 S15 S17 S18 S2 S21 S22 S23 S24 S25 S26 S27 S28 S3 S30 S31 S33 S34 S35 S36 S37 S4 S5 S6 S7 S8 S9
W1 W10 W11 W12 W13 W14 W15 W16 W17 W18 W19 W2 W20 W21 W22 W23 W24 W25 W26 W27 W28 W29 W3 W30 W31 W32 W33 W34 W35 W36 W37 W4 W5 W6 W7 W8 W9
HUMAN
SOIL
GROUNDWATER

Full of life, amazing diversity!

<u>scale</u> img.

	# of samples	# of sequences	Richness	Diversity		Evenness
			OTUs per sample	Shannon's (H)	Fisher α	J
Ground water	37	1,751,074	2,071 (185-4,373)	5.37 (2.13-6.95)	468.9 (23.1-1257.2)	0.71 (0.41-0.85)
Soil	32	1,407,413	2,397 (190-3,937)	5.98 (2.72-6.89)	571.6 (34.1-956.8)	0.78 (0.51-0.87)

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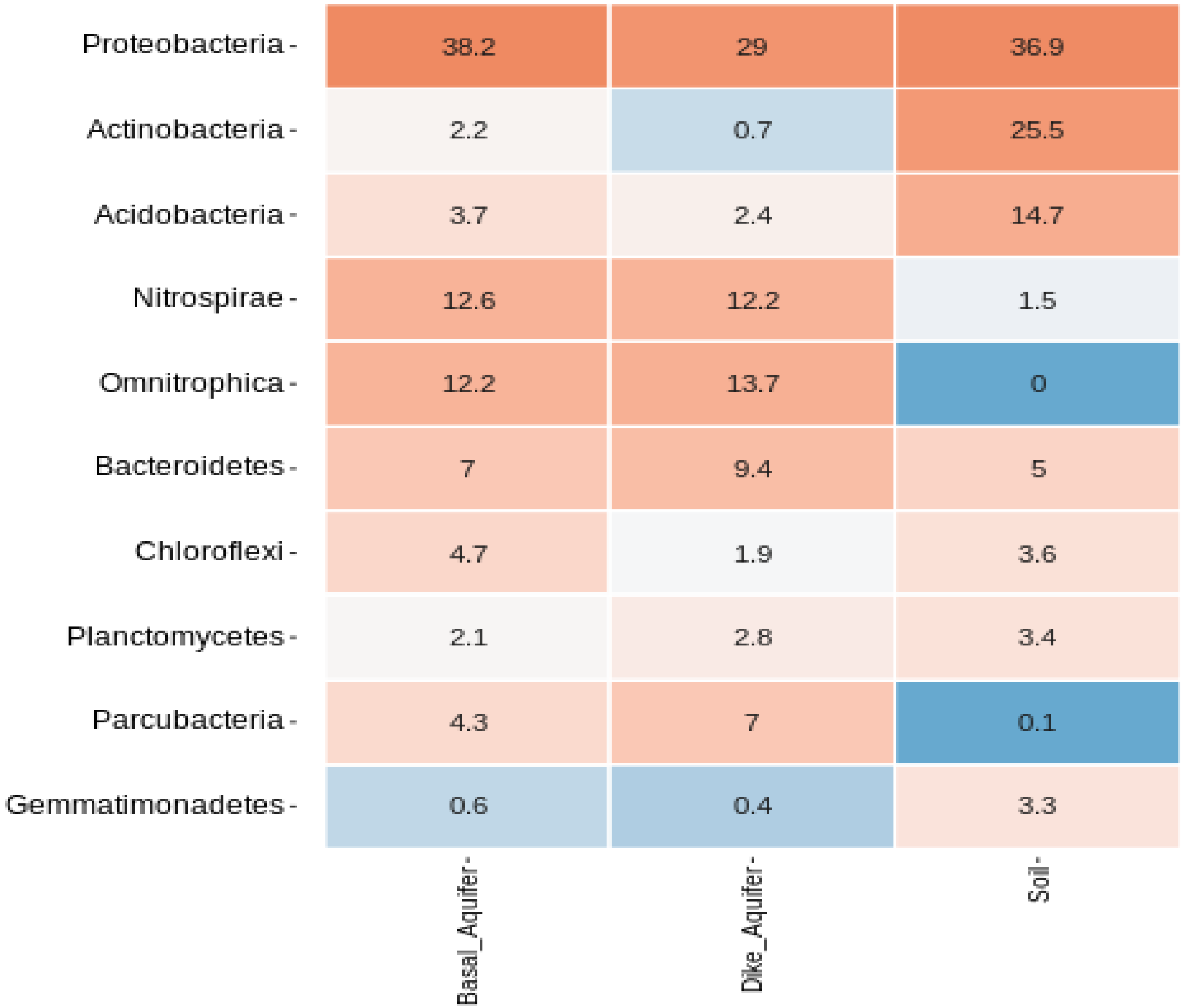

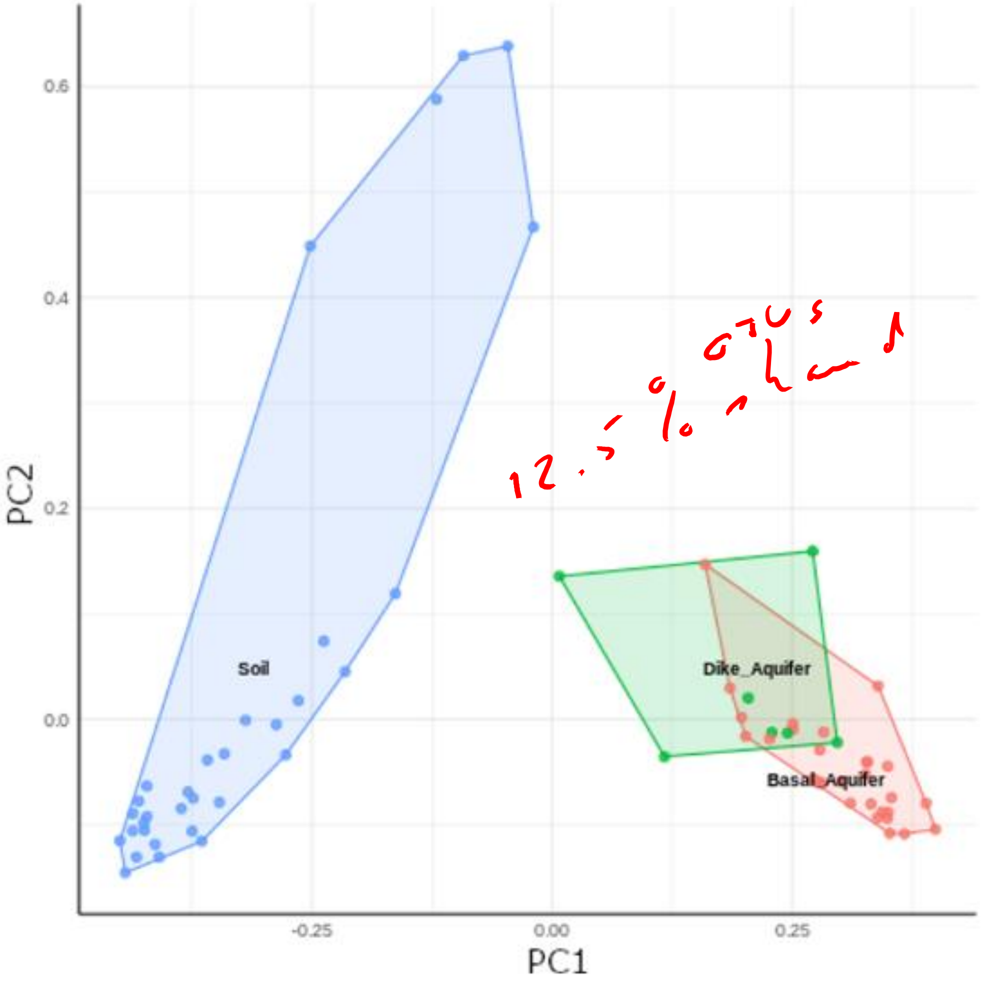

Relative abundance (%) of top ten phyla in the groundwater and soil samples.

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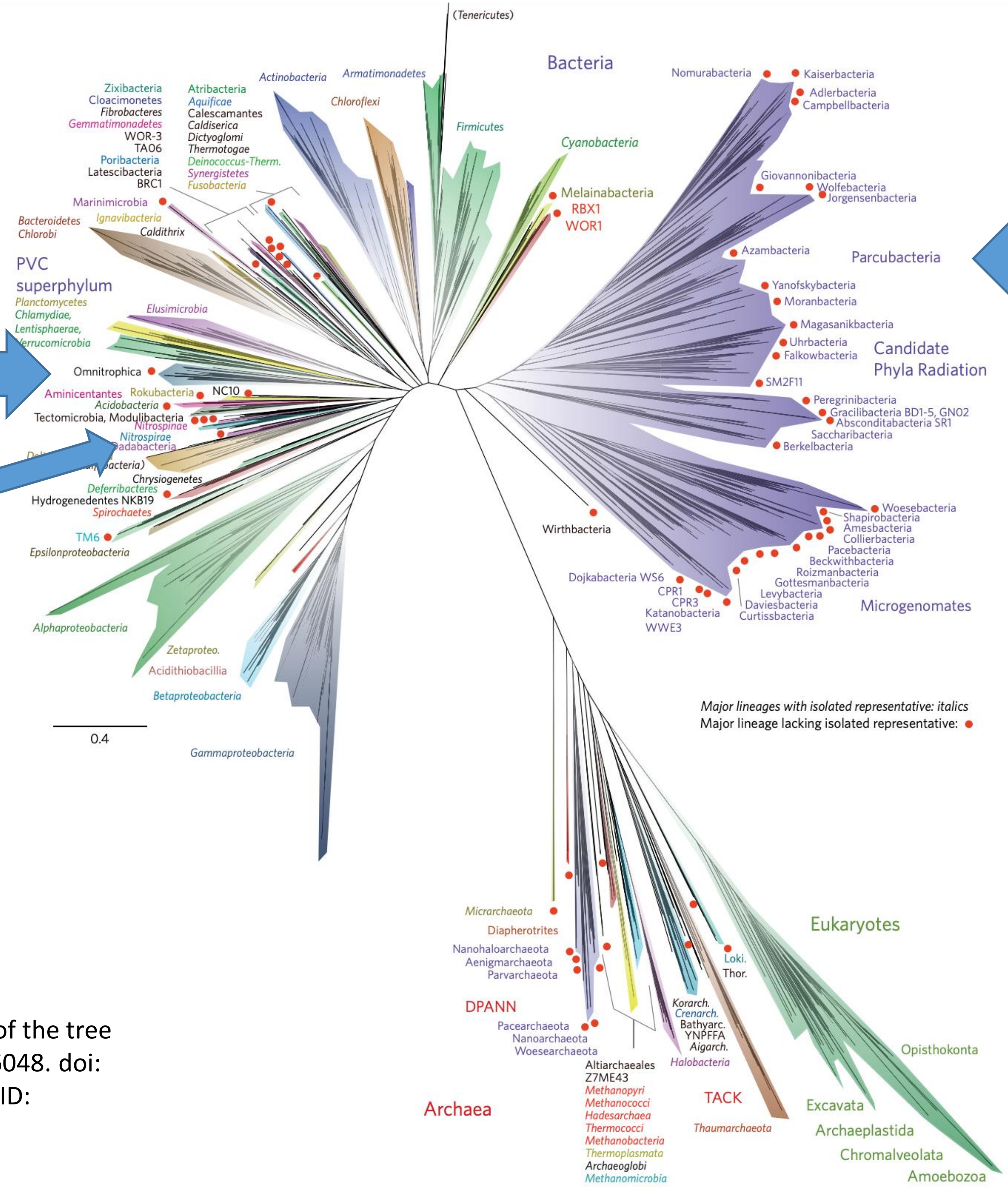

