Microorganisms in Freshwaters

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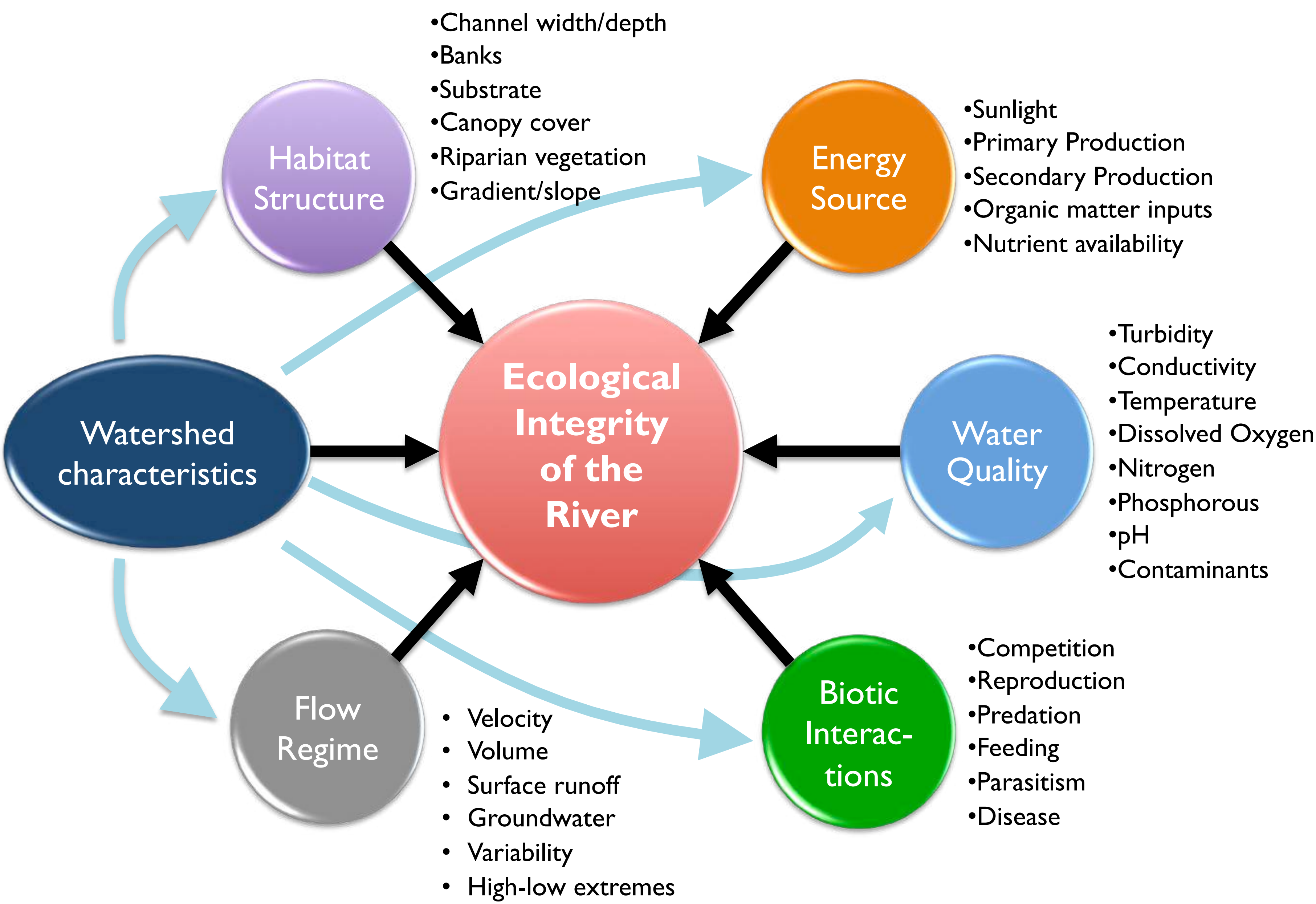
STROUD
WATER RESEARCH CENTER



•Channel width/depth
•Banks
•Substrate
•Canopy cover
•Riparian vegetation
•Gradient/slope
Habitat Structure
Energy Source
•Sunlight
•Primary Production
•Secondary Production
•Organic matter inputs
•Nutrient availability
Watershed characteristics
Ecological Integrity of the River
Water Quality
•Turbidity
•Conductivity
•Temperature
•Dissolved Oxygen
•Nitrogen
•Phosphorous
•pH
•Contaminants
Flow Regime
• Velocity
• Volume
• Surface runoff
• Groundwater
• Variability
• High-low extremes
Biotic Interac-tions
•Competition
•Reproduction
•Predation
•Feeding
•Parasitism
•Disease

Outline

- **Introduction**
  - Microorganisms (abundance, mass, type, size, diversity etc.)
- **Microbes in natural watersheds and their significance**
  - Microbes in natural watersheds (planktonic and biofilms)
  - Ecological significance
  - Pathogens and potential public health concerns
  - Microbial health in streams and fecal indicator bacteria
- **Agriculture and urban impacts**
  - Sources (agriculture vs. urban)
  - Prevention and treatment
- **Effects and efficacy of remediation and restoration (case studies)**
  - BMPs (United Water/Suez)
  - Collaborative efforts (Yellow Water River and Upper Fishtrap Creek)

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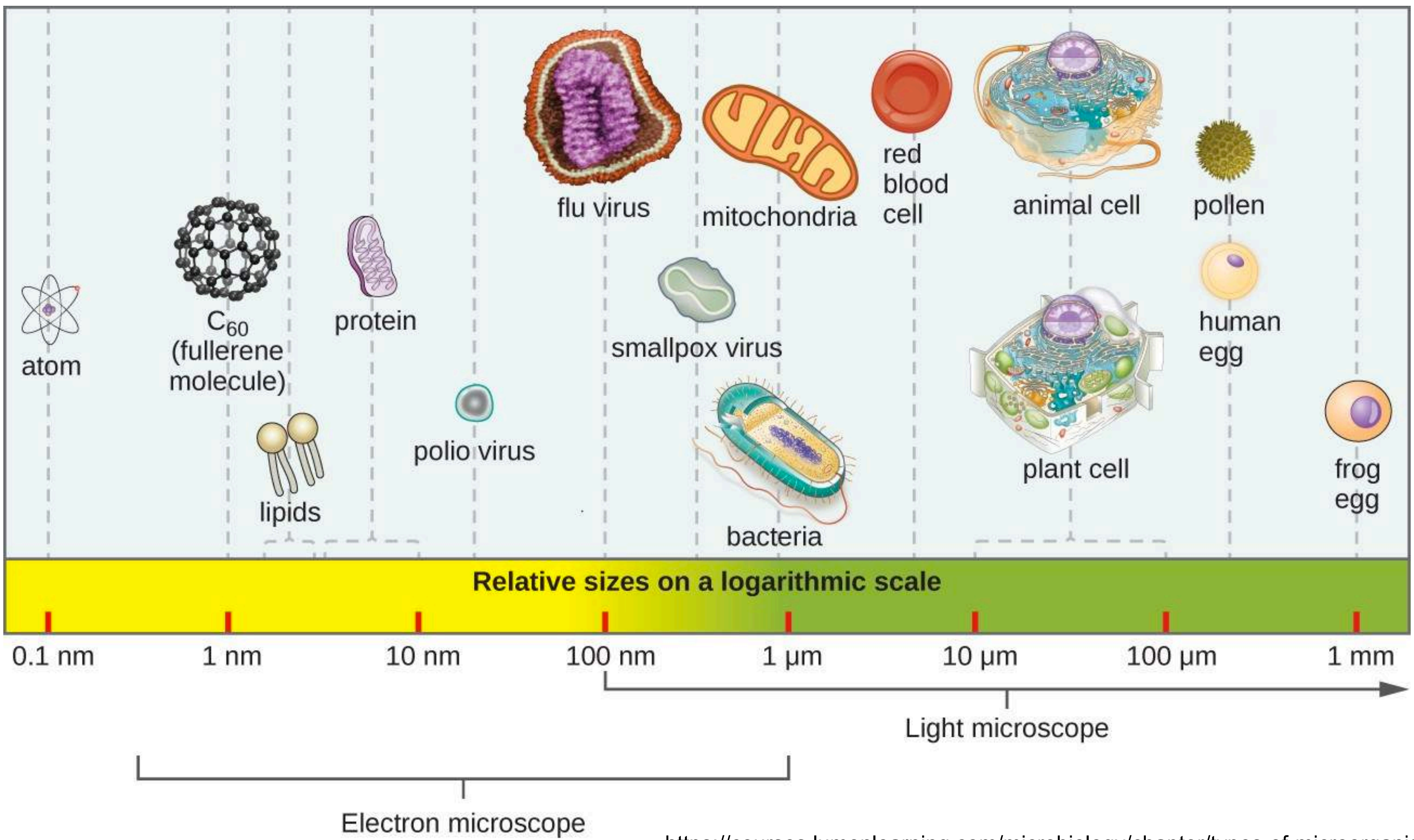
Micro-organisms

Microscopic; Too small to be seen by unaided eyes

Living organisms, independent



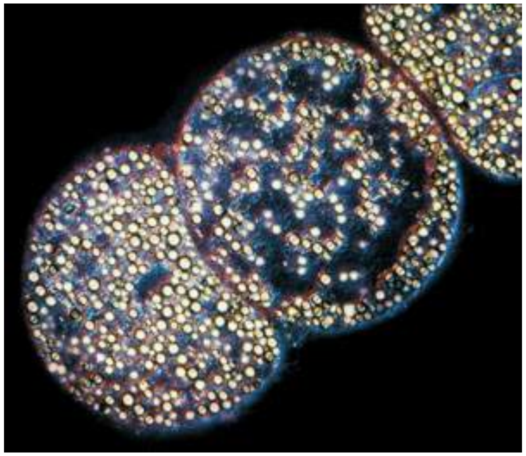
https://courses.lumenlearning.com/microbiology/chapter/types-of-microorganisms/

Different Size

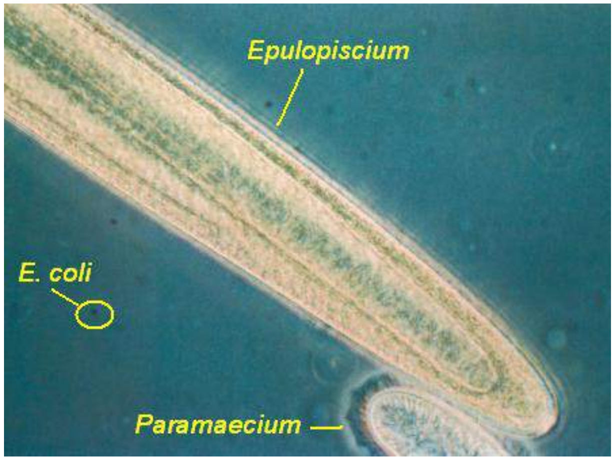
Individual cells: Cocci-most 0.1-0.2 µm in diameter

***E. coli/Bacillus*: 0.2 µm wide, 1-5 µm long.**

A few unusually large cells:



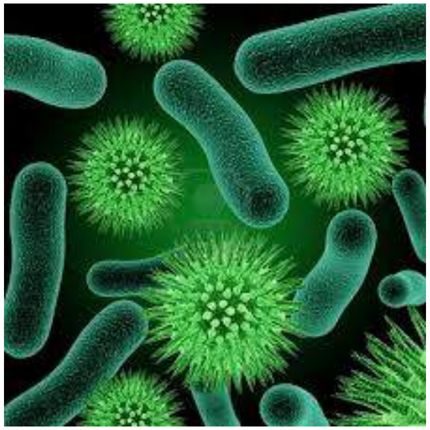
Thiomargarita namibiensis
(100-300 µm in diameter)

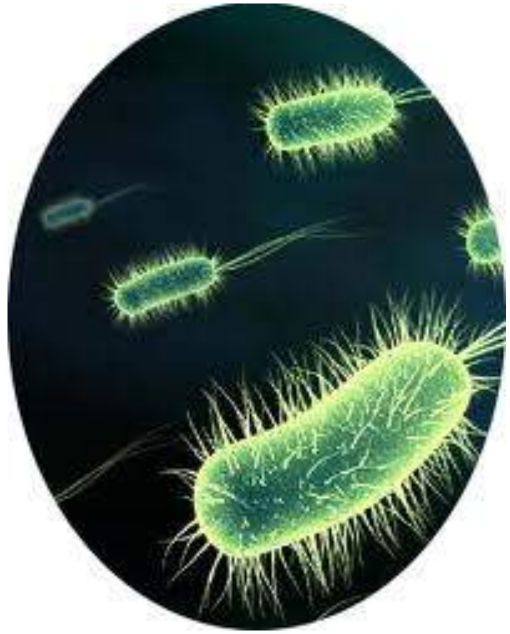


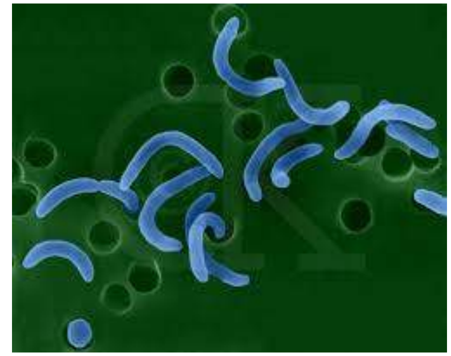
Epulopiscium fishelsoni
(80 µm *dia*, 200-700 µm long)