Figure 1 | A current view of the tree of life, encompassing the total diversity represented by sequenced genomes. The tree includes 92 named bacterial

red. genome numbs? diverse

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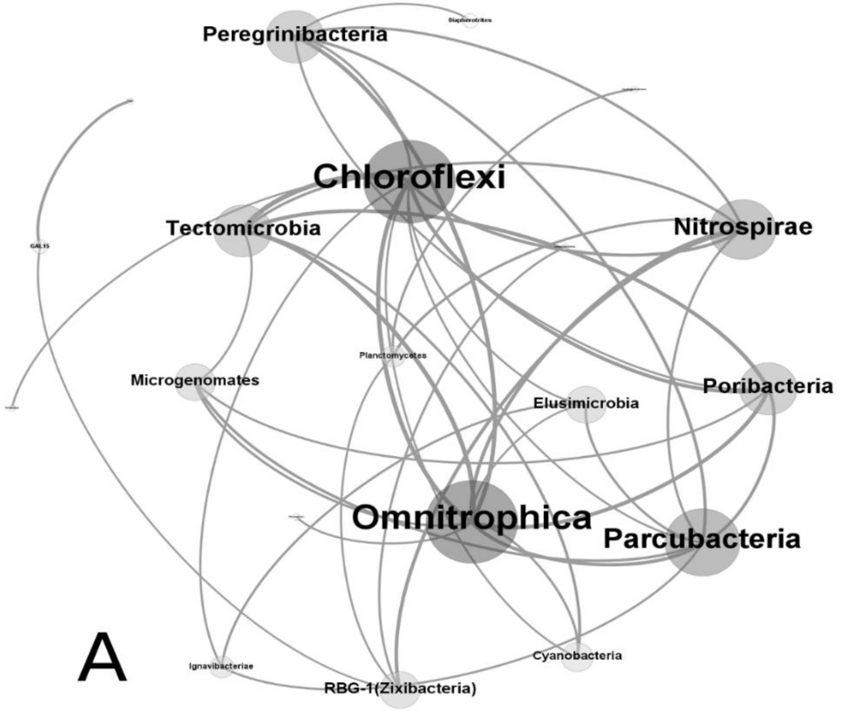

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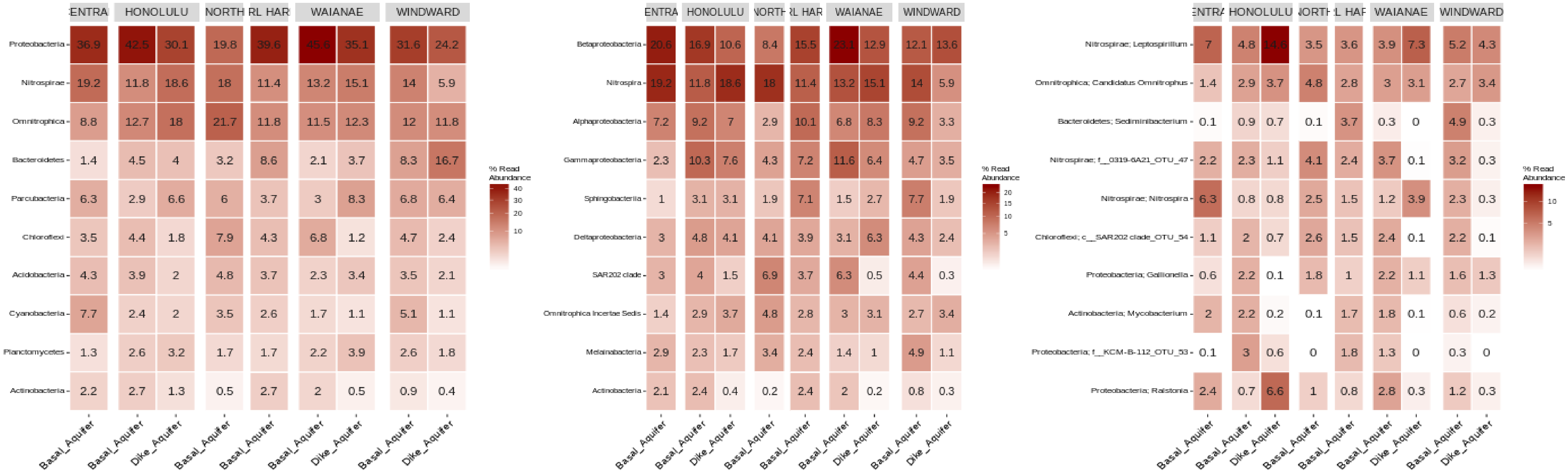
Top 10 phyla, classes, genera



Top ten phyla (left), classes (middle), and genera (right) based on the sequence abundance in the OAHU aquifers (ENTRA – central, HONOLULU – Honolulu, NORTH – North, RL HAR – Pearl Harbor, WAIANAE – Waianae, and WINDWARD – Windward aquifers)

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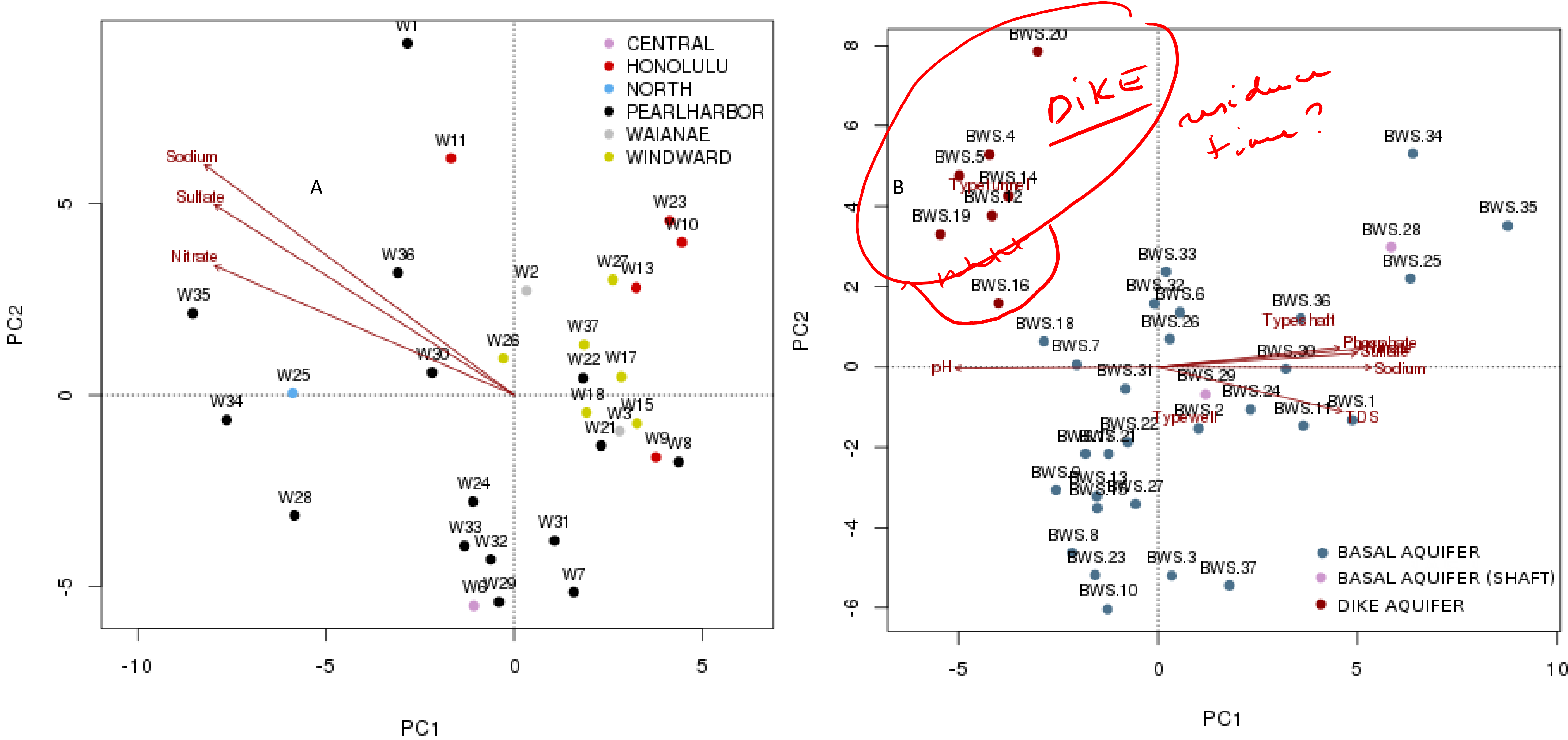

CHEMOAUTOTROPHS!

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Core Groundwater Microbiome

Aquifer Type	Portion of core OTUs[1] (%)	Portion of core sequences (%)
Basal Aquifer	71 OTUs 0.63 %	31.8% (16.2%-62.0%)
Dike Aquifer	29 OTUs 0.31 %	15.4% (0.1%-31.5%)

14+ OTUs

limited

abundant

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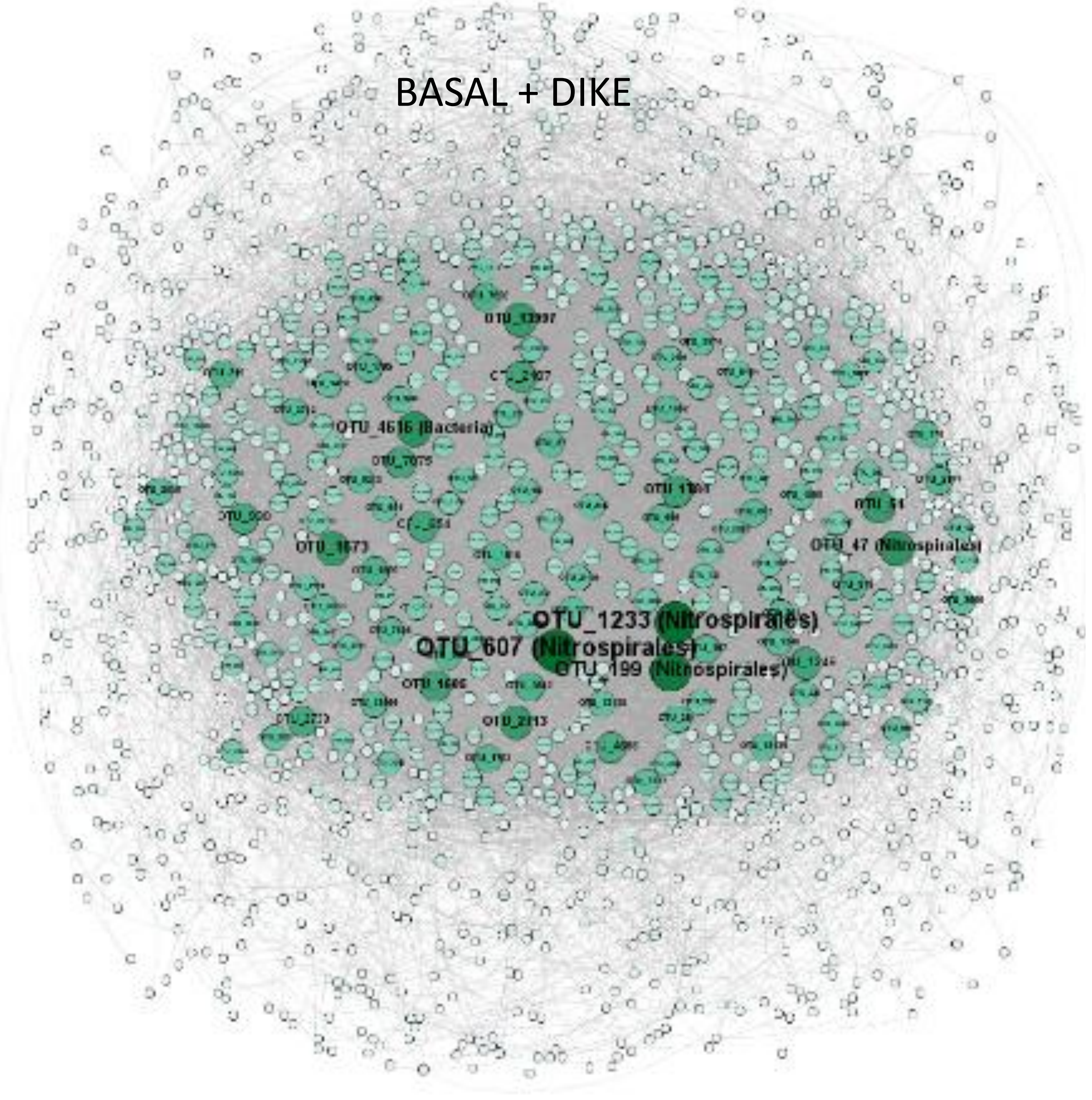
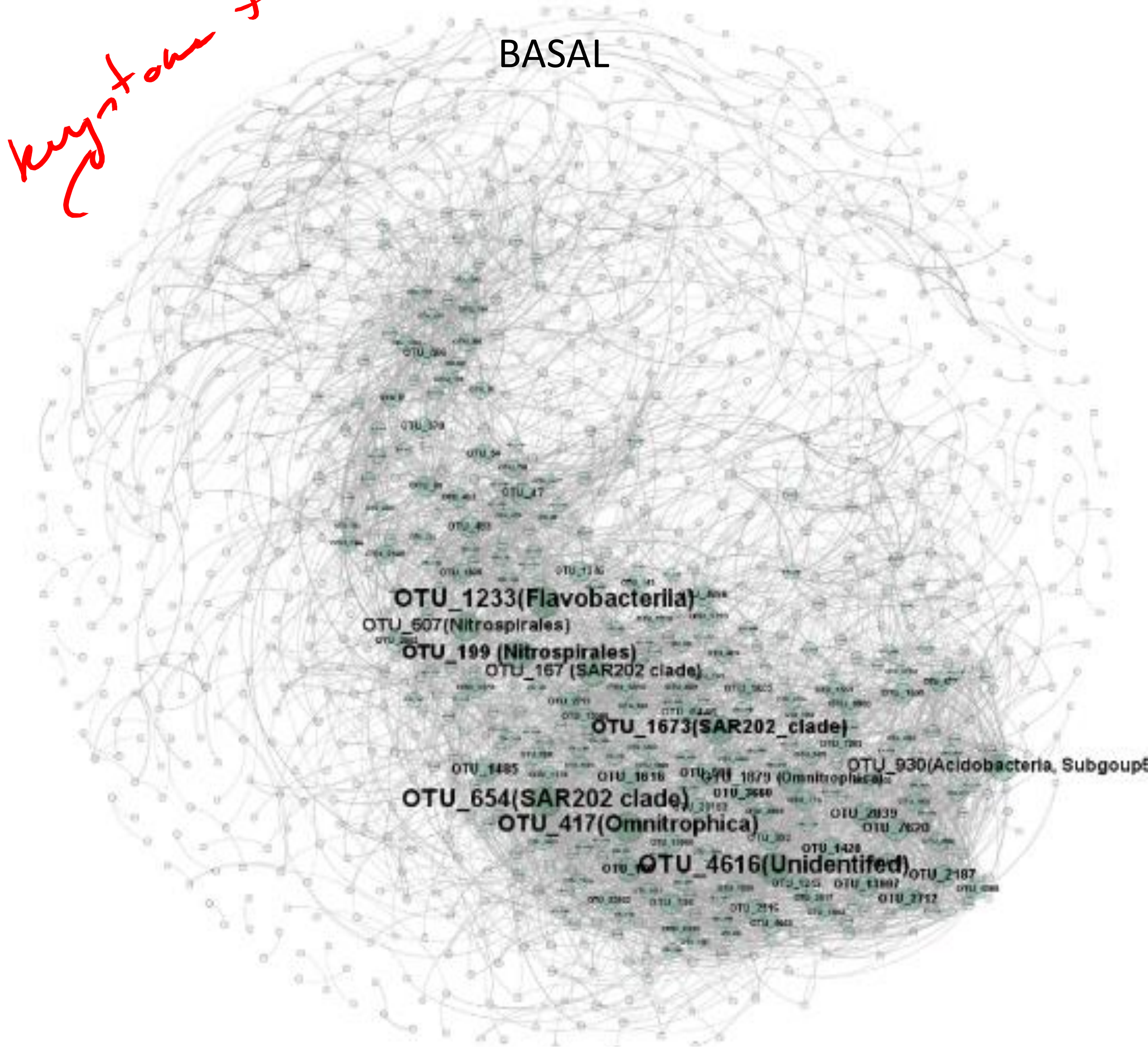
Core Groundwater Microbiome