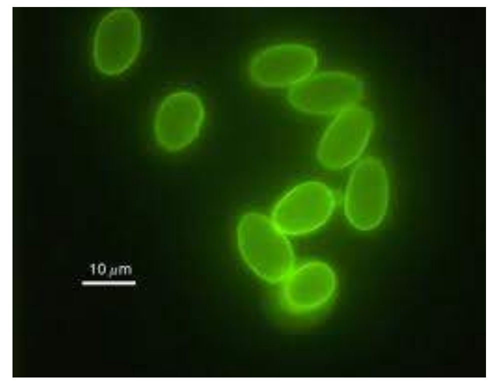
Some form filaments, some in sheaths

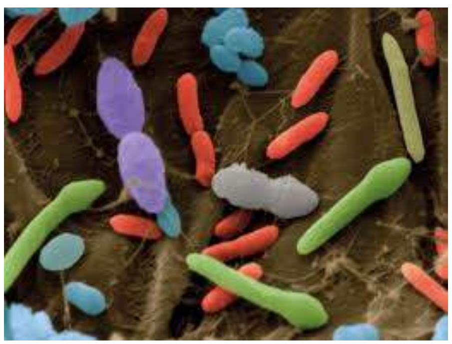
Different type

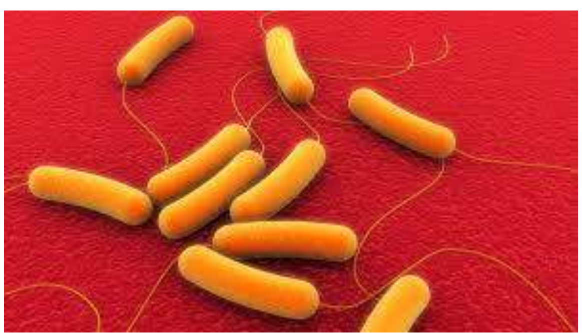


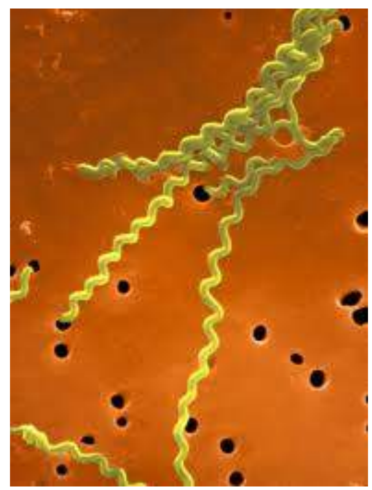






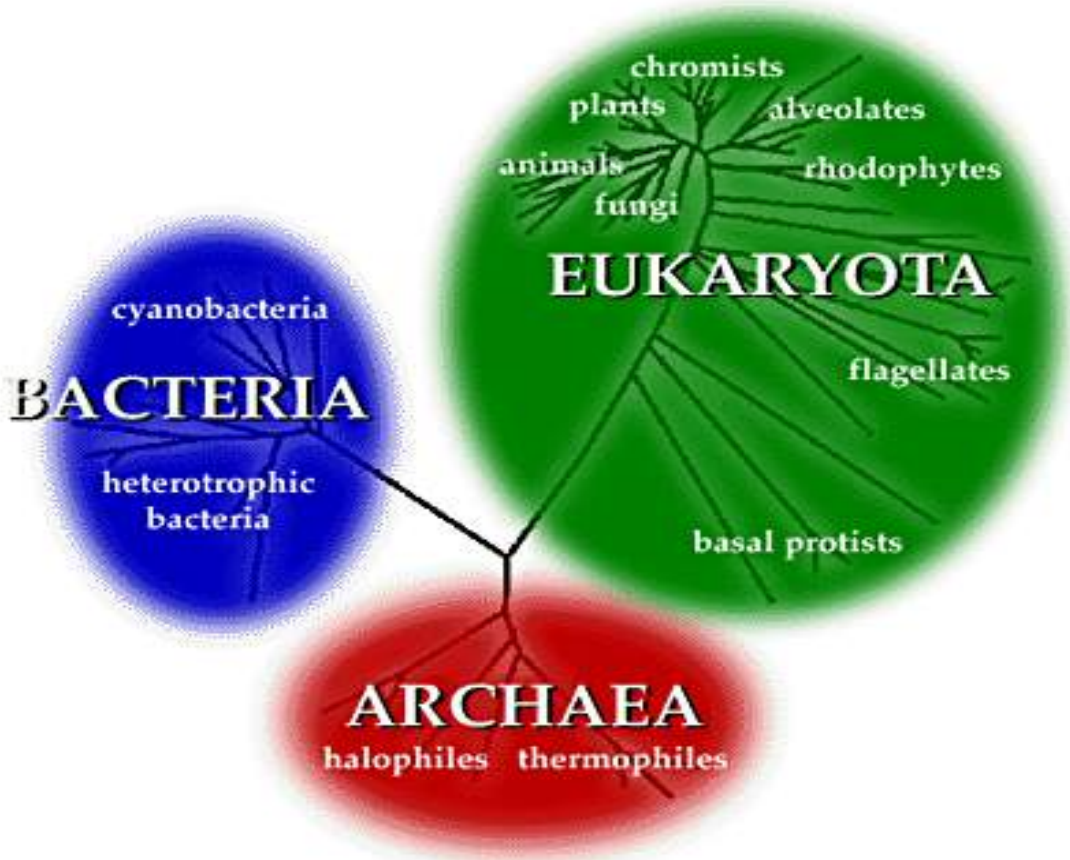






Miscellaneous online resources

High diversity



Bacteria + Archaea: 90 million

vs. Eukaryota: ~8.7 ± 1.3 million

Woese and Fox, 1977; Pace, 1990; Woese et al., 1990; Mora et al. 2011

Abundance and Mass

Earth is a microbial planet

Population size:

Human: 7.73 × 10^9 (estimated Sep 2019)

Microbes: 1.2 × 10^{31} (including in both water and soils)

~ 1.5 × 10^{20} microbes for every human

Biomass

Humans (@70 kg) = 0.05 Gt C

Microbes = ~ 77.2 Gt C

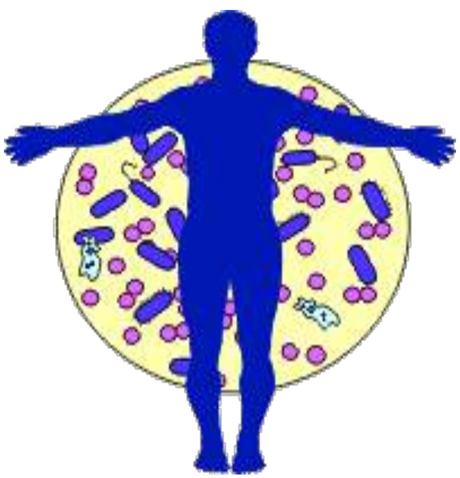
Microbes "outweigh" humans ~ > 1,500 to 1

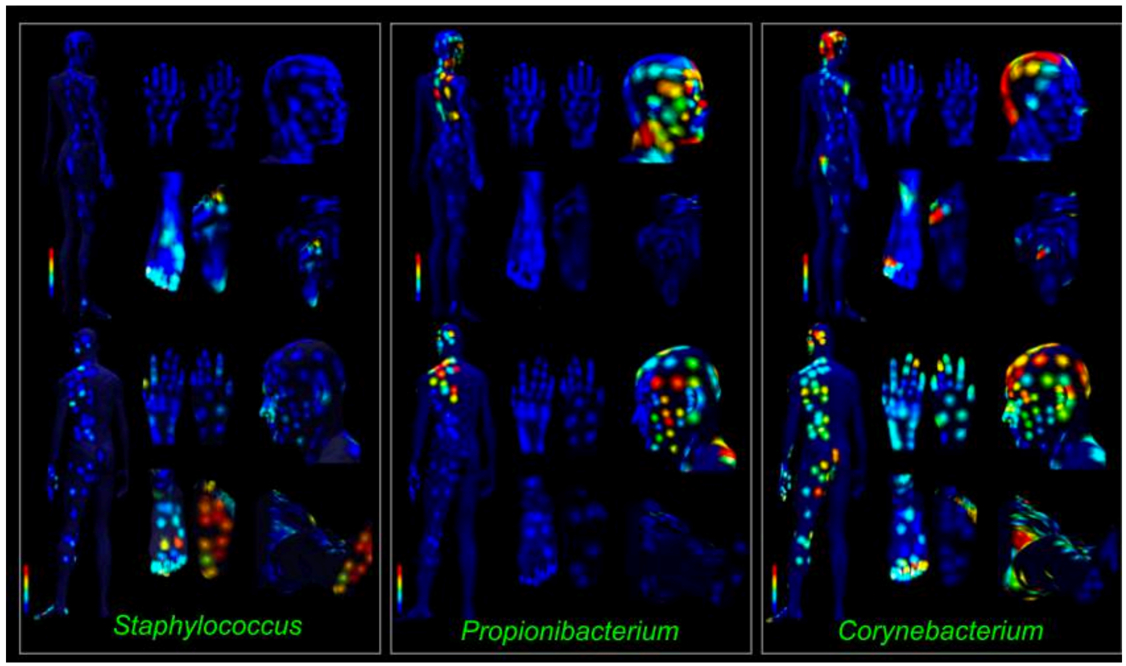
Microbes are second largest pool of living C (after plants) and the largest pool of living N and P

Whitman et al. 1998. PNAS. 95:6578-83

Human microbiome: a good analogy

Human is a microbial "body"





Bouslimani et al. 2015

Cells in human body: 37.2 trillion; Bacteria: 1-10 times more

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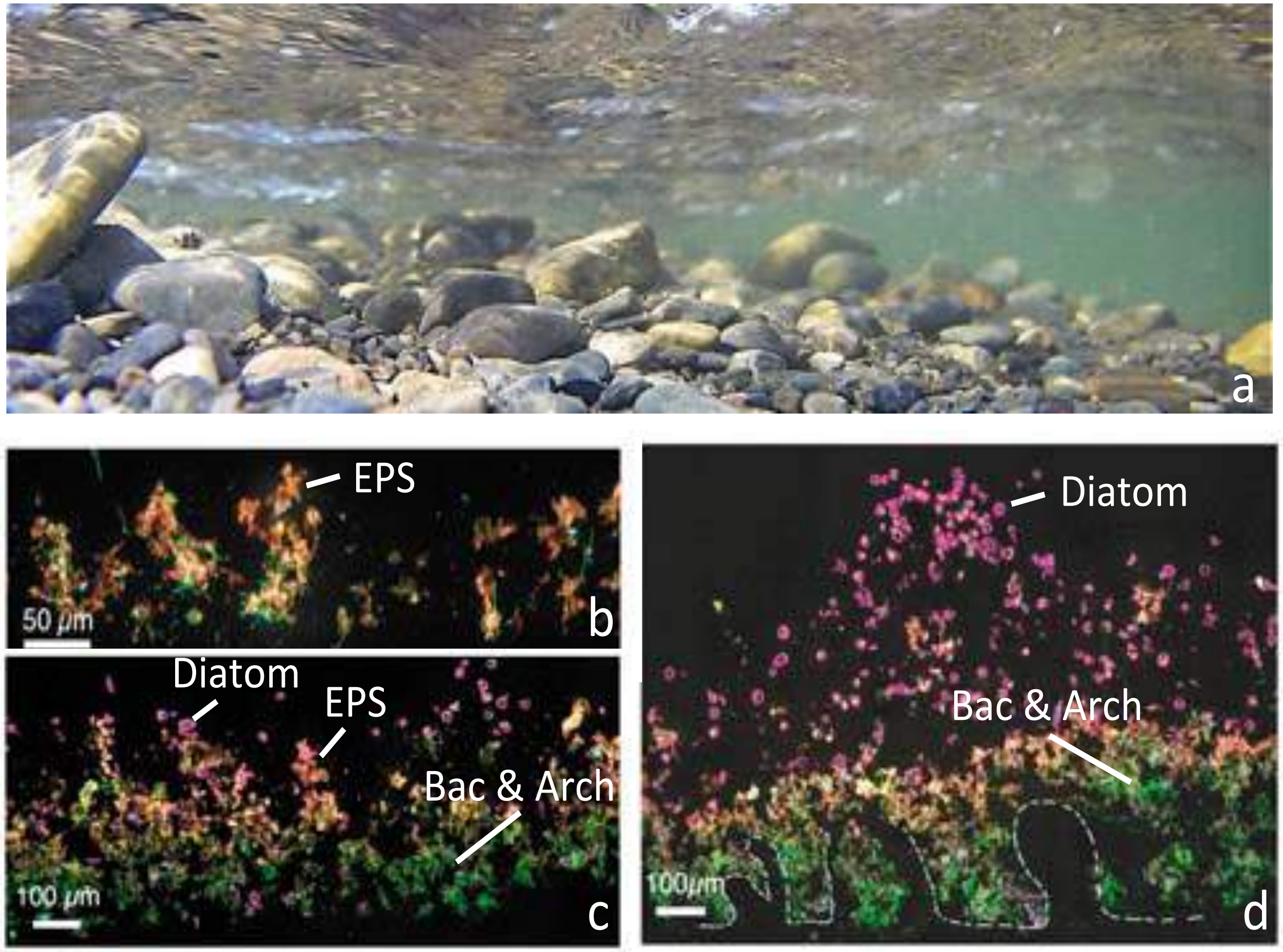
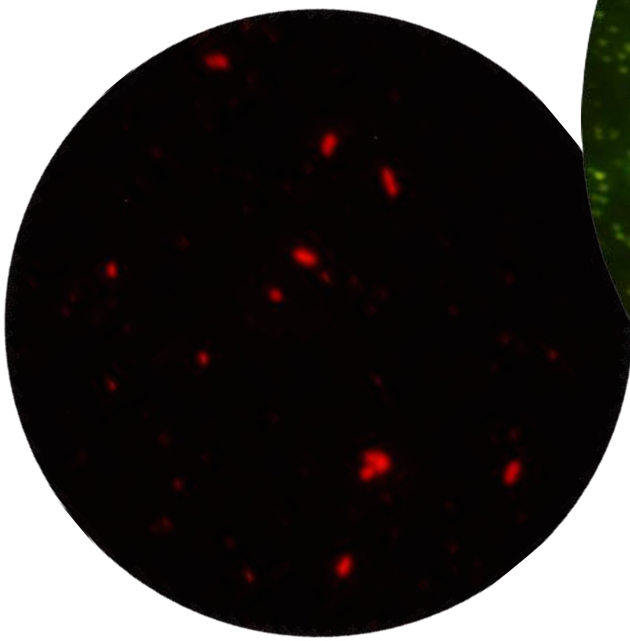
Microbes living in freshwater



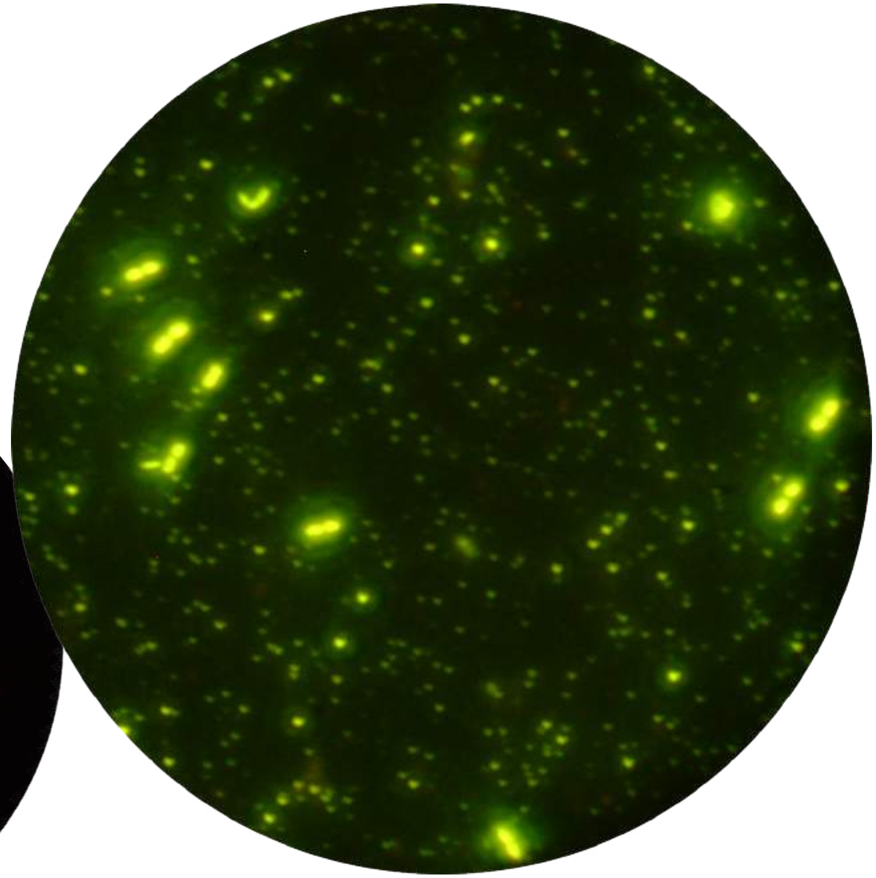
Planktonic (free-floating) *vs.* Benthic (biofilm); 1 million cells/ml water

Battin et al. 2003

Under microscope

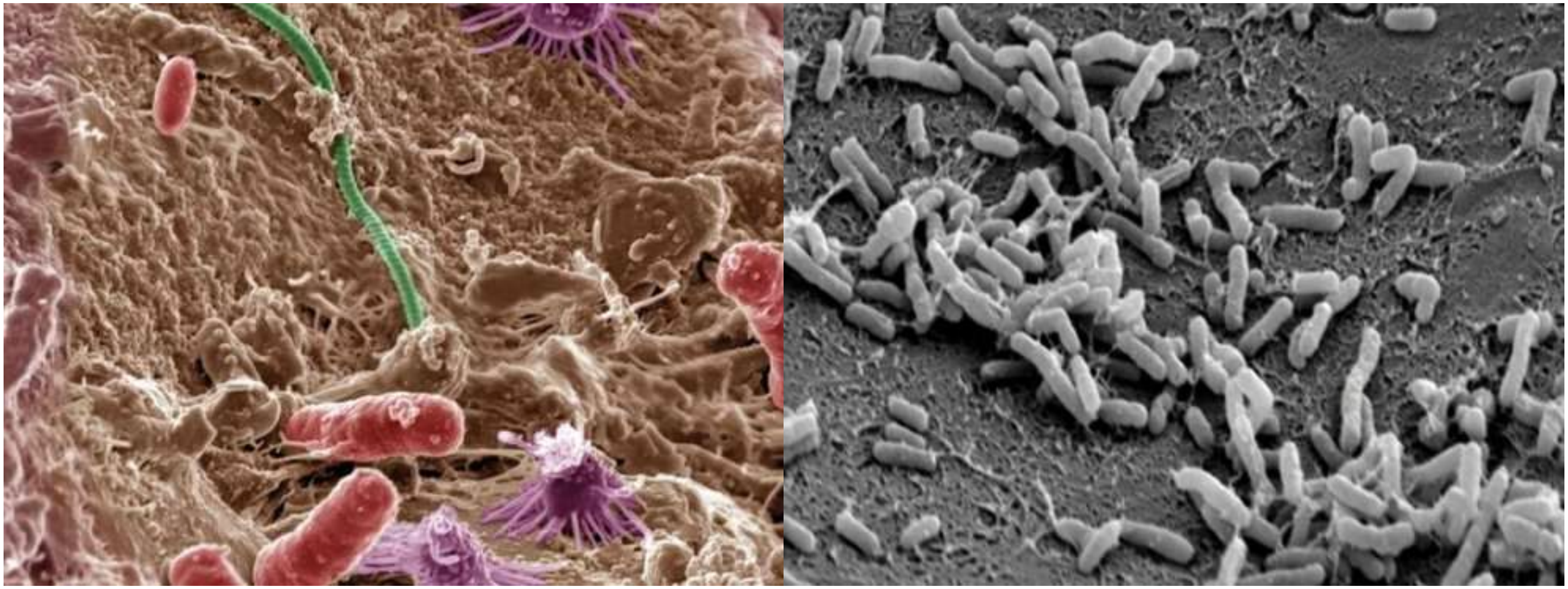


Photosynthetic pigments



Epifluorescence (DNA staining)

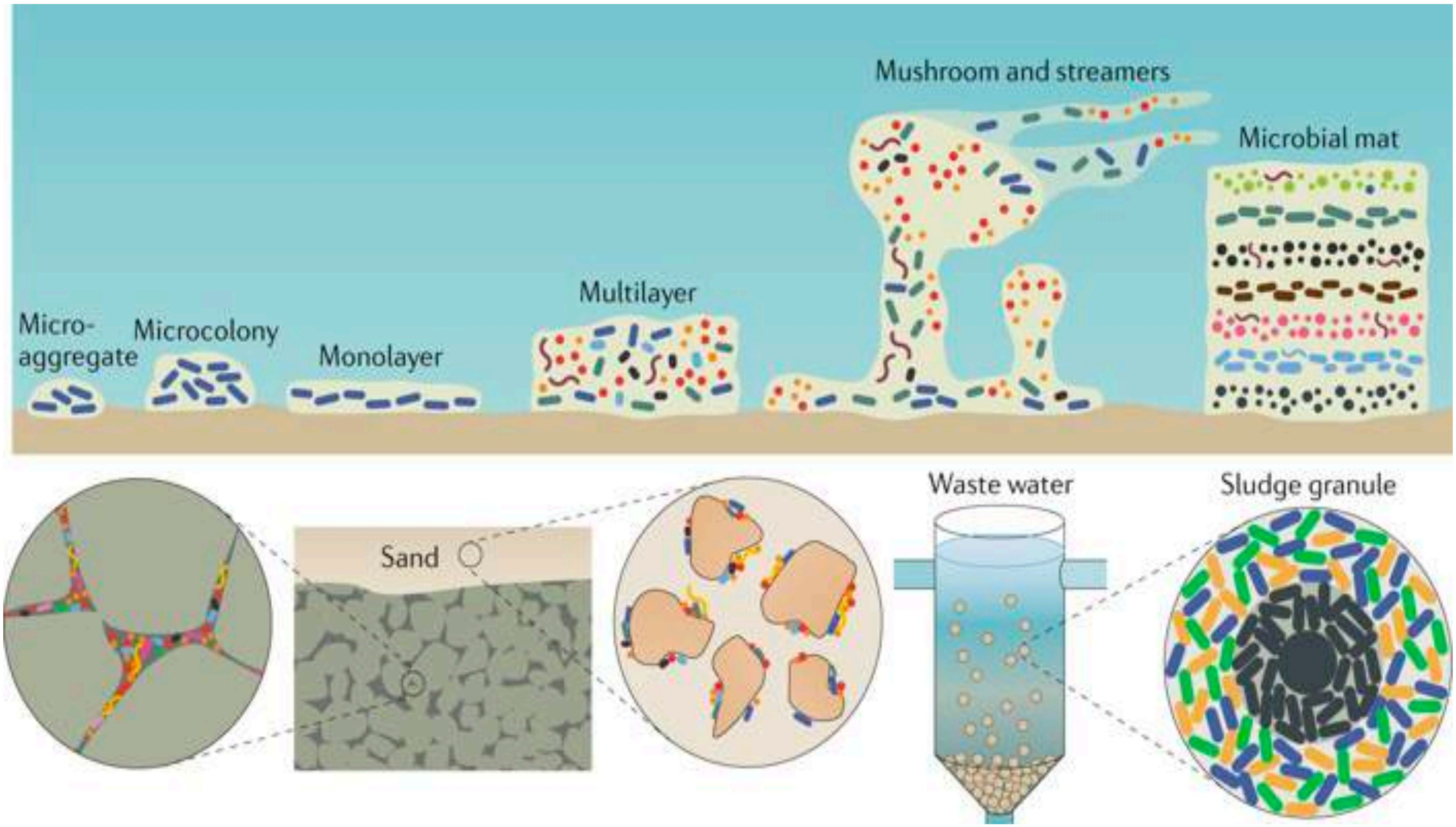
Microbes living in soil/sediments



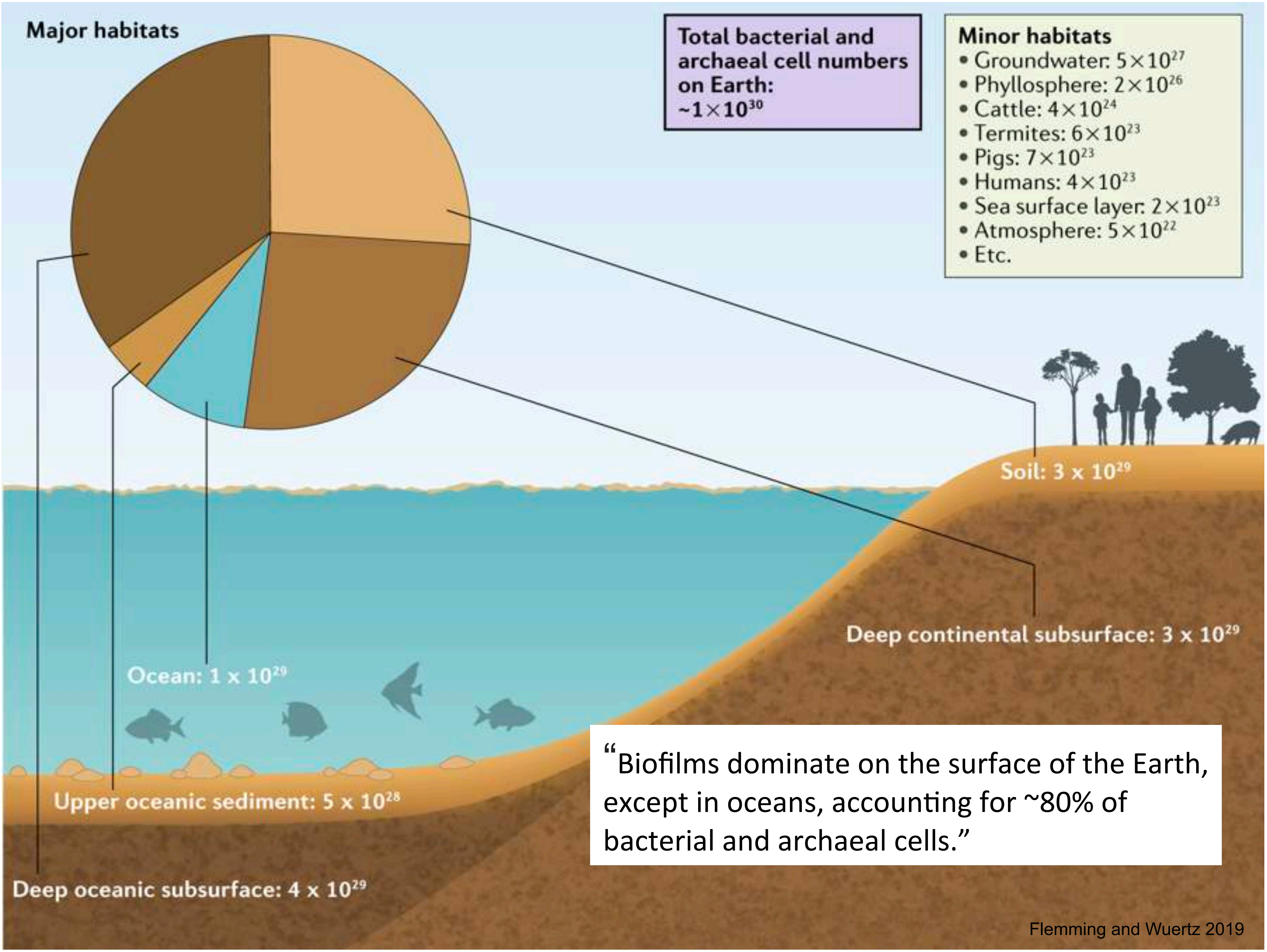
Microbes growing in soils; up to 1 billion cells/g dry soil

https://www.pitchcare.com/news-media/functioning-healthy-soil.html

Biofilms: “microbial skin”