keystone taxa





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HI Groundwater Quality: indicators

- 16.2% of groundwater samples were positive for total coliforms (GM: 0.6 MPN/100ml; Range: <1 to 18.9 MPN/100ml)

- No *E. coli* or *C. perfringens* or F+ coliphages were detected in the groundwater

- No sewage-specific markers were detected in the groundwater

Do not get scared, it is before chlorination!

GW Study Summary

- GW not sterile, but distinct and diverse bacterial communities
- High abundance of chemoautotrophs
- A few core OTUs, but abundant
- SO_4, NO_3, and Na correlated significantly with gw. bacterial community composition
- Relatively good groundwater quality
- Likely large adaptive potential

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Why water quality matters?

Human health concern

Environmental concern

Economy

$$$,$$$,$$$

Public health hospital costs from USA drinking water exposures

- CDC estimate drinking water disease costs > $970 m/y
  - Less so fecal pathogens, largely Legionnaires' disease, *otitis externa*, and non-tuberculous mycobacteria causing >40 000 hospitalizations/year

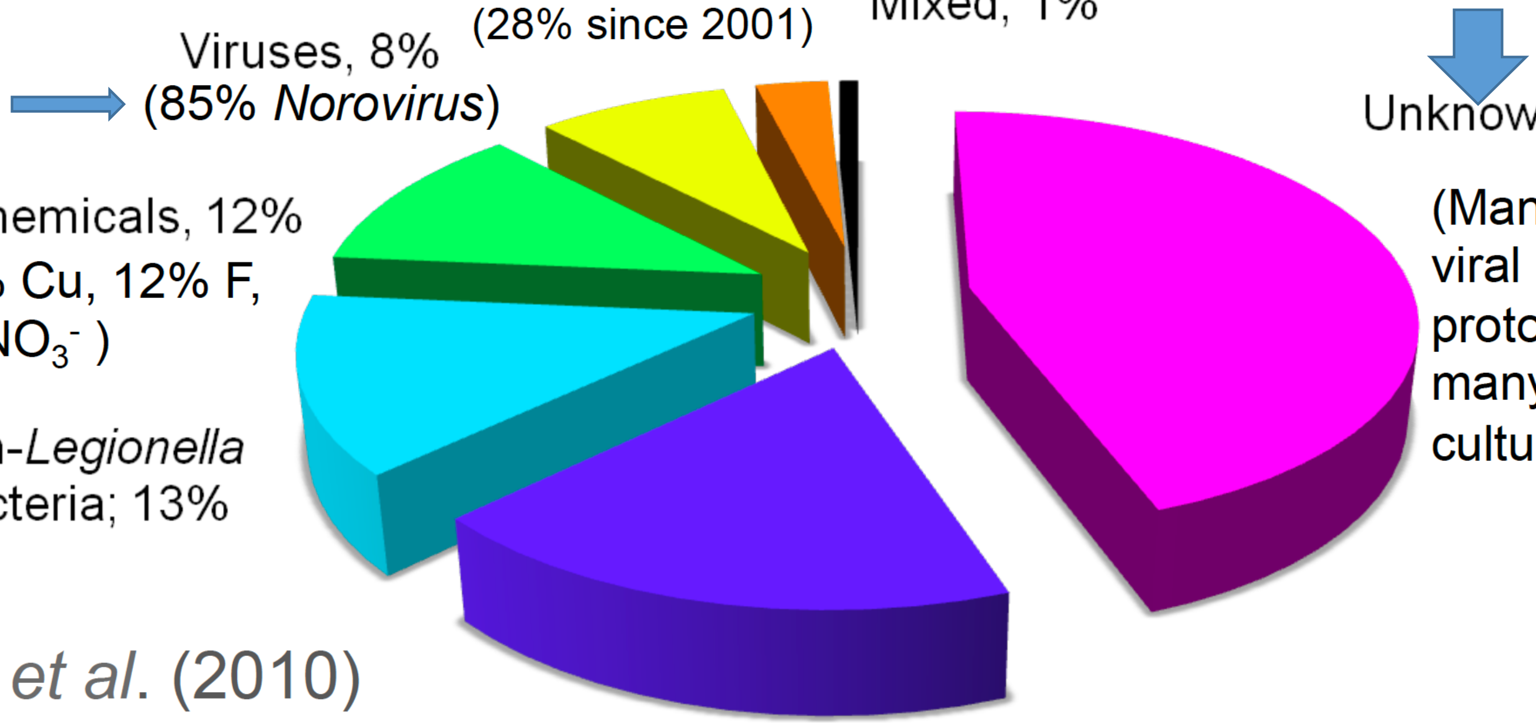
Disease	Annual costs
Cryptosporidiosis	**$46M**
Giardiasis	**$34M**
Legionnaires' disease	**$434M**
NTM infection/Pulmonary	**$426M/ $195M**

not fecal!

Collier *et al.* (2012) Epi Inf 140: 2003-2013

NTM –non-tuberculosis mycobacteria

Etiological agents and percentages for 780 drinking water outbreaks, 1971-2006 USA



Craun *et al*. (2010)
CMR 23:507-528

(403,000 cases from a single outbreak of *Cryptosporidium hominis* in Milwaukee (WI) April 1993, but only 9% of outbreaks vs. *Giardia* 86%)

Top 10 Causes – Outbreaks in Public Water Systems in the US (CDC.gov)

- *Giardia*
- *Legionella*
- Norovirus
- *Shigella*
- *Campylobacter*
- Copper
- *Salmonella*
- Hepatitis A
- *Cryptosporidium*
- *E. coli*, excess fluoride (tie)

Drinking water source

In US:

68% surface water

32% groundwater

<1% other

In Hawaii:

>99% groundwater

https://www.otonabeeconservation.com/new-road-signs-identify-municipal-drinking-water-protection-zones/

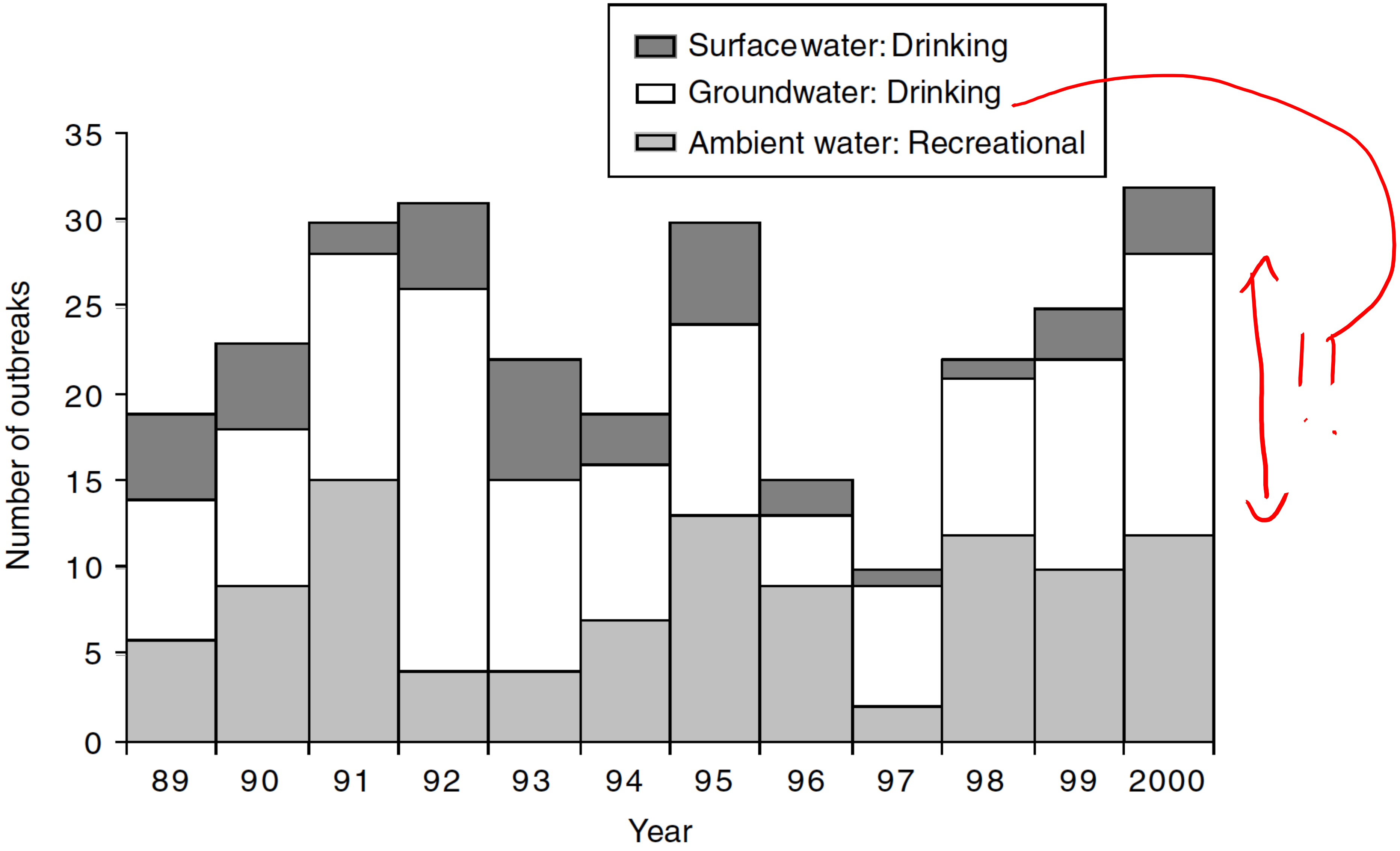
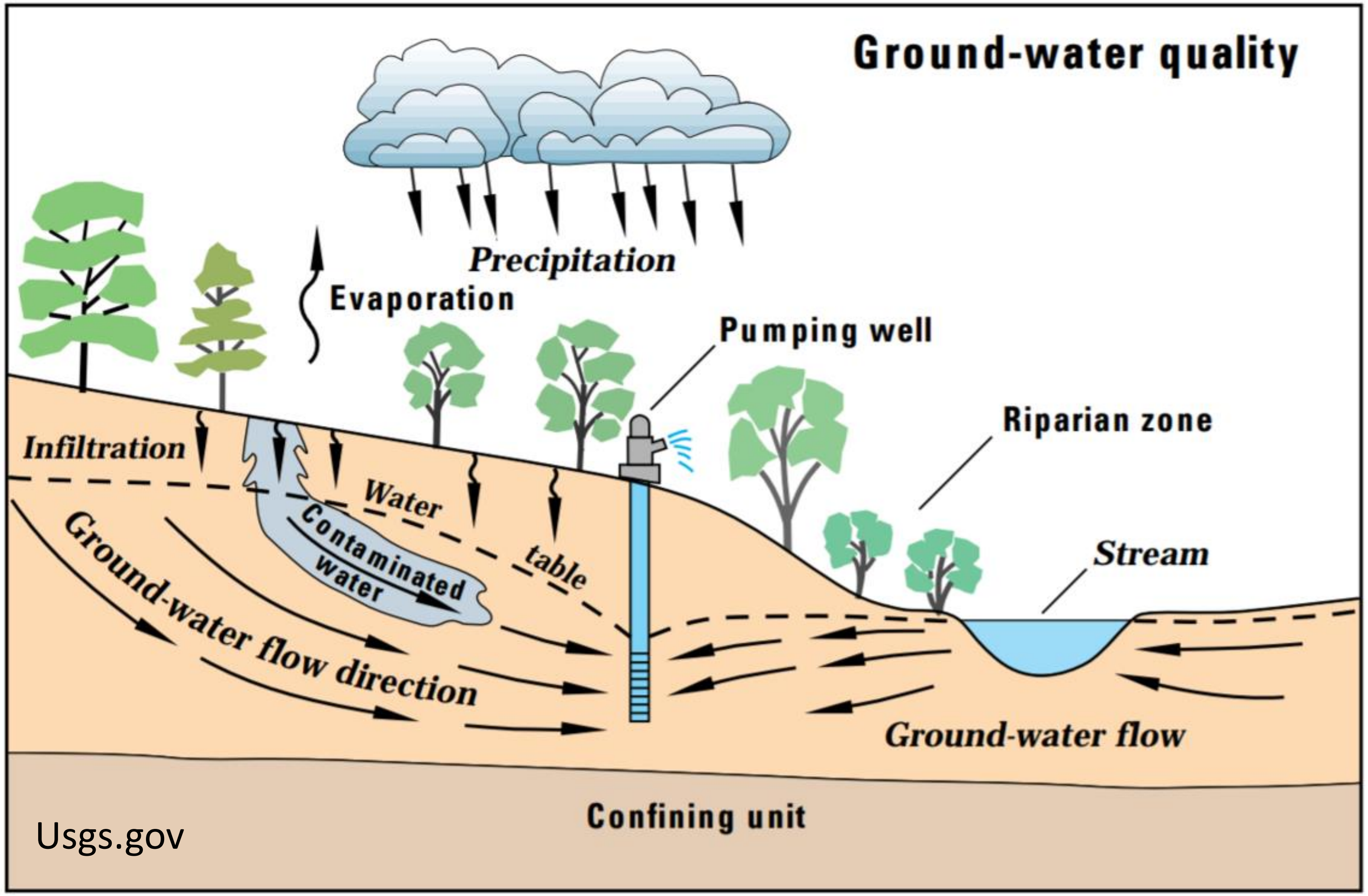


FIGURE 1-2 Number of waterborne disease outbreaks by year and water type for the United States: 1989-2000 (n = 278). SOURCE: Outbreak data through 2000 from the CDC's National Waterborne Diseases Outbreak Surveillance System.

NRC 2004 Indicators of waterborne Pathogens

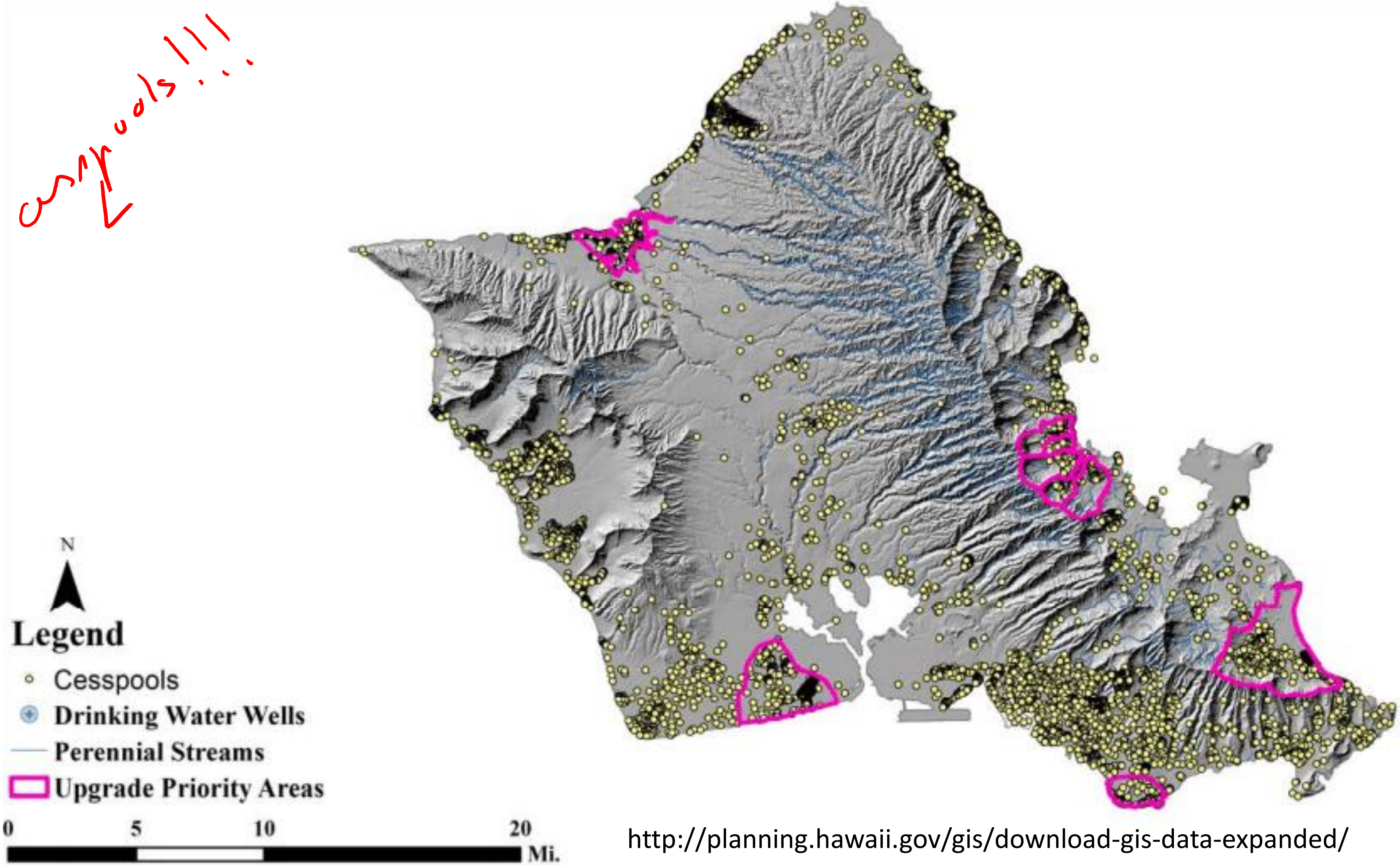
How can GW get contaminated?

Groundwater under the direct influence of surface water (GWUDISW or GWUDI)



- Permeability
- Location and depth of the well
- Aquifer composition
- Proximity to pollution source
- Well and spring construction

cesspools!!!



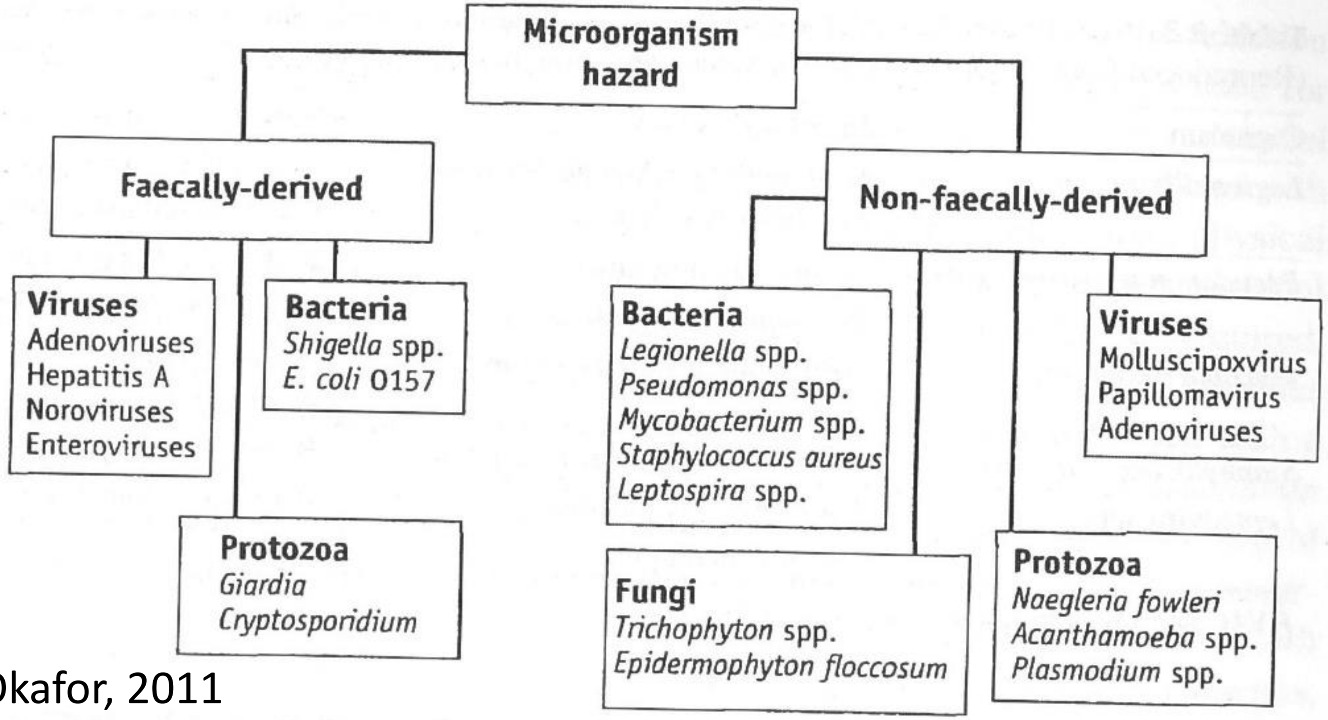
http://planning.hawaii.gov/gis/download-gis-data-expanded/