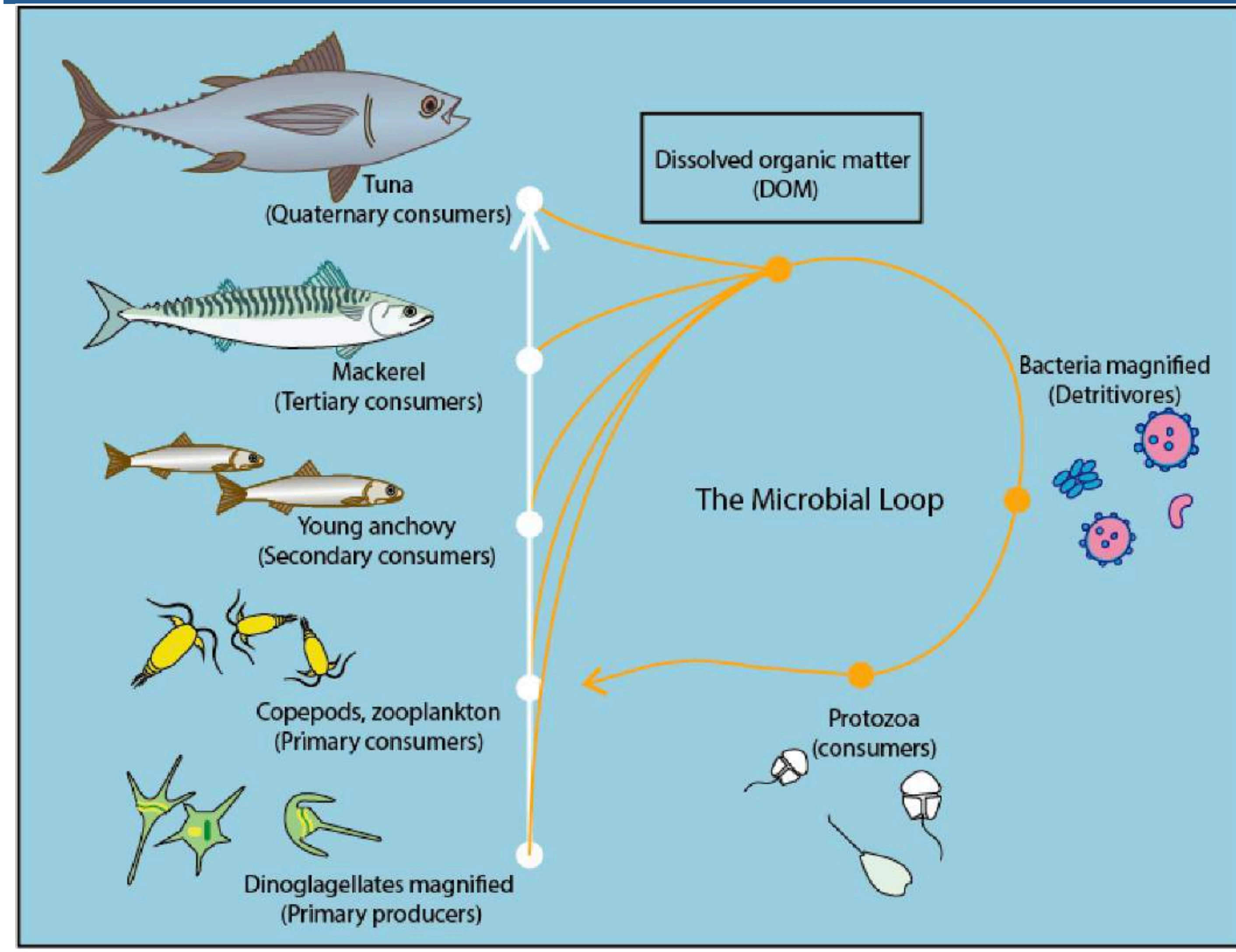
Flemming and Wuertz 2019



"Biofilms dominate on the surface of the Earth, except in oceans, accounting for ~80% of bacterial and archaeal cells."

Flemming and Wuertz 2019

Significance in ecosystems

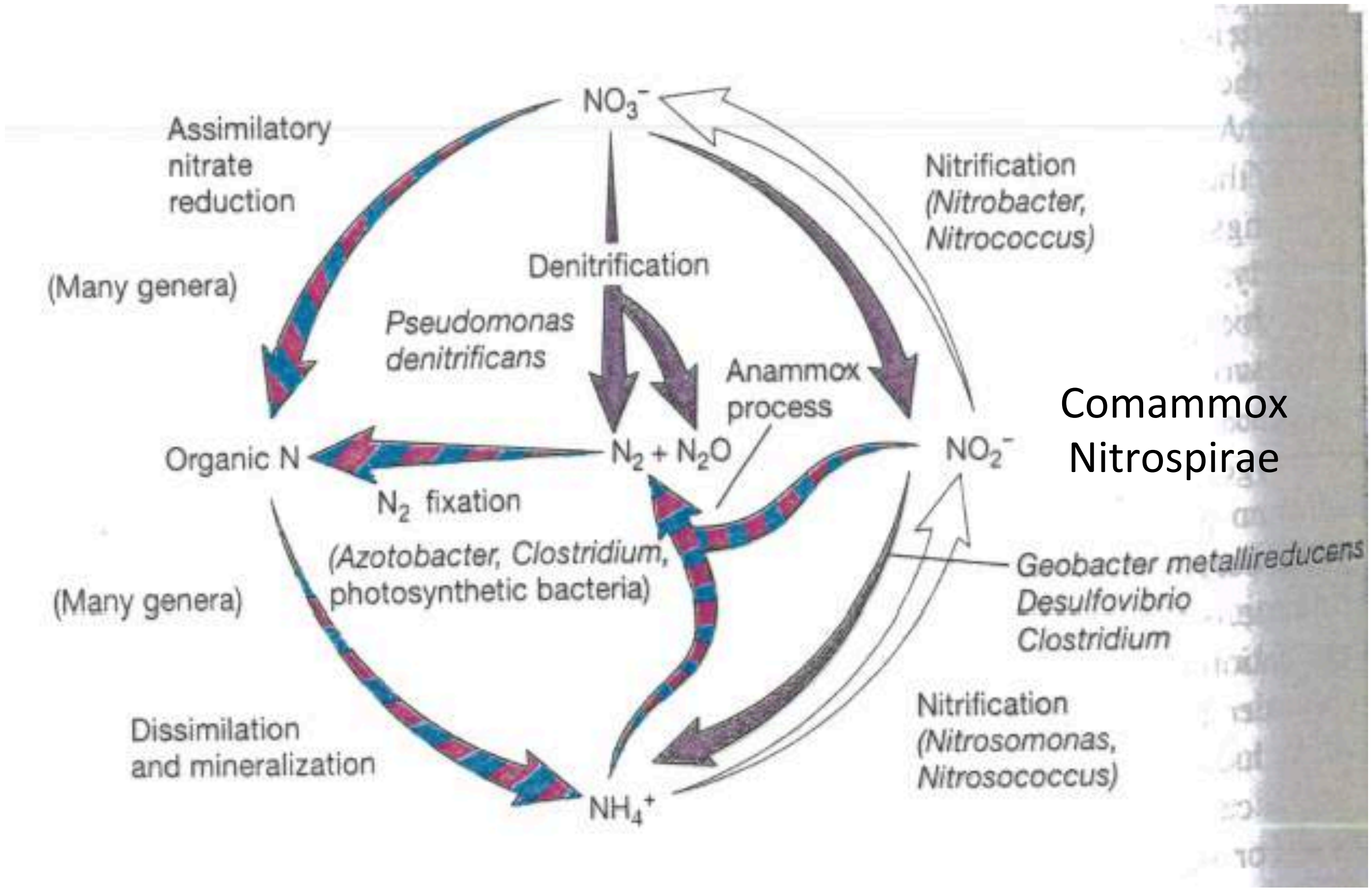


Food web and transformation of dissolved organic matter (microbial loop)

http://www.plankton-bloom.com/ocean-processes/marine-snow/

Nutrient cycling

Significance: Transformation of key elements including Carbon, Nitrogen, Phosphorus, Sulfur, etc.



Public Health Concerns

Water-representative taxa and problems

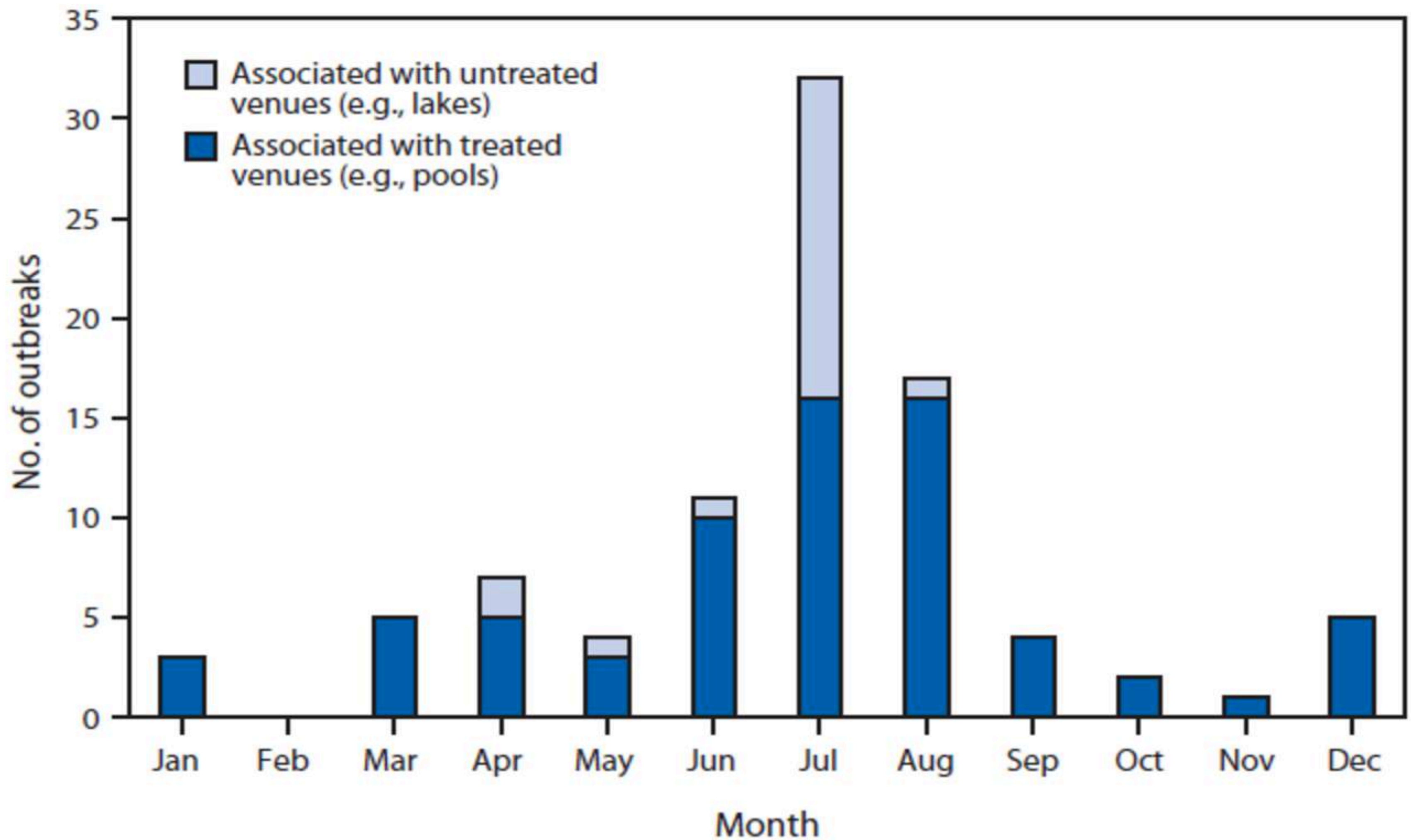
- *Pseudomonas aeruginosa*: ear infection, bathing beaches
- *Clostridium botulinum*: food poisoning
- *Legionella pneumophilia*: respiratory infection and death Legionaire's disease
- *Salmonella*, *Shigella*, *Vibrio* et al.:

  gi (gastrointestinal) tract, diarrhea and dysentery
- *Streptococcus*, *Vibrio vulnificus* et al.:

  Necrotizing fasciitis
- *Cryptosporidium*, *Giardia* etc.