Koch postulates (1890) to define a pathogen

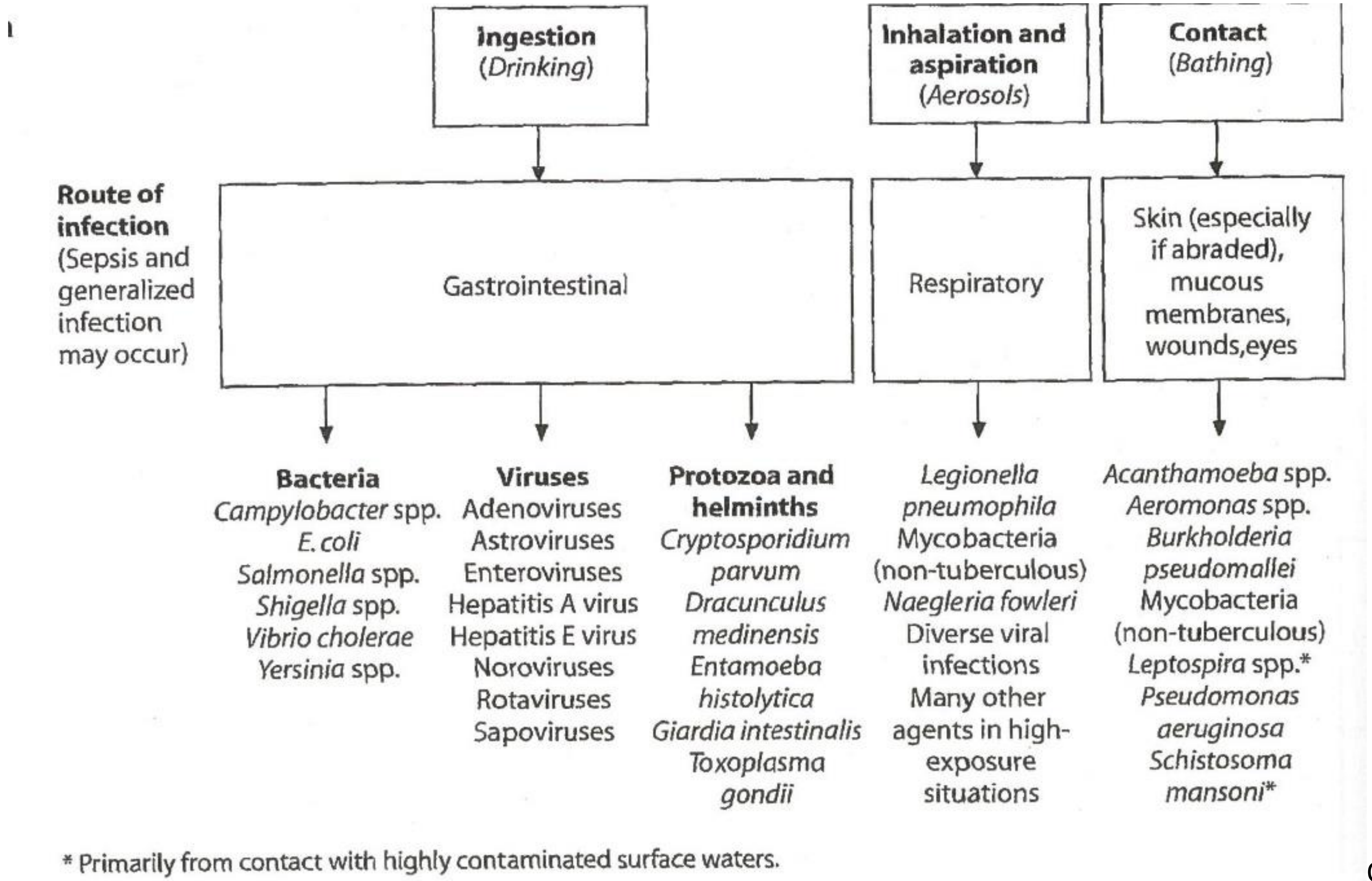
- The bacteria must be present in every case of the disease.
- The bacteria must be isolated from the host with the disease and grown in pure culture.
- The specific disease must be reproduced when a pure culture of the bacteria is inoculated into a healthy susceptible host.
- The bacteria must be recoverable from the experimentally infected host.

Pathogens (the health concern)

- Pathogens
- Infection (vs. disease)
- Infectious dose
- Frank pathogen vs opportunistic pathogen
- Incubation time
- Origin: host AND or the environment
- Die-off
- Enteric pathogens
- Water-borne disease



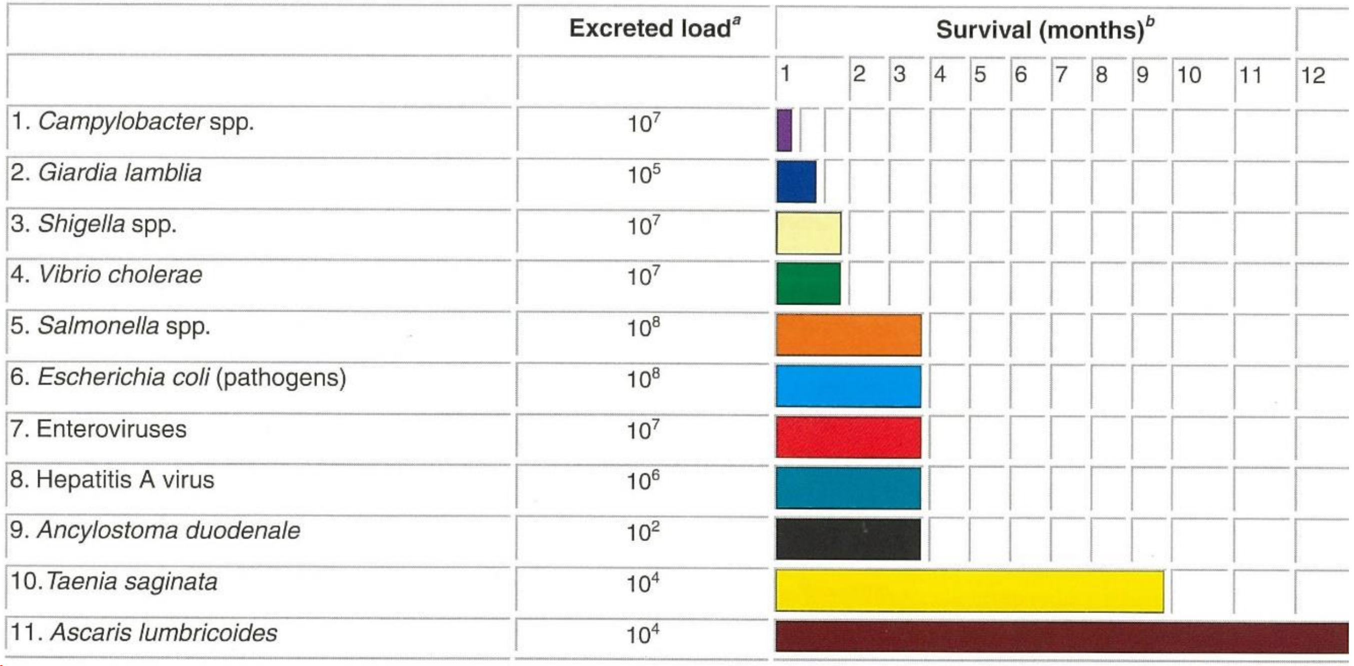
Okafor, 2011


Ingestion
(Drinking)
Inhalation and aspiration
(Aerosols)
Contact
(Bathing)
Route of infection
(Sepsis and generalized infection may occur)
Gastrointestinal
Respiratory
Skin (especially if abraded), mucous membranes, wounds, eyes
Bacteria
Campylobacter spp.
E. coli
Salmonella spp.
Shigella spp.
Vibrio cholerae
Yersinia spp.
Viruses
Adenoviruses
Astroviruses
Enteroviruses
Hepatitis A virus
Hepatitis E virus
Noroviruses
Rotaviruses
Sapoviruses
Protozoa and helminths
Cryptosporidium parvum
Dracunculus medinensis
Entamoeba histolytica
Giardia intestinalis
Toxoplasma gondii
Legionella pneumophila
Mycobacteria (non-tuberculous)
Naegleria fowleri
Diverse viral infections
Many other agents in high-exposure situations
Acanthamoeba spp.
Aeromonas spp.
Burkholderia pseudomallei
Mycobacteria (non-tuberculous)
Leptospira spp.*
Pseudomonas aeruginosa
Schistosoma mansoni*
* Primarily from contact with highly contaminated surface waters.

Okafor, 2011

Survival

- In general, viral and protozoan pathogens survive longer than bacteria
- Survival depends from the environment (temperature, moisture, etc)



[a] Typical average number of organisms/g feces
[b] Estimated average life of infective stage at 20–30°C.
(Modified from Feachem *et al.*, 1983).

FIGURE 22.26 Survival times of enteric pathogens in water, wastewater, soil, and on crops.

Gerba in Mayer, et al, 2009

TABLE 22.13 Environmental Factors Affecting Enteric Pathogen Survival in Natural Waters

Factor	Remarks
Temperature	Probably the most important factor; longer survival at lower temperatures; freezing kills bacteria and protozoan parasites, but prolongs virus survival.
Moisture	Low moisture content in soil can reduce bacterial populations.
Light	UV in sunlight is harmful.
pH	Most are stable at pH values of natural waters. Enteric bacteria are less stable at pH >9 and pH <6.
Salts	Some viruses are protected against heat inactivation by the presence of certain cations.
Organic matter	The presence of sewage usually results in longer survival.
Suspended solids or sediments	Association with solids prolongs survival of enteric bacteria and virus.
Biological factors	Native microflora is usually antagonistic.

TABLE 22.1 Incubation Time for Common Enteric Pathogens

Agent	Incubation period	Modes of transmission	Duration of illness
Adenovirus	8–10 days	Fecal–oral–respiratory	8 days
Campylobacter jejuni	3–5 days	Food ingestion, direct contact	2–10 days
Cryptosporidium	2–14 days	Food or water ingestion, direct and indirect contact	Weeks to months
Escherichia coli			
ETEC	16–72 h	Food or water ingestion	3–5 days
EPEC	16–48 h	Food or water ingestion, direct and indirect contact	5–12 days
EHEC	72–120 h	Food/ingestion, direct or indirect contact	2–15 days
Giardia lamblia	7–14 days	Food or water ingestion, direct and indirect contact	Weeks to months
Norovirus	24–48 h	Food or water ingestion, direct and indirect contact, aerosol?	1–2 days
Rotavirus	24–72 h	Direct and indirect contact	4–6 days
Hepatitis A	30–60 days	Hepatitis	2–4 weeks
Salmonella	16–72 h	Food ingestion, direct and indirect contact	2–7 days
Shigella	16–72 h	Food or water ingestion, direct and indirect contact	2–7 days
Yersinia enterocolitica	3–7 days	Food ingestion, direct contact	1–3 weeks

Gerba in Mayer, et al, 2009

TABLE 22.2 Concentration of Enteric Pathogens in Feces

Organism	Per gram of feces
Protozoan parasites	10^6–10^7
Helminths	
Ascaris	10^4–10^5
Enteric viruses	
Enteroviruses	10^3–10^7
Rotavirus	10^{10}
Adenovirus	10^{11}
Enteric bacteria	
Salmonella spp.	10^4–10^{10}
Shigella	10^5–10^9
Indicator bacteria	
Coliform	10^7–10^9
Fecal coliform	10^6–10^9

Gerba in Mayer, et al, 2009

Table 8.1 Waterborne pathogens and their significance in water supplies (From Anonymous 2006a. With permission)

Pathogen	Health significance	Persistence in water supplies[a]	Resistance to chlorine[b]	Relative infectivity[c]	Important animal source
Bacteria					
Burkholderia pseudomallei	Low	May multiply	Low	Low	No
Campylobacter jejuni, C. coli	High	Moderate	Low	Moderate	Yes
Escherichia coli – Pathogenic[d]	High	Moderate	Low	Low	Yes
E. coli – Enterohaemorrhagic	High	Moderate	Low	High	Yes
Legionella spp.	High	Multiply	Low	Moderate	No
Non-tuberculous mycobacteria	Low	Multiply	High	Low	No
Pseudomonas aeruginosa[e]	Moderate	May multiply	Moderate	Low	No
Salmonella typhi	High	Moderate	Low	Low	No
Other salmonellae	High	May multiply	Low	Low	Yes
Shigella spp.	High	Short	Low	Moderate	No
Vibrio cholerae	High	Short	Low	Low	No
Yersinia enterocolitica	High	Long	Low	Low	Yes
Viruses					
Adenoviruses	High	Long	Moderate	High	No
Enteroviruses	High	Long	Moderate	High	No
Hepatitis A virus	High	Long	Moderate	High	No
Hepatitis E virus	High	Long	Moderate	High	Potentially
Noroviruses and sapoviruses	High	Long	Moderate	High	Potentially
Rotaviruses	High	Long	Moderate	High	No
Protozoa					
Acanthamoeba spp.	High	Long	High	High	No
Cryptosporidium parvum	High	Long	High	High	Yes
Cyclospora cayetanensis	High	Long	High	High	No
Entamoeba histolytica	High	Moderate	High	High	No
Giardia intestinalis	High	Moderate	High	High	Yes
Naegleria fowleri	High	May multiply[f]	High	High	No
Toxoplasma gondii	High	Long	High	High	Yes
Helminths					
Dracunculus medinensis	High	Moderate	Moderate	High	No
Schistosoma spp.	High	Short	Moderate	High	Yes

Note: Waterborne transmission of the pathogens listed has been confirmed by epidemiological studies and case histories. Part of the demonstration of pathogenicity involves reproducing the disease in suitable hosts. Experimental studies in which volunteers are exposed to known numbers of pathogens provide relative information. As most studies are done with healthy adult volunteers, such data are applicable to only a part of the exposed population, and extrapolation to more sensitive groups is an issue that remains to be studied in more detail

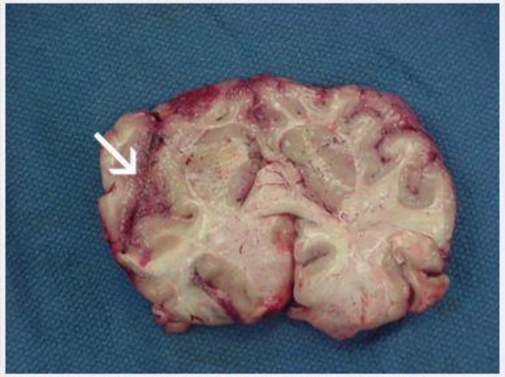
[a]Detection period for infective stage in water at 20°C: short, up to 1 week; moderate, 1 week to 1 month; long, over 1 month
[b]When the infective stage is freely suspended in water treated at conventional doses and contact times. Resistance moderate, agent may not be completely destroyed
[c]From experiments with human volunteers or from epidemiological evidence
[d]Includes enteropathogenic, enterotoxigenic and enteroinvasive
[e]Main route of infection is by skin contact, but can infect immunosuppressed or cancer patients orally
[f]In warm water

Okafor, 2011

https://www.cdc.gov/parasites/naegleria/index.html

Naegleria fowleri

- amoeboflagellate
- World-wide in freshwater, sediments, soils
- >35°C amoeba transforms to flagellated form which allows it to swim
- Swims to nose, and follows nerve to brain, produces toxin that liquefies brain
- Massive headache, death in 4-6 days, 95-97% fatality
- Prognosis after death…(amphotericin B if diagnosed early)



Focal hemorrhage and necrosis in frontal cortex due to *Naegleria fowleri*

8 Texas cities were alerted to a brain-eating amoeba found in water supply

By Lauren M. Johnson and Artemis Moshtaghian, CNN
Updated 5:49 PM ET, Sat September 26, 2020

Multiple agencies working to clear Texas town's water supply of brain-eating amoeba

BY STEFAN STEVENSON
SEPTEMBER 29, 2020 02:42 PM

Naegleria fowleri, or "brain-eating amoeba", is an incredibly rare and deadly microorganism that can be found in warm freshwater. BY MCCLATCHY

'Brain-eating' amoeba in Texas city's water supply kills 6-year-old

By Rachael Rettner - Senior Writer 4 days ago

After the boy's death, officials detected the deadly amoeba in the city's water supply.

WVLT 8 KNOXVILLE, TN

News Weather Sports Eye on Education Election Central 2020 Watch WVLT News Submit Photos & Vide

Frost Advisory Is In Effect

Texas officials say 2-3 months until water safe after brain-eating amoeba kills 6-year-old

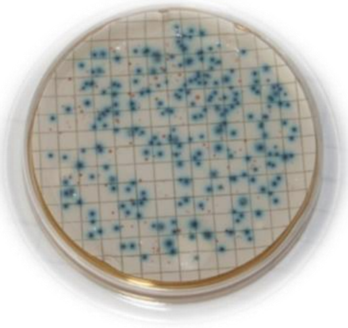
Officials said they will continue to test the city's water once the process is complete to make sure it's safe.

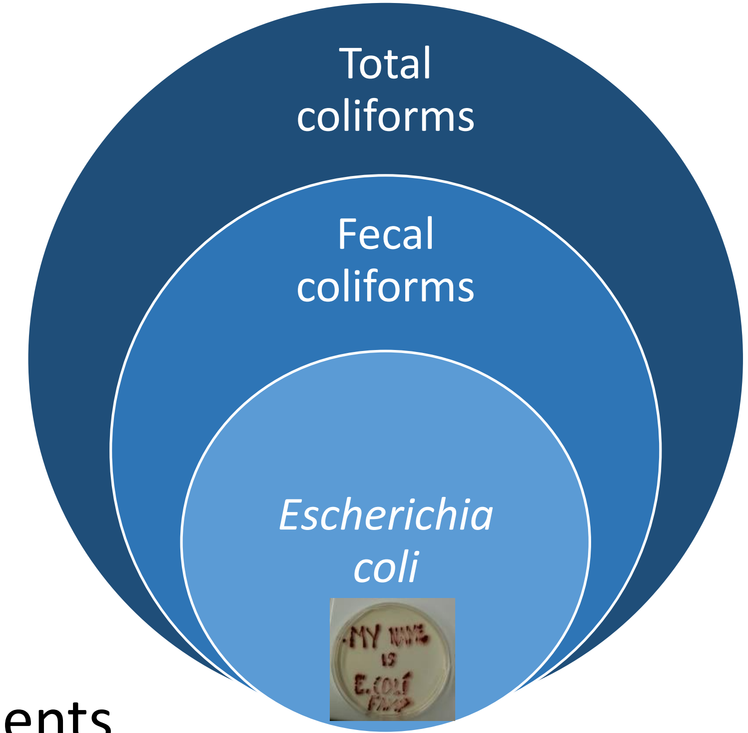
What and Why we measure?

- Too many potential pathogens, methods tedious, difficult, and time consuming
- Microbial indicators
- What do microbial indicators indicate?

Some of the microbial indicators

- Total coliforms
- Fecal coliforms
- *Escherichia coli*
- Enterococci





(Drawn not in proportions; taxonomically nonsense)

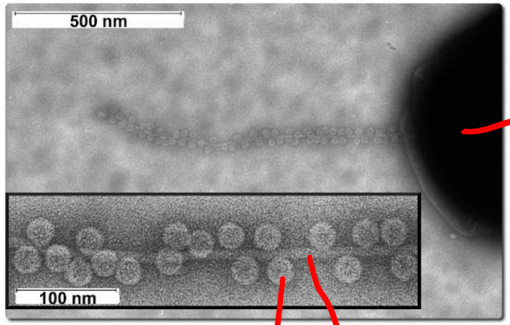
Problems:

- Regrowth in aquatic and/or soil environments
- Regrowth in distribution system (TC)
- **If regrowth - Not indicative of health threat or risk**
- **No relationship with protozoa or viruses or environmental pathogens**
- *E. coli* die-off rapid (faster than pathogens) in marine environments

Some examples of alternative microbial indicators

F+ and somatic coliphages

- Pros: good proxy for viruses
- Cons: concentrations relatively low in sewage, methods tedious



E. coli cell

E.coli

pilus

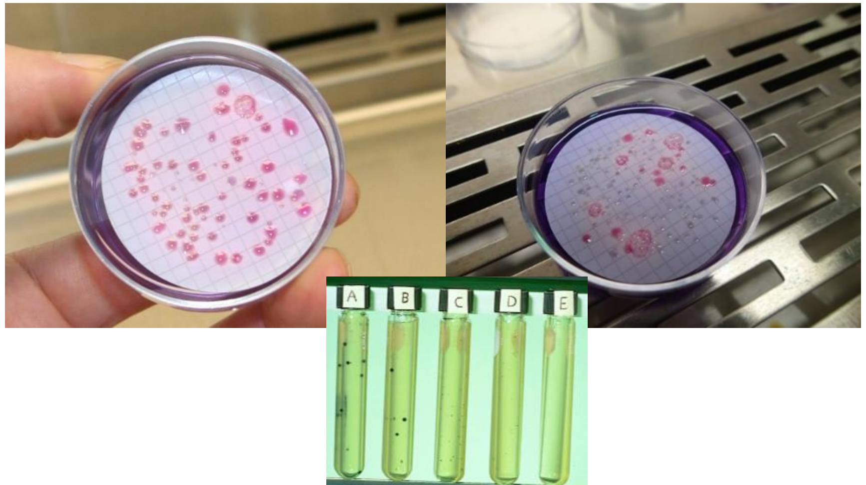
phage

plaque – eaten by phages

Clostridium perfringens (spores)

div ~8-10min fast

- Pros: obligate anaerobe
- Cons: spores can survive long in sediments



Safe Drinking Water Act

- Total Coliform Rule -treatment efficiency and distribution system integrity
- Surface Water Treatment Rule - treatment efficiency and distribution system integrity
- **Groundwater Rule – fecal contamination in source groundwater**
- Drinking Water Contaminant Candidate List (CCL) – treatment and distribution system

Regulations dependent from how many people are served

Public Water System - >15 service connections or serves an average 25 or more people at least 60 days a year

Hawaii Drinking Water Regulations

- HAR Title 11 Chapter 20 (2017)
- Not just microbiology, also includes organic and inorganic contaminants
- Regulates sampling frequency, depends how many people served
- Regulates until water reaches your house, you are responsible for pluming (lead) and its integrity (entry of contaminants)
- Distribution system is not compliant if *E.coli* positive sample follows total coliform positive sample or total coliform positive samples follows *E. coli* positive sample. Failure to collect is also considered as system failure
- *E.coli* positive sample triggers boil-water notice
- Drinking Water samples should also not contain *Cryptosporidium, Giardia lambia* and *Legionella* as well as free from enteric viruses (coliphage as proxy). Used to evaluate treatment.

TOTAL COLIFORM MONITORING FREQUENCY

Population Served			Minimum Samples/Month
25	to	1,000	1
1,001	to	2,500	2
2,501	to	3,300	3
3,301	to	4,100	4
4,101	to	4,900	5
4,901	to	5,800	6
5,801	to	6,700	7
6,701	to	7,600	8
7,601	to	8,500	9
8,501	to	12,900	10
12,901	to	17,200	15
17,201	to	21,500	20
21,501	to	25,000	25
25,001	to	33,000	30
33,001	to	41,000	40
41,001	to	50,000	50
50,001	to	59,000	60
59,001	to	70,000	70
70,001	to	83,000	80
83,001	to	96,000	90
96,001	to	130,000	100
130,001	to	220,000	120
220,001	to	320,000	150
320,001	to	450,000	180
450,001	to	600,000	210
600,001	to	780,000	240
780,001	to	970,000	270
970,001	to	1,230,000	300
1,230,001	to	1,520,000	330
1,520,001	to	1,850,000	360
1,850,001	to	2,270,000	390
2,270,001	to	3,020,000	420
3,020,001	to	3,960,000	450
3,960,001	or more		480

20-50

3244

§11-20-9.1

Outside the US

TABLE 23.9 Drinking Water Criteria of the European Union

Tap water	
Escherichia coli	0/100 ml
Fecal streptococci	0/100 ml
Sulfite-reducing clostridia	0/20 ml
Bottled water	
Escherichia coli	0/250 ml
Fecal streptococci	0/250 ml
Sulfite-reducing clostridia	0/50 ml
Pseudomonas aeruginosa	0/250 ml

From European Union, 1995.

The water serving Your Location has been tested and meets all Federal and State standards. 13411

The water quality monitoring results are presented below.

The water sources serving this address are:

Source Name	Origin of Water	Treatment	Region
a) Beretania Pumping Station	Groundwater	Chlorination	1
b) Kaimuki Pumping Station	Groundwater	Chlorination	1

Unregulated Contaminants (Do not have designated maximum limits but require monitoring)

Contaminant	Tested By	Sample Year	Unit	Highest Average	Range Minimum	Range Maximum	Health Advisory	Found in Sources
1-Butanol	(2)	2018	ppb	6.250	5.600	6.900	NYA	a
Chlorate	(2)	2017	ppb	72.500	53.000	77.000	210.000	All Sources
Chloride	(2)	2019	ppm	100.000	93.000	100.000	250 **	All Sources
Chromium, Hexavalent	(2)	2017	ppb	2.000	2.000	2.000	13.000	All Sources
Dieldrin	(2)	2019	ppb	0.044	0.044	0.044	0.200	b
Sodium	(2)	2017	ppm	52.000	37.000	52.000	60.000	All Sources
Strontium	(2)	2017	ppb	155.000	79.000	180.000	4000.000	All Sources
Sulfate	(2)	2019	ppm	16.000	10.000	16.000	250 **	All Sources
Vanadium	(2)	2017	ppb	19.500	12.000	21.000	21.000	All Sources

** Secondary Maximum Contaminant Levels (SMCLs) are standards established as guidelines to assist public water systems in managing the aesthetic quality (taste, odor and color) of drinking water. EPA does not enforce SMCLs.

NO!

Microbial Contaminants (2)

System Name	Contaminant	Number of positive E. coli samples found	Violation (Yes/No)	Number of assessments required to perform	Major sources in drinking water
Honolulu-Windward-Pearl Harbor	E. Coli	1	No	0	Human and animal fecal waste

re-testing

Level 1 Assessment: A Level 1 assessment is a study of the water system to identify potential problems and determine (if possible) why total coliform bacteria have been found in our water system.

Level 2 Assessment: A Level 2 assessment is a very detailed study of the water system to identify potential problems and determine (if possible) why an E. coli MCL violation has occurred and/or why total coliform bacteria have been found in our water system on multiple occasions.

of expected risk to health. MCLGs allow for a margin of safety.

GAC	Granular Activated Carbon Filtration
Health Advisory	An estimate of acceptable drinking water levels for a chemical substance based on health effects information. Health advisory is not a legally enforceable standard.
CFU/100ml	Colony forming units per 100 milliliter
mrem/yr	Millirems Per Year (A Measure of Radiation)
pCi/L	Picocuries Per Liter (A Measure of Radioactivity)
ppb	Parts Per Billion or Micrograms Per Liter"
ppm	Parts Per Million or Milligrams Per Liter
ppt	Parts Per Trillion or Nanograms Per Liter
NQ	Not Quantifiable (< means "less than")
NYA	Not Yet Available
N/A	Not Applicable
ND	Not Detected
*	EPA considers 50 pCi/L to be the level of concern for beta particles
(1)	Analysis by the State of Hawaii Department of Health.
(2)	Analysis by the Honolulu Board Of Water Supply. Questions, call 808-748-5370.
LRAA	Locational running annual average is the average of sample analytical results for samples taken at a particular monitoring location during the previous four calendar quarters.
MRDL	Maximum residual disinfectant level: The highest level of a disinfectant allowed in drinking water.
MRDLG	Maximum residual disinfectant level goal: The level of a drinking water disinfectant below which there is no known or expected risk to health.