Public Health Risk

- **May cause a variety of diseases**



Outbreaks of illness associated with recreational water (2011-2012)

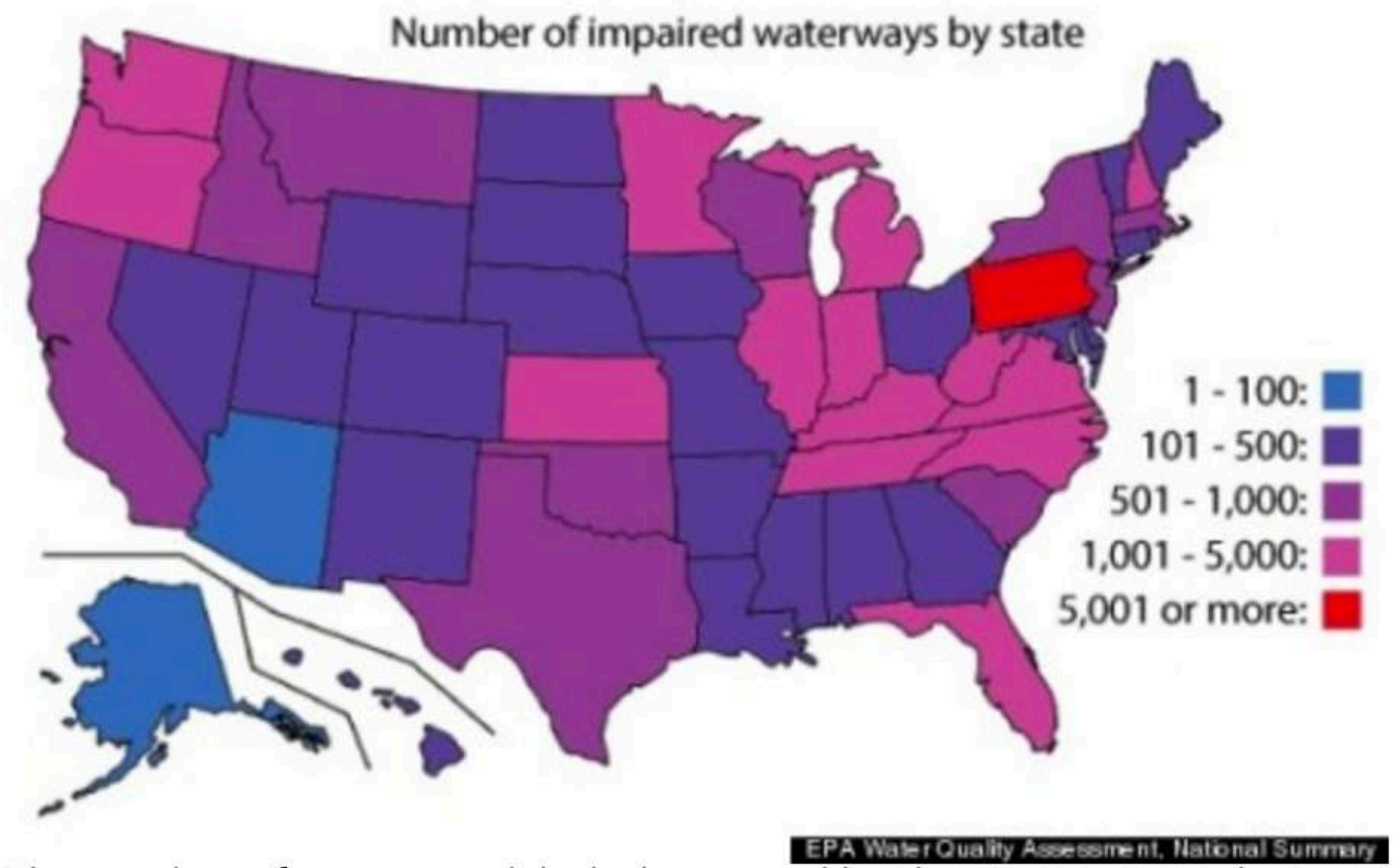
Hlavsa et al. 2015 CDC

Bacterial monitoring for water quality

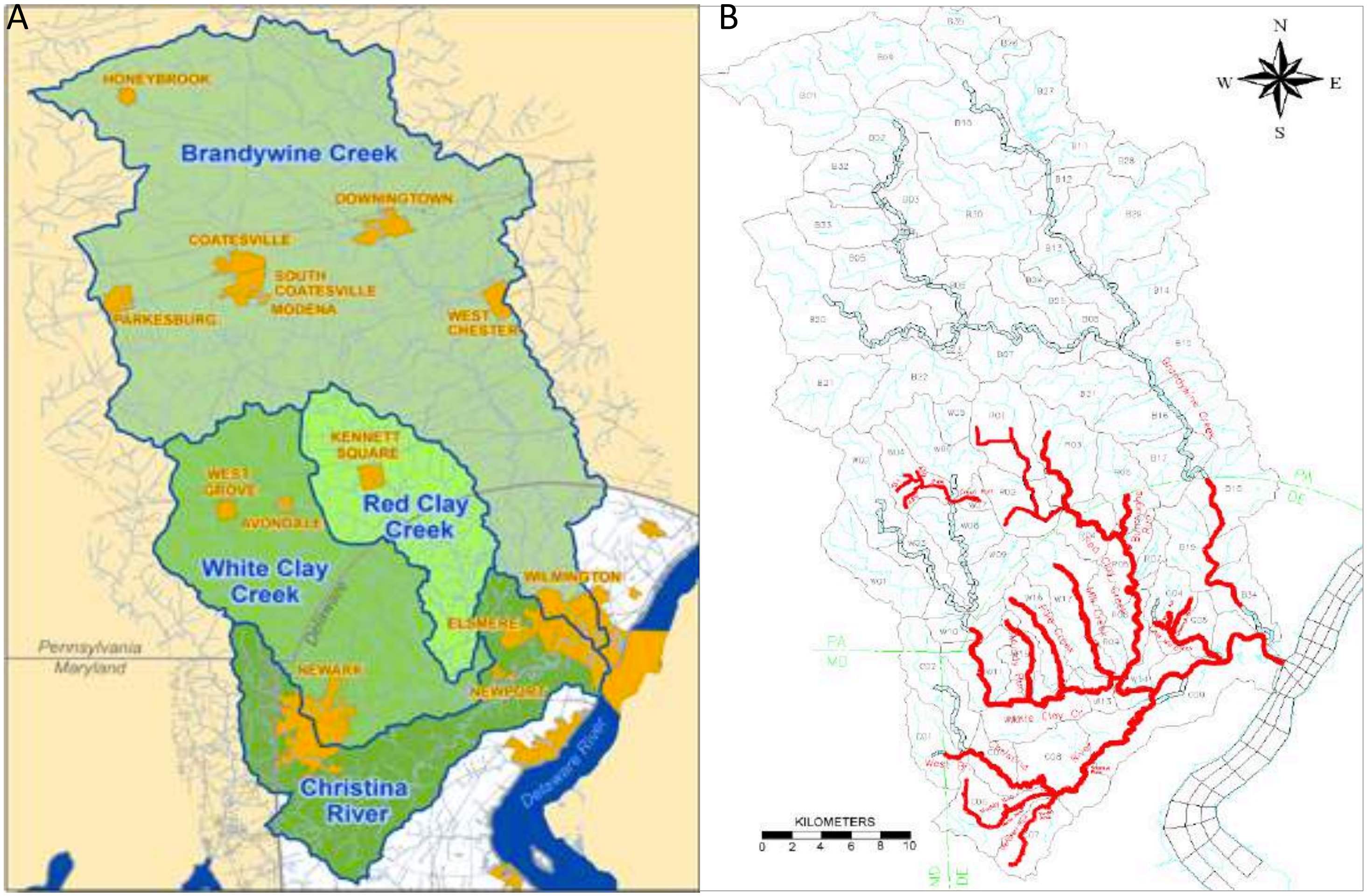
- **Clean Water Act (CWA): "to restore and maintain the chemical, physical, and biological integrity of the Nation's waters"**
- **Impaired waters and TMDL (Total Maximum Daily Load) program**
- **Among all the TMDLs (including nutrients, sediments etc.), microbial contaminants (e.g. pathogenic bacteria) are ranked No. 1 causes for water quality degradation (U.S. EPA).**

(39% rivers and streams, 13% of lakes, reservoirs and ponds; 30% of assessed bays and estuaries)

Impaired waterways in US



Brandywine-Christina Basin



A: The Brandywine-Christina Basin includes Brandywine, White Clay, Red Clay and Christina river subwatersheds (adapted from Water Resource Agency, Univ. of Delaware). B: Stream segments impaired by bacteria (highlighted in red) by PA DEP and DNREC (adapted from U.S. EPA Brandywine-Christina Basin nutrients and bacterial TMDL).

Fecal Indicator Bacteria monitoring

- Total coliform → Fecal coliform → *E. coli /Enterococcus*
  - Public health agencies have used total coliforms and fecal coliforms as indicators since 1920s
    - non-fecal origin bacterial groups
    - coliforms can regrow in natural environments
    - Still being used in many states and agencies
  - *E. coli* and *Enterococcus*
    - More specific bacterial groups
    - Commonly used in these days
    - Recommended by US EPA (2012)

What bacteria should I monitor?

Depends on what you want to know; Consumption vs. recreation

- **Health risk from recreational water contact**:

  Best indicators in freshwater*: E. coli* and *Enterococci*; for salt water, *Enterococci*

- **Water supply or meets state water quality standards**

  Total and/or fecal coliform

State Bacteria Monitoring

Water contact (WC): Swimming season- *E.coli* and fecal coliform; non-swimming season- fecal coliform.