Residual Chlorine

System Name	Sample Year	Unit	Lowest Monthly Average	Highest Monthly Average	Running Annual Average	MRDL	MRDLG
Honolulu-Windward-Pearl Harbor	2019	ppm	0.29	0.35	0.3	4	4

Lead/Copper Testing (2)

Contaminant	Sample Year	Unit	90th Percentile Reading	Action Level	# Samples Above Action Level
Copper	2018	ppm	0.029	1.300	0
Lead	2018	ppb	<1.000	15.000	0

No violations found for calendar year 2019

Growth of indicator bacteria (total coliforms, *E. coli*, and enterococci) in Hawaiian soils

- Known for 25+ years
- High concentrations measured

by many scientist:

Hardina & Fujioka, 1991

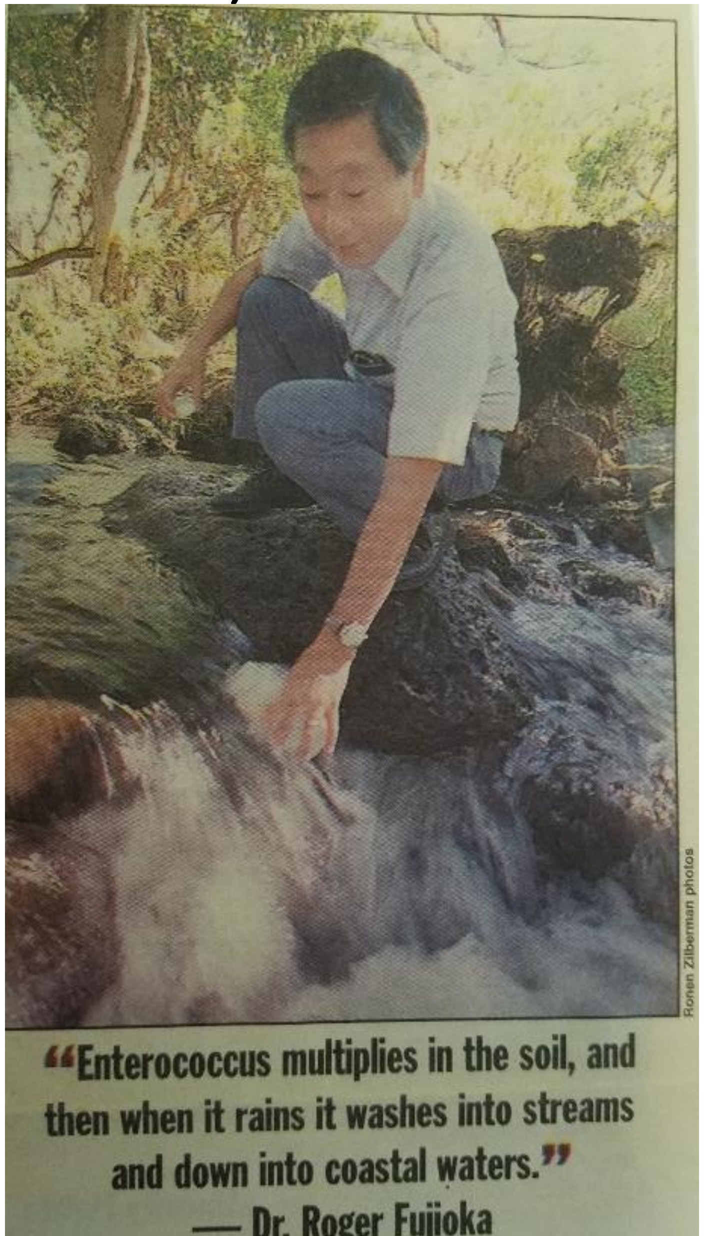
Luther & Fujioka, 2004

Byappanahalli et al., 2011

Goto & Yan, 2011

Kirs et al, 2017

<u>It is not a theory or hypothesis</u>

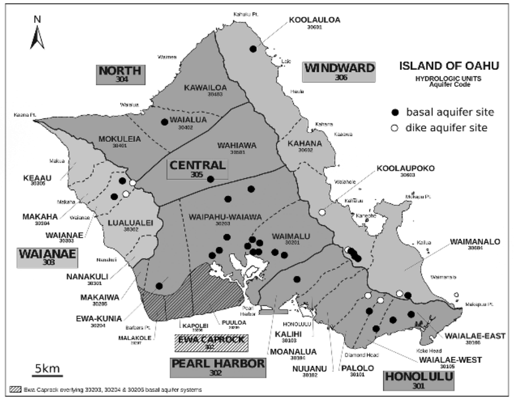


Growth of indicator bacteria in Hawaiian soils - consequences

- All current indicator bacteria can grow in Hawaii soils
- Transported by rain
- Impacts water quality

What do high indicator bacteria truly mean?

Soil samples around 34 groundwater wells on Oahu (fenced, no public access)



- All soil samples were positive for total coliforms and enterococci.
- Geometric mean concentrations of both organisms were 1403 MPN/g and 49 MPN/g respectively, but frequently exceeded >2,419.6 MPN/g of soil.
- *E. coli* was detected in 55% of samples and the concentrations varied from <1 to >2,419.6 MPN/ g.

Indicator bacteria in soils at Lyon arboretum

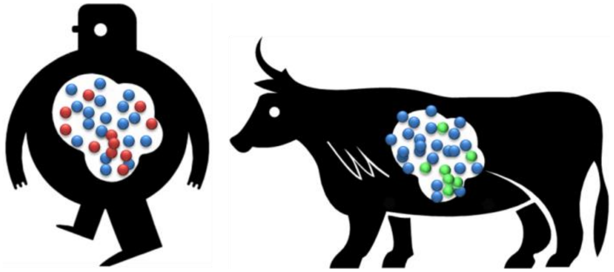
per 1 gram!!!.

Adam Cannon (REU student)

Organism	Units	Native 1	Native 2	Non-native 1	Non-native 2
Enterococci	MPN/gram (min,max)	92 (<10,241)	164.2 (<10,670)	61.4 (20,141)	250.6 (10,613)
Total coliforms	MPN/gram (min,max)	14562 (6440,21640)	111140 (14450,>241960)	29987.5 (4060,>241960)	37592.5 (6700,86640)
E. coli	MPN/gram (min,max)	5 (<10,<10)	25.2 (<10,169)	7 (<10,10)	442.3 (51,1259)
C. perfringens	CFU/gram (min,max)	250 (0,1000)	120 (0,500)	100 (0,200)	560 (0,1100)
Somatic coliphage	PFU/gram (min,max)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)
F+ coliphage	PFU/gram (min,max)	4 (0,20)	8 (0,40)	0 (0,0)	1.67 (0,10)
Pathogenic *Leptospira spp*.	Positive samples/All samples	0/5	0/6	1/5	1/6
Human-associated *Bacteroides*	Positive samples/All samples	0/5	0/6	0/5	0/6

Microbial Source Tracking (MST):

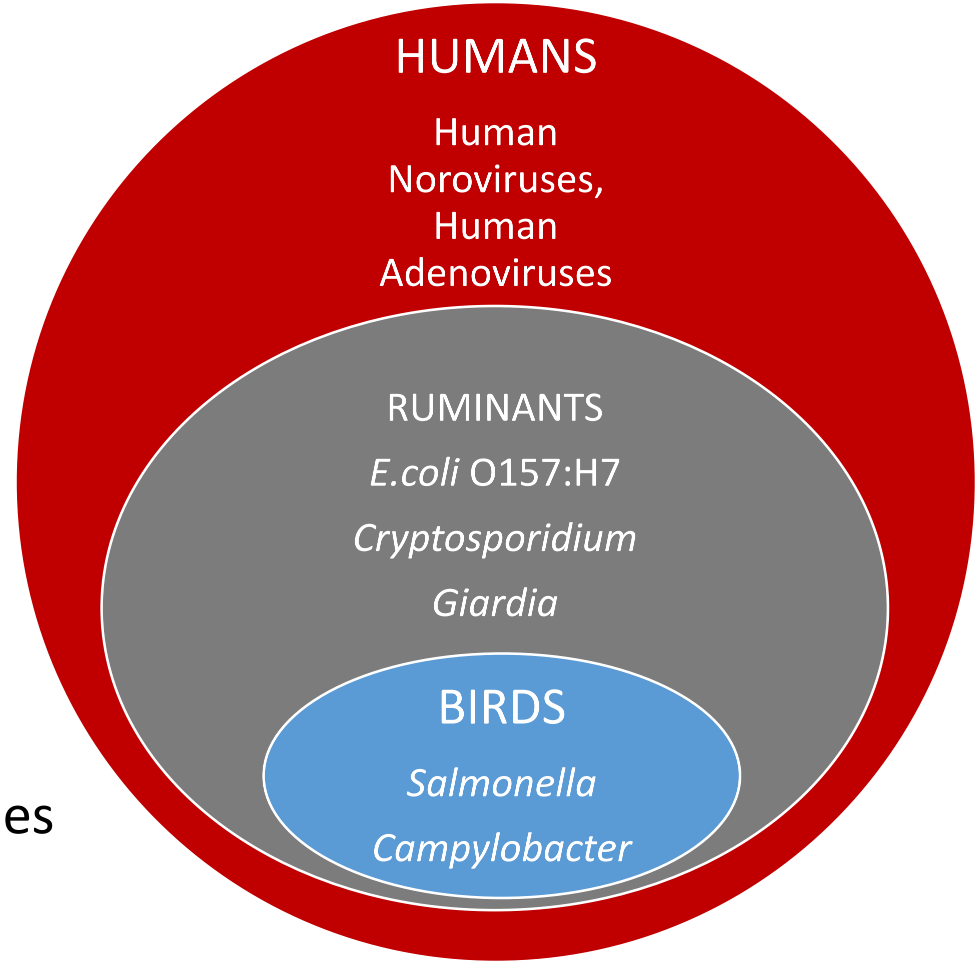
Set of tools to identify the source of fecal contamination in ground and surface waters



Importance:

- Health risk
- Management
- Mediating Conflicts

Key component in QMRA studies



Plan

Lecture 1

- Crash course: microbiology
- Groundwater microbiology

Lecture 2

- Pathogens and groundwater quality
- **Drinking water treatment**

Drinking Water Treatment

- Water sources: groundwater, surface water, rainwater….wastewater
- Slow sand filters were the earliest methods used
- In 1881, Koch showed that chlorine can be used as disinfectant
- First continuous chlorination of public water supply for the first time 1905 (London), regular use in US 1908 (Chicago)