Drinking Water supply (PWC): Total coliform

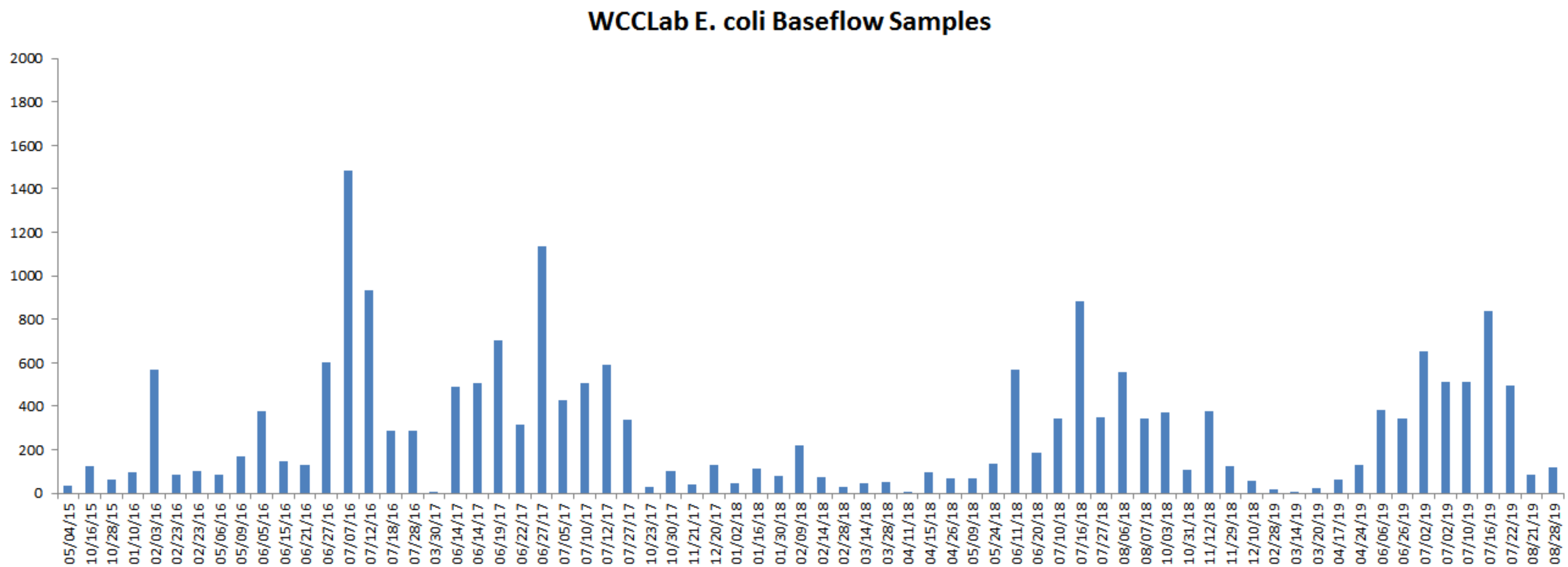
Total and fecal coliform

Primary: *E.coli* and *Entero*
Secondary: Fecal coliform

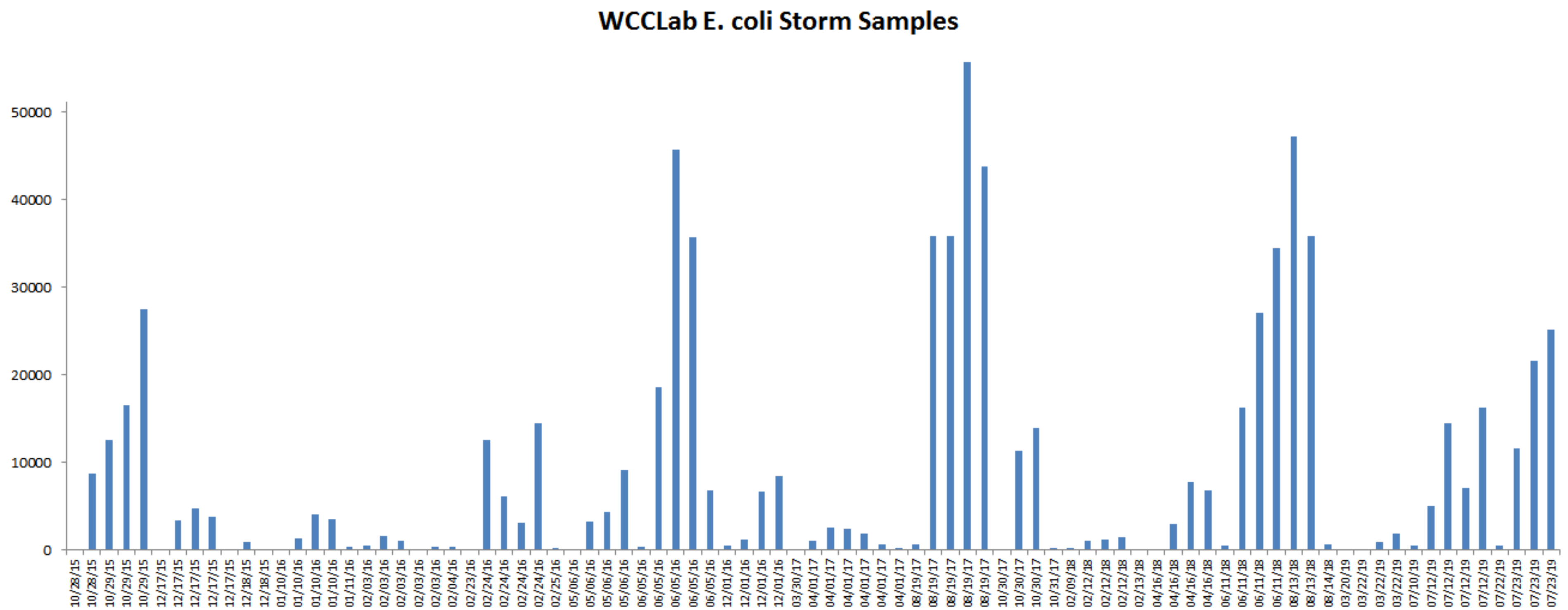
E.coli and *Entero*

Primary: *Entero*
Secondary: *Entero*

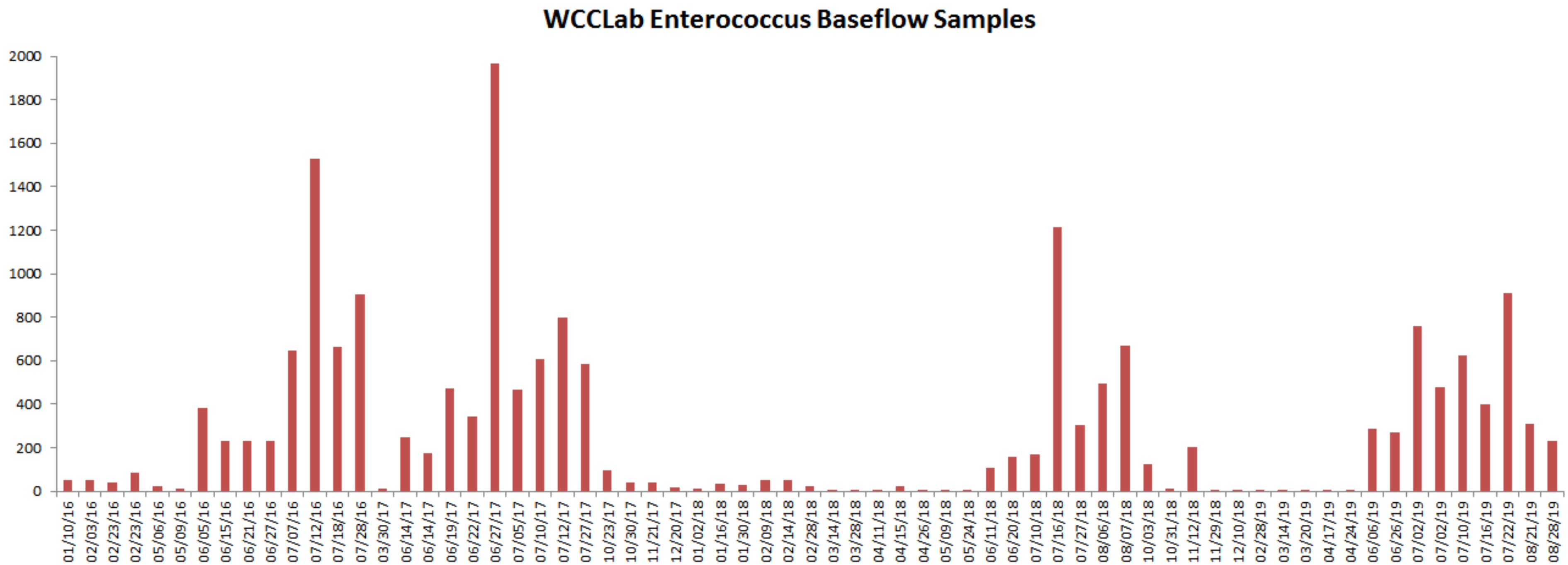
E. coli monitoring at WCC



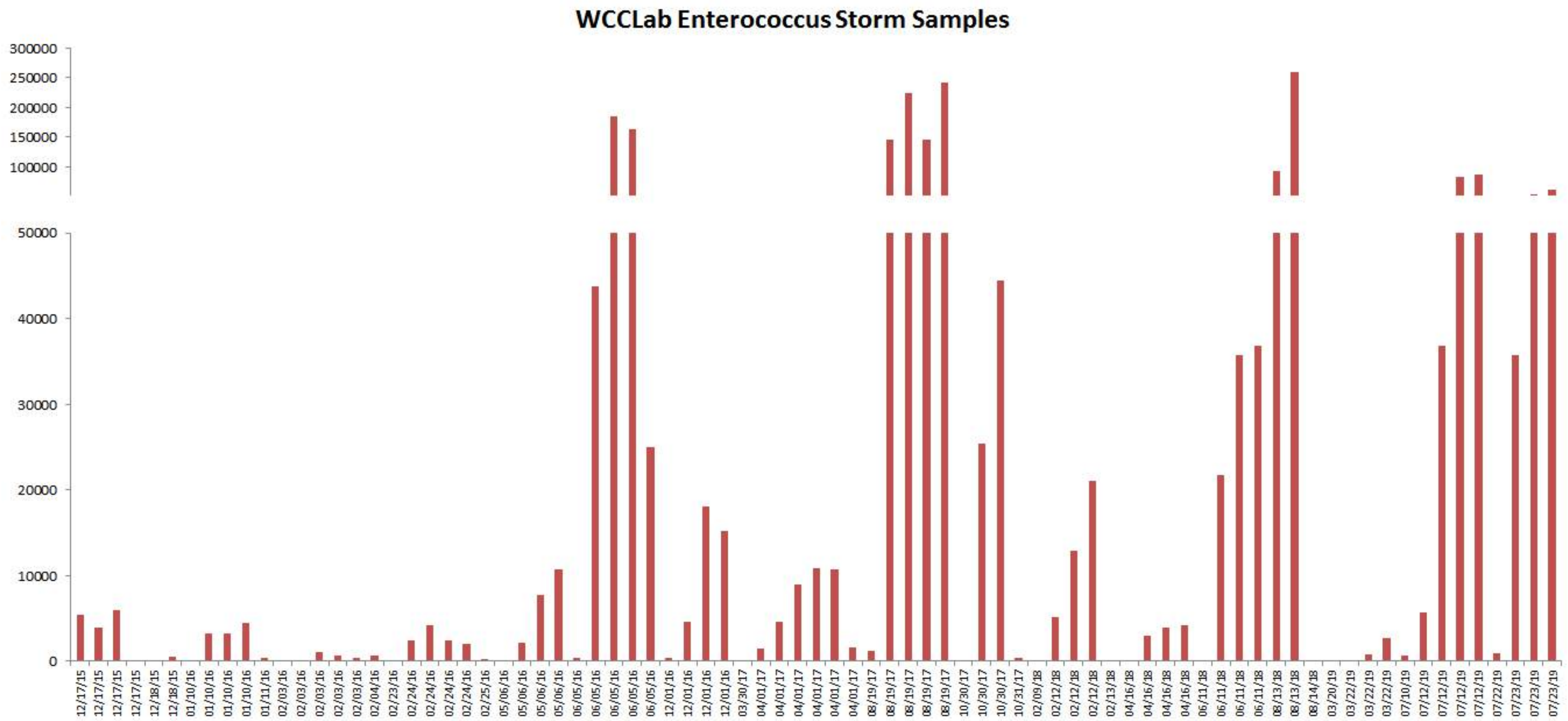
E. coli monitoring at WCC



Enterococci monitoring at WCC



Enterococci monitoring at WCC



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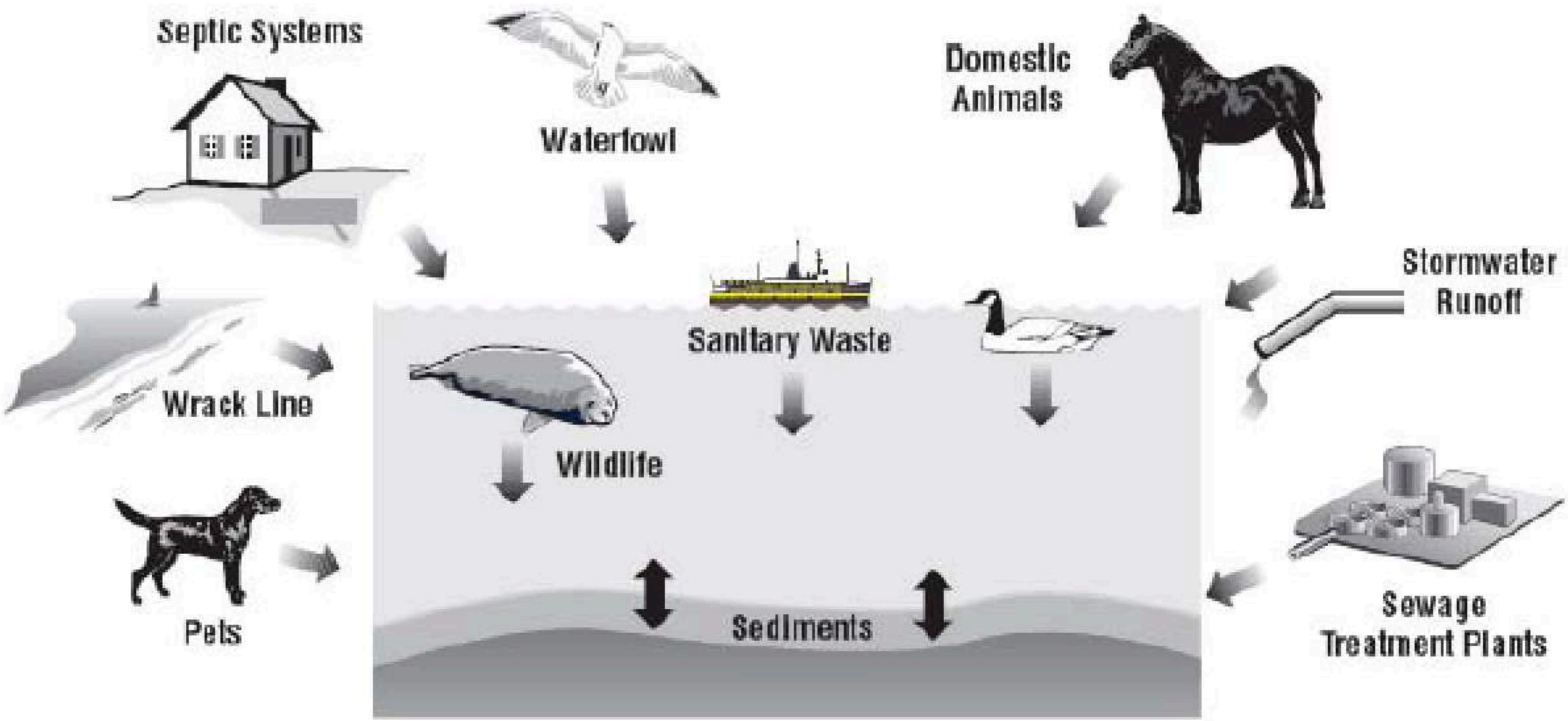
Agriculture and urban