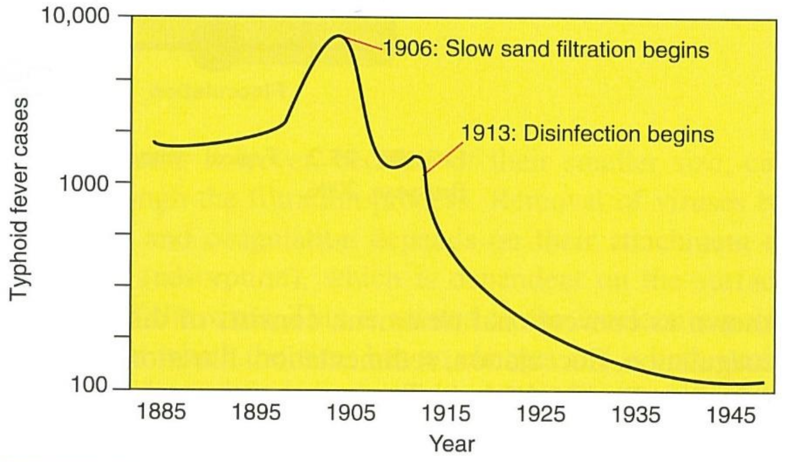
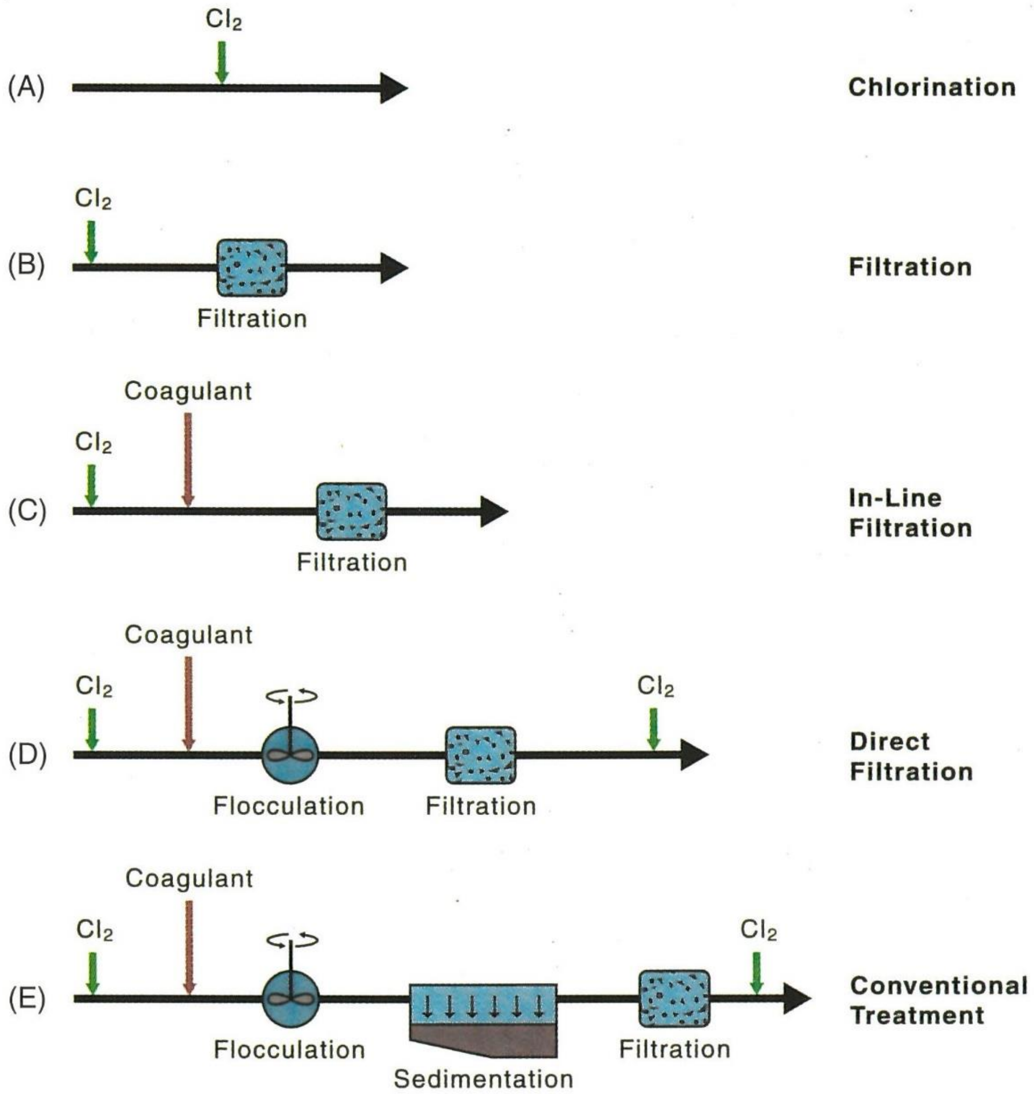


FIGURE 25.1 Impact of water filtration and chlorination on typhoid fever death rate in Albany, New York. From Logsdon and Lippy, 1982.

Gerba & Pepper, 2015

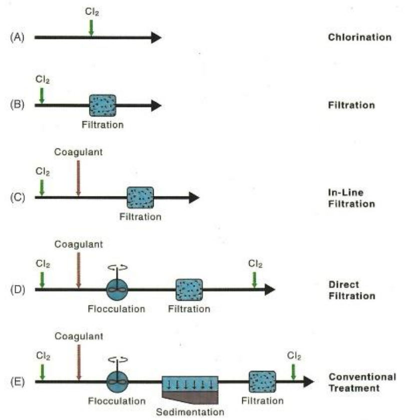
Drinking Water Treatment

- Treatment process trains (options):



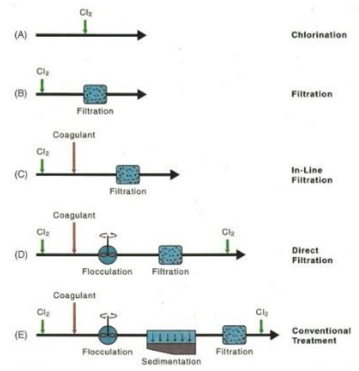
Gerba & Pepper in Mayer, et al, 2009

Drinking Water Treatment



1. Chlorination (chlorine or chlorine dioxide) or Ozone ((or bromine, iodine, UV))

- Strong oxidizers
- Chlorine when added as gas forms hypochlorous acid (HOCl) and hydrochloric acid (HCl)
- To prevent regrowth, treatment with ozone is typically followed by chlorination as ozone leaves no residue.
- Ozone treatment is more expensive than chlorination, can produce suspected carcinogens



Drinking Water Treatment

2. Sedimentation

- Coagulant is added to enhance removal of dissolved suspended solids during the sedimentation and filtration
- Flocculation (stirring) enhances coagulation
- Frequently used coagulants are alum, ferric sulfate and ferric chloride or polyelectrolytes
- Gravitational settling

3.Filtration

- Rapid sand and/or anthracite filters (50-75 cm) (mostly US), backwashed on regular basis
- Slow sand filtration (60-120 cm and gravel 30-50 cm)) (mostly Europe), biofilm formation important
- Filtration especially important for removal of *Giardia* and *Cryptosporidium*

Drinking Water Treatment

TABLE 25.2 Coagulation, Sedimentation, Filtration: Typical Removal Efficiencies and Effluent Quality

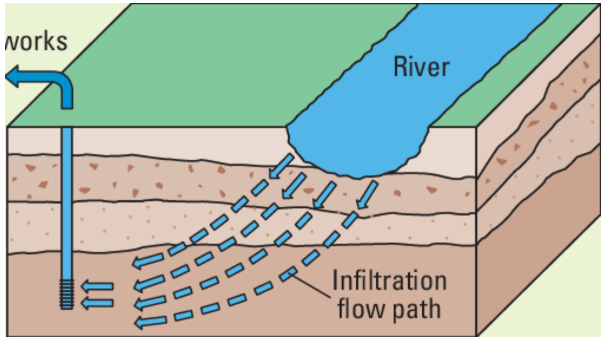
Organisms	Coagulation and sedimentation (% removal)	Rapid filtration (% removal)	Slow sand filtration (% removal)
Total coliforms	74–97	50–98	>99.999
Fecal coliforms	76–83	50–98	>99.999
Enteric viruses	88–95	10–99	>99.999
Giardia	58–99	97–99.9	>99
Cryptosporidium	90	99–99.9	99

From U.S. EPA, 1988.

Gerba in Mayer, et al, 2009

Drinking Water Treatment – Other options

- Riverbank filtration

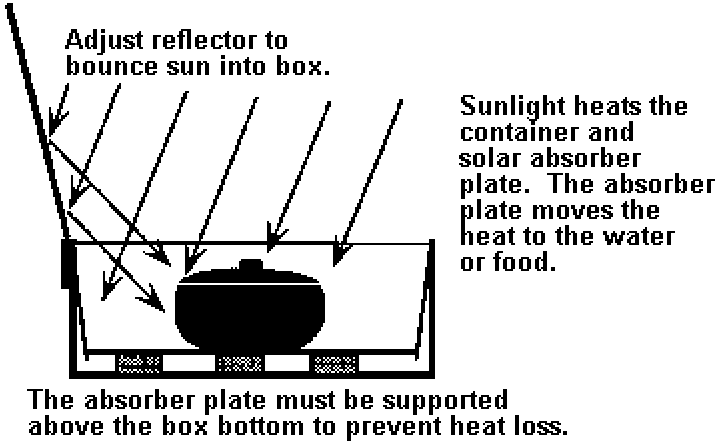


Ryan, et al., 2002

- Solar pasteurization

Ray and Jain, 2011

CEDESOL Foundation



Solarcooking.org

Temperature	Results
55°C(131°F)	Worms, protozoa cysts D =~ 1min
60°C(140°F)	*E. coli*, rotavirus, *Salmonella typhi*, *Vibrio cholera*, *Shigella* D value ~1min
65°C(149°F)	Hepatitis A virus D value =~1min

D – value – 90% of contaminant removal

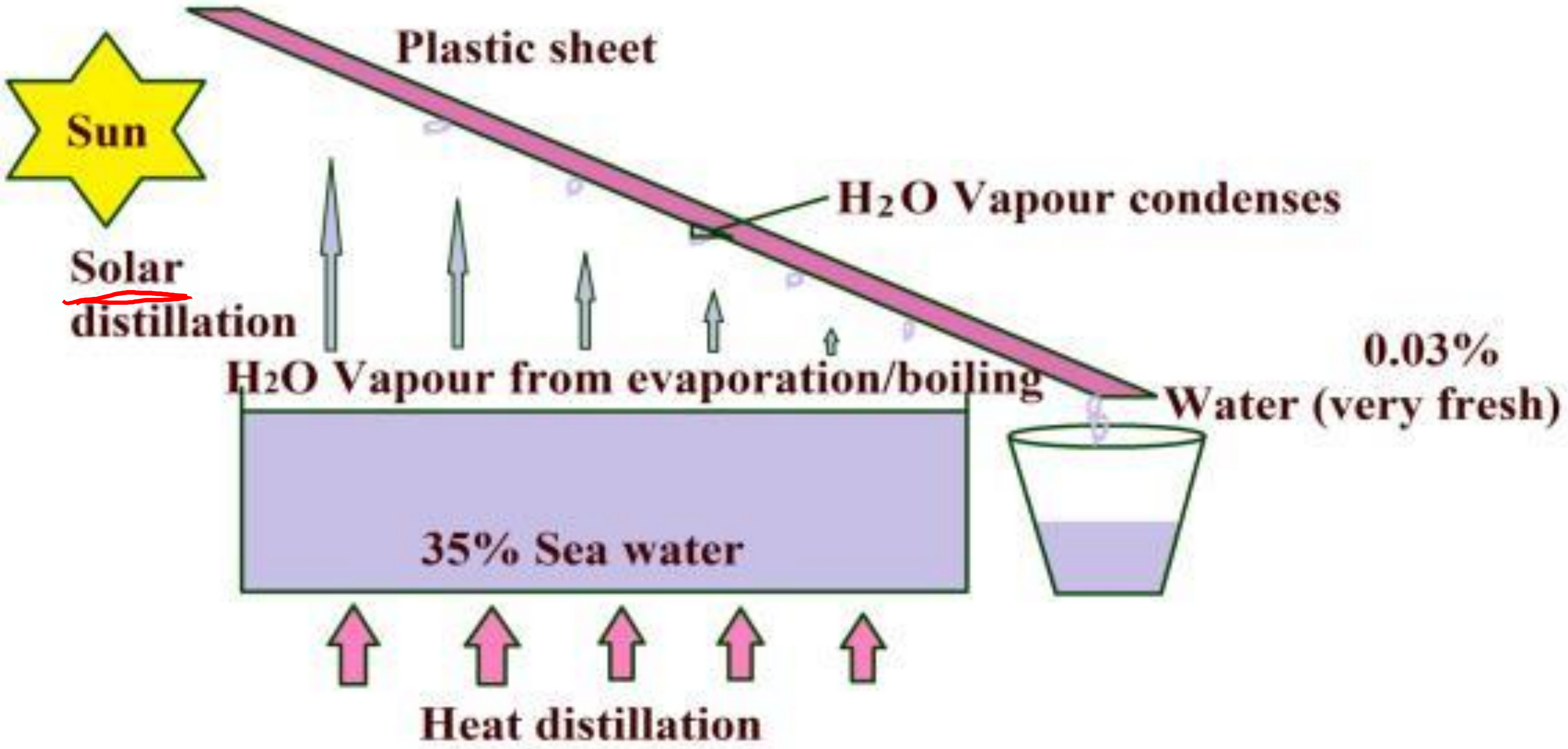
Drinking Water Treatment – Other options

Boil

Drinking Water Treatment – Other options

Distillation ... earliest probably 1200BC in Mesopotamia

- Solar distillation



Panchal, 2012

Several barriers to protect end-user

- Source water protection
- Water plant process and disinfection
- Distribution system residual disinfection
- Security

Water Distribution Systems and Biofilms

- Dissolved organic compounds can cause problems – taste, odor, enhanced chlorine demand, and bacterial colonization (biofilms)
- Biofilms can have aerobic and anaerobic zones
- Biofilms controlled by temperature, water hardness, pH, redox potential, dissolved carbon and residual disinfectant.
- <u>**Microorganisms more resistant to disinfectants in biofilms**</u>
- Heterotrophic plate count can be used to indicate deteriorating water quality in the distribution system
- Water-based pathogens such as *Legionella* can form biofilms

Typical water that leave treatment plant HPC: 10 CFU/ml

Average household tap has HPC: ~3,000 HPC /ml (Pepper et al., 2004)

Summary

- Health risk from a wide array of microbial pathogens may exist if drinking water is sourced and used untreated from the GWUDI wells.
- Indicator bacteria concept and its shortcomings
- Drinking water treatment options
- Biofilms in the distribution systems