Forested


Wastewater treatment plant

Source of bacterial contaminants



Point and non-point sources

Source of bacterial contaminants

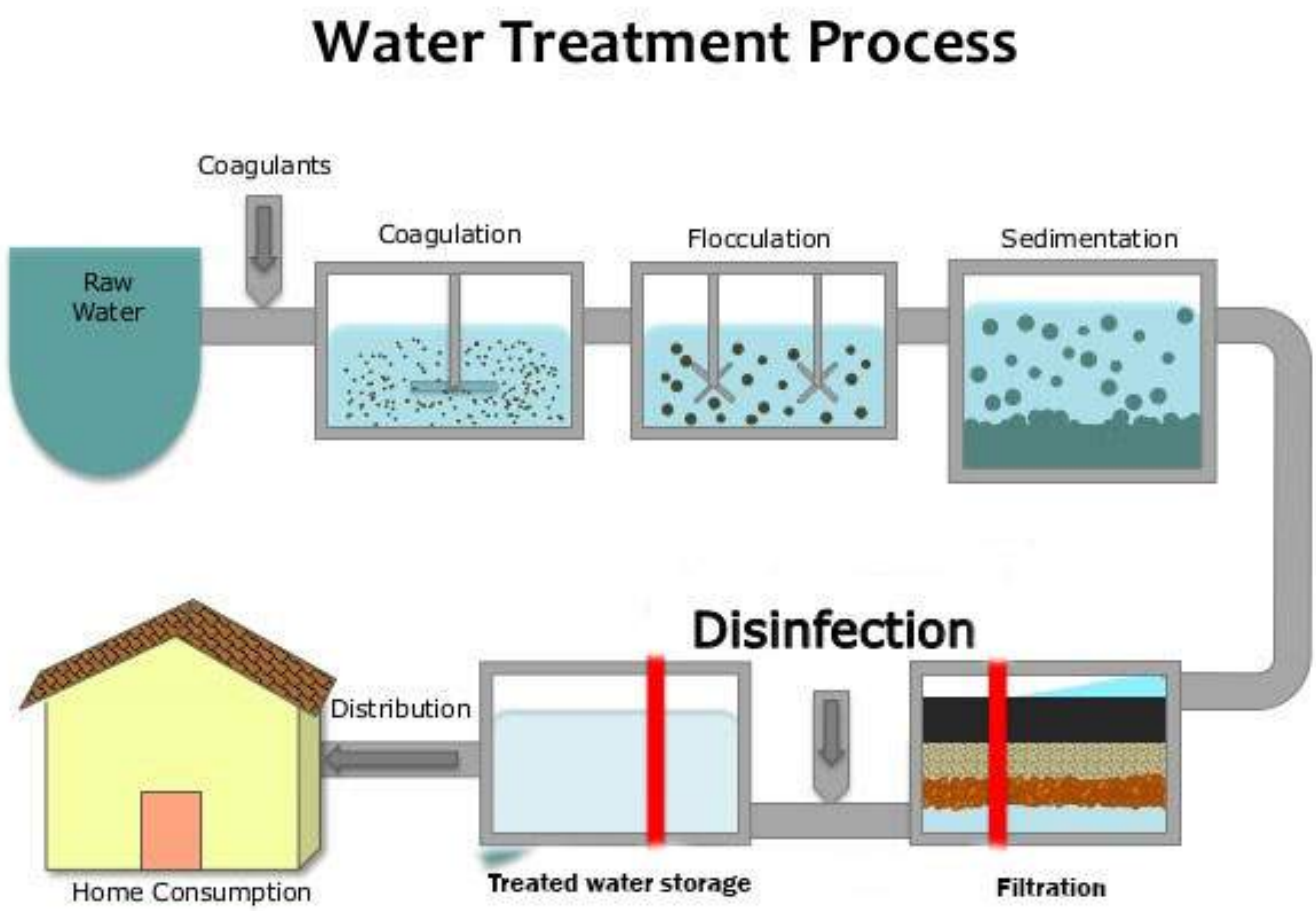
Sewage disposal systems: sewer breaks, sewer overflows, and sewer misconnections; on site septic systems

Agriculture: animal waste runoff, manure storage, vegetative buffer strips; livestock

Stormwater runoff: impervious surfaces, lacking catch basins and settling basins, inappropriate landscaping

Wildlife: birds and small mammals; direct contact or watershed runoff; *Giardia*, *Cyrptosporidium*, *Salmonella*, *Camphylobacter*, *E. coli* etc.

Water treatment



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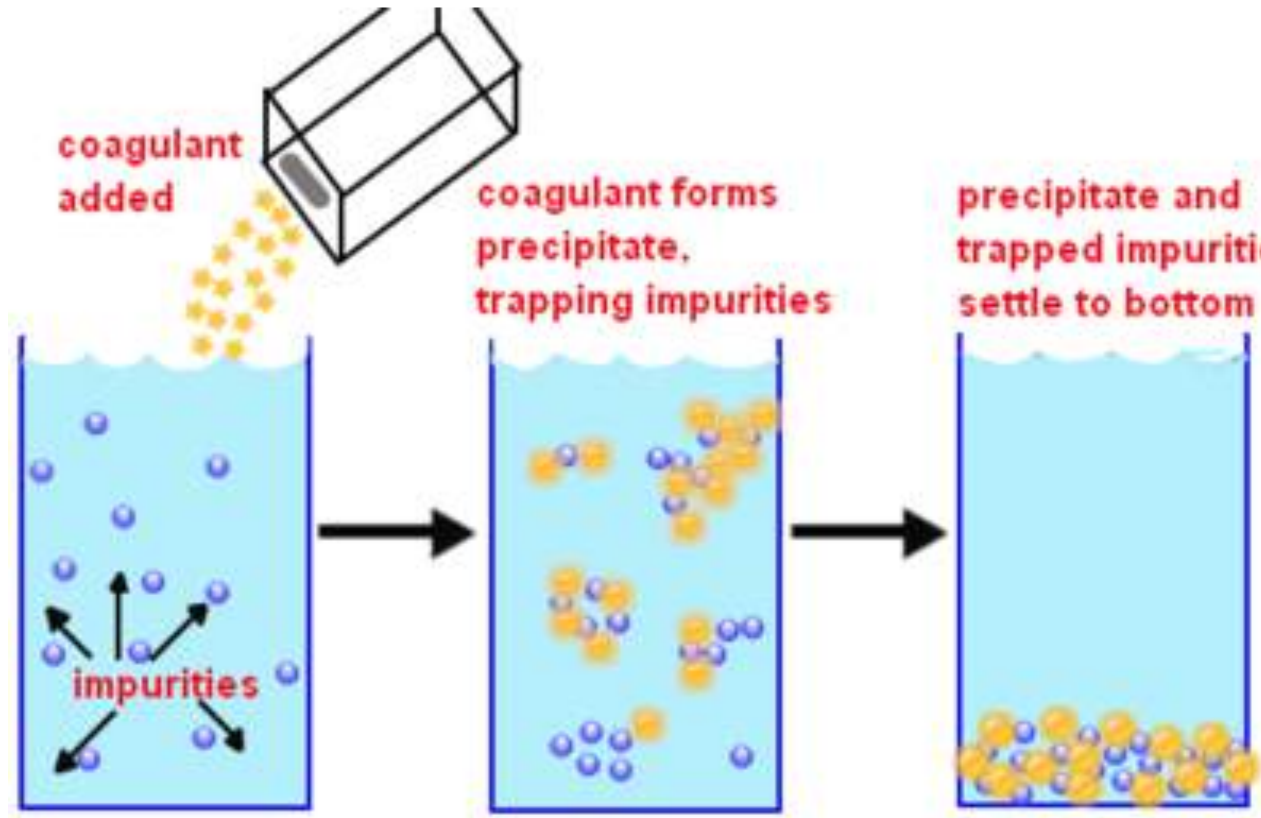
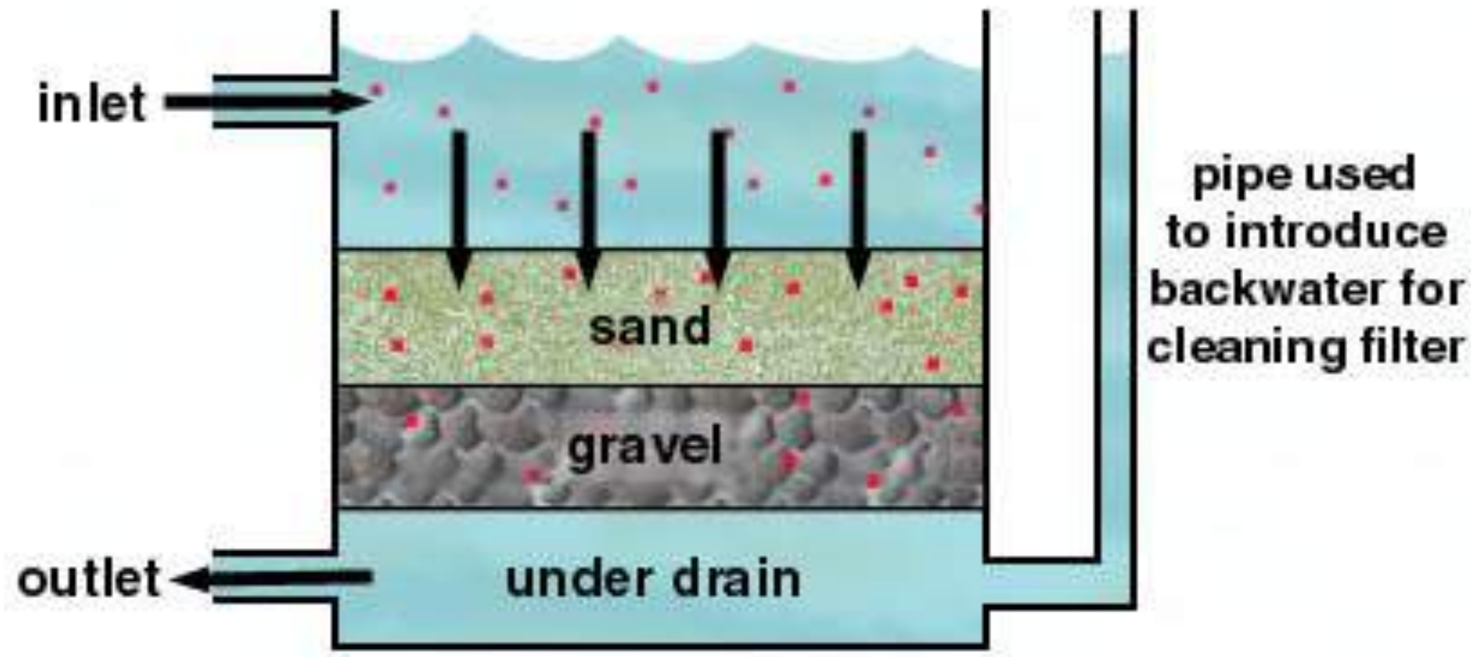


Coagulation

http://www.chemistry.wustl.edu/~edudev/LabTutorials/Water/PublicWaterSupply/PublicWaterSupply.html

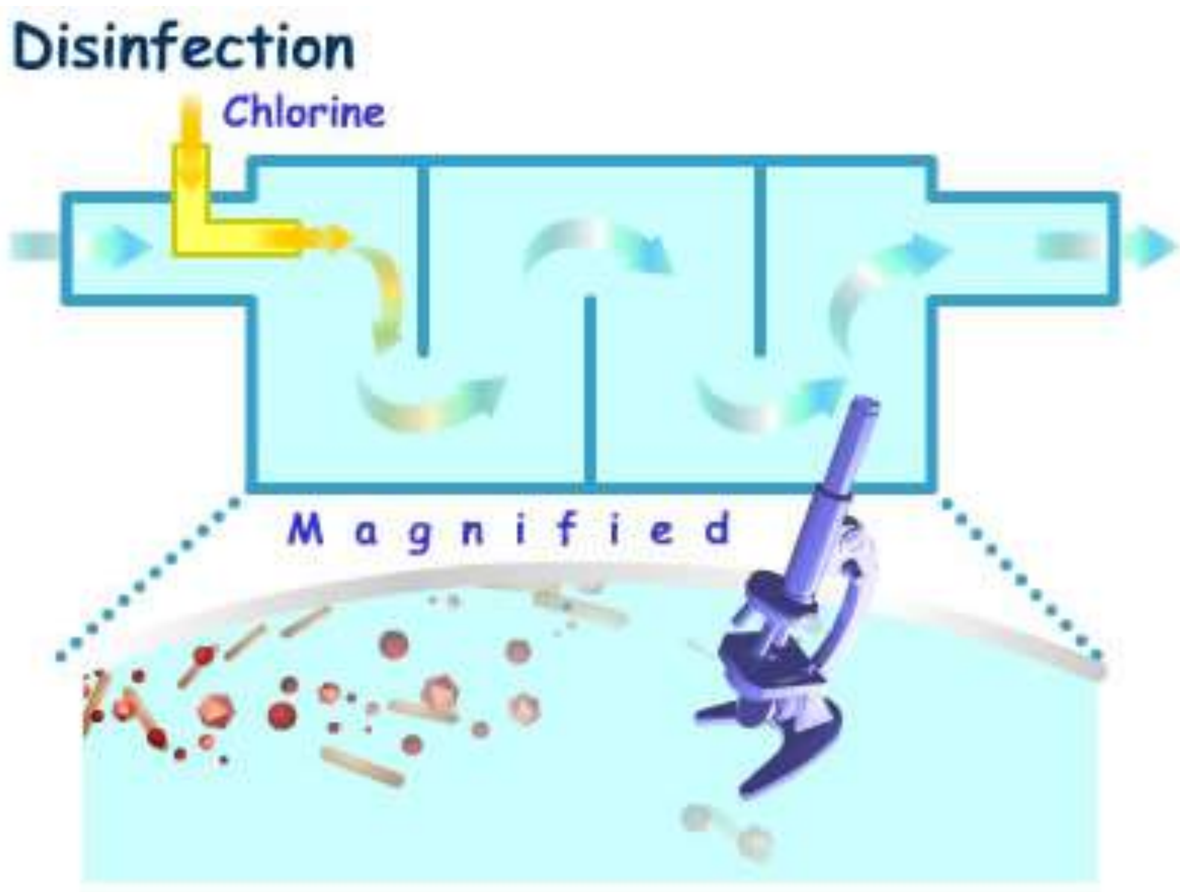
Water treatment



Filtration

http://www.chemistry.wustl.edu/~edudev/LabTutorials/Water/PublicWaterSupply/PublicWaterSupply.html

Water treatment



Disinfection

http://techalive.mtu.edu/meec/module03/Sources-SurfaceWater.htm

Prevention

Disinfection has proven effective and efficient against bacteria and enteric viruses, but protozoa such ***Giardia*** and especially ***Cryptosporidium*** are very resistant to chlorination alone!

The most important and cost effective protection for water suppliers is to **prevent** pathogen entry into source water.

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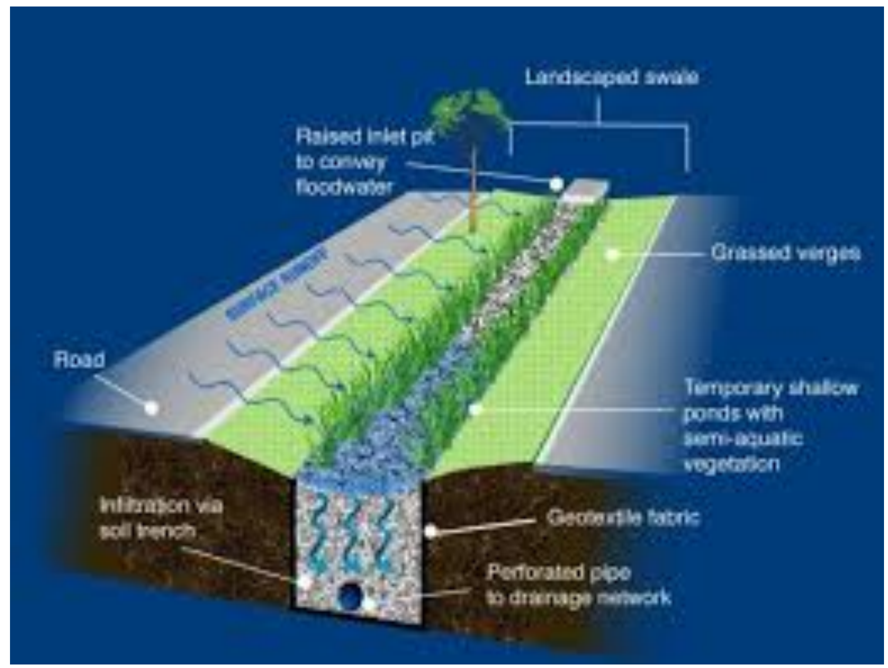
Remediation and restoration

Different approaches and BMPs

Restoration efforts: Pasture management, runoff management, riparian protection, manure management etc.

- **Buffer strips**
- **Constructed/storm water wetland**
- **Sand filters**
- **Retention/detention ponds**
- **Biofiltration**

Buffers can reduce bacteria by 43 to 57%, especially in agricultural watersheds



Biofiltration can reduce >99% of the microbes

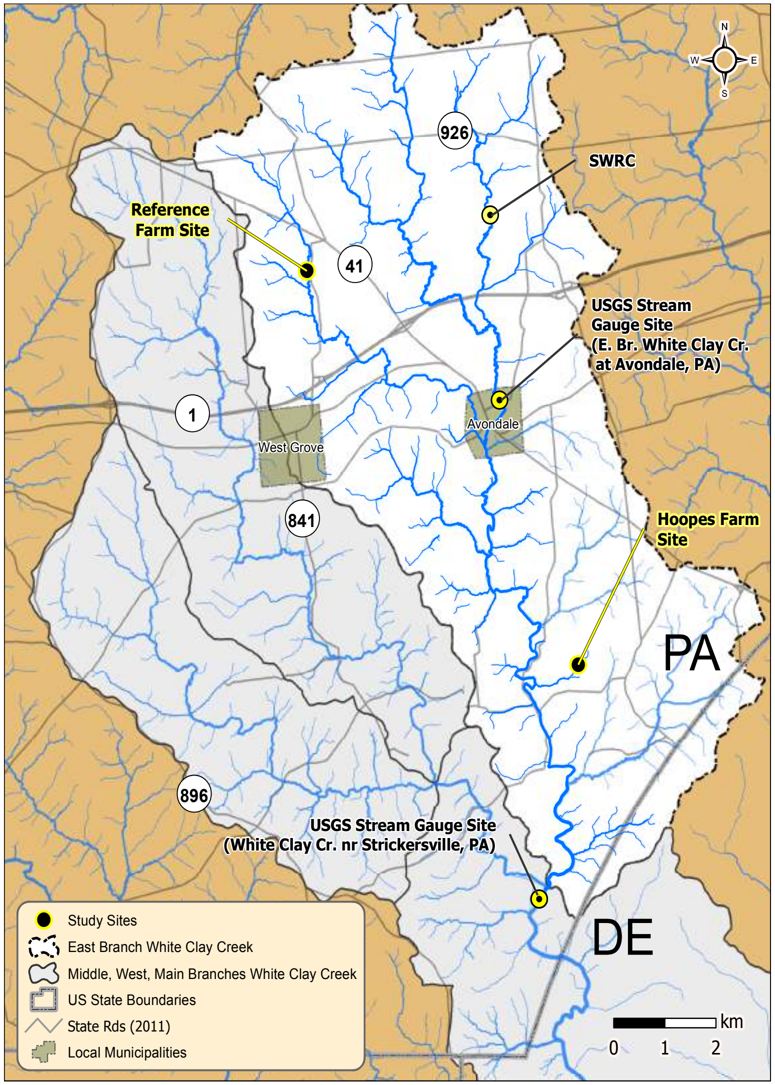
(A. Boyer, DNREC, DE)

Retention/detention ponds can reduce bacteria by 44 to 99%

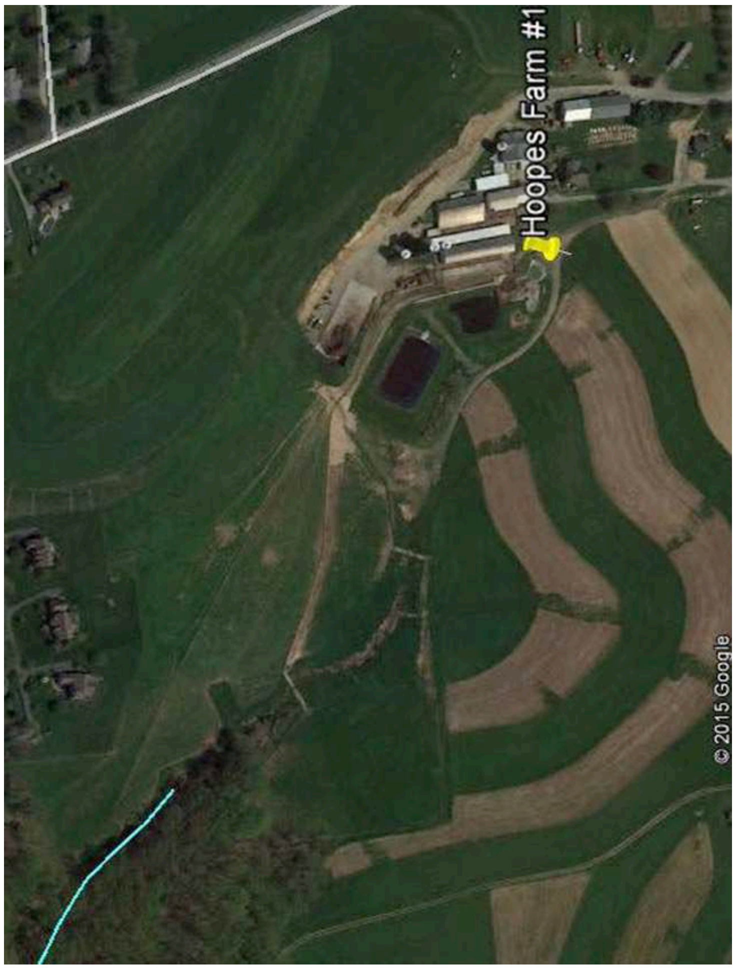
Constructed/storm water wetlands can reduce bacteria by 78 to 90%

(A. Boyer, DNREC, DE)

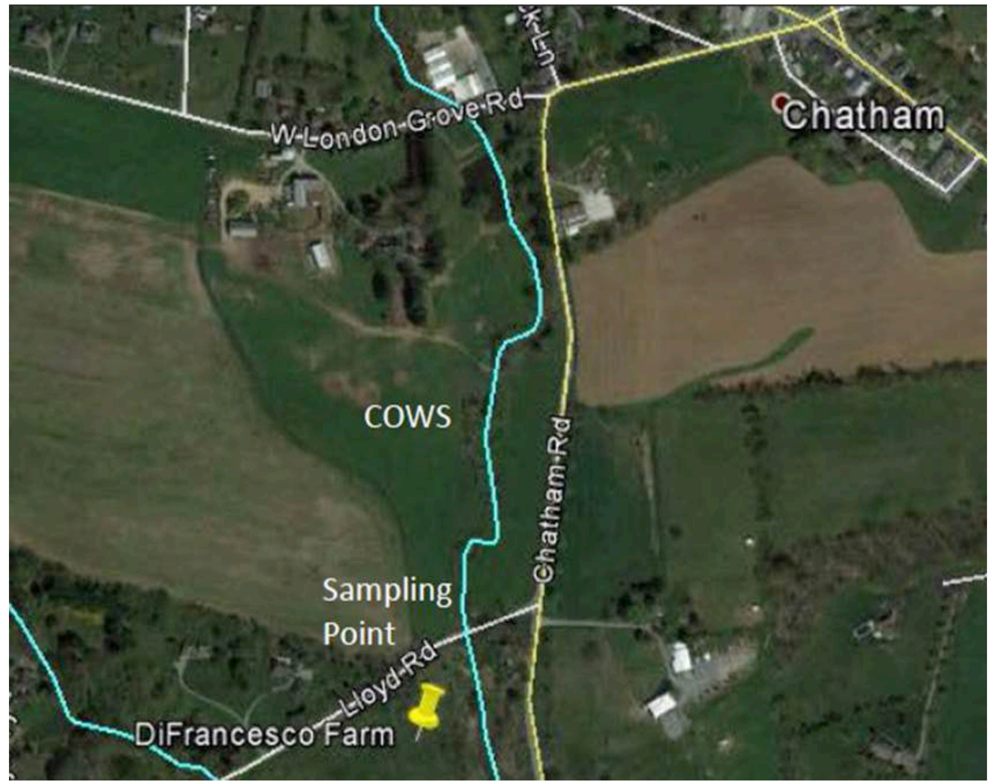
Case study : United water/Suez



Case study : United water/Suez

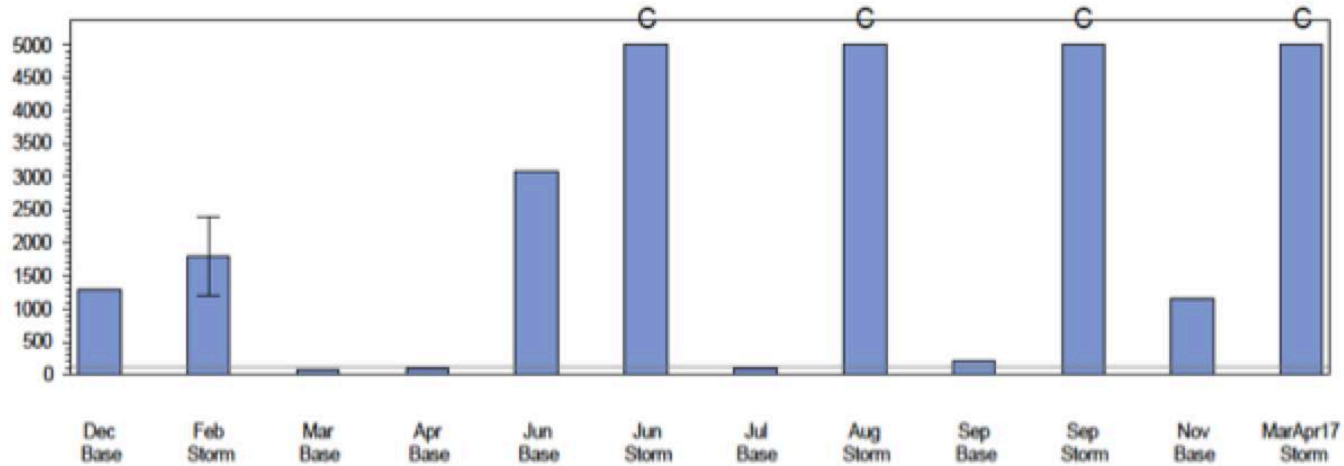


BMP site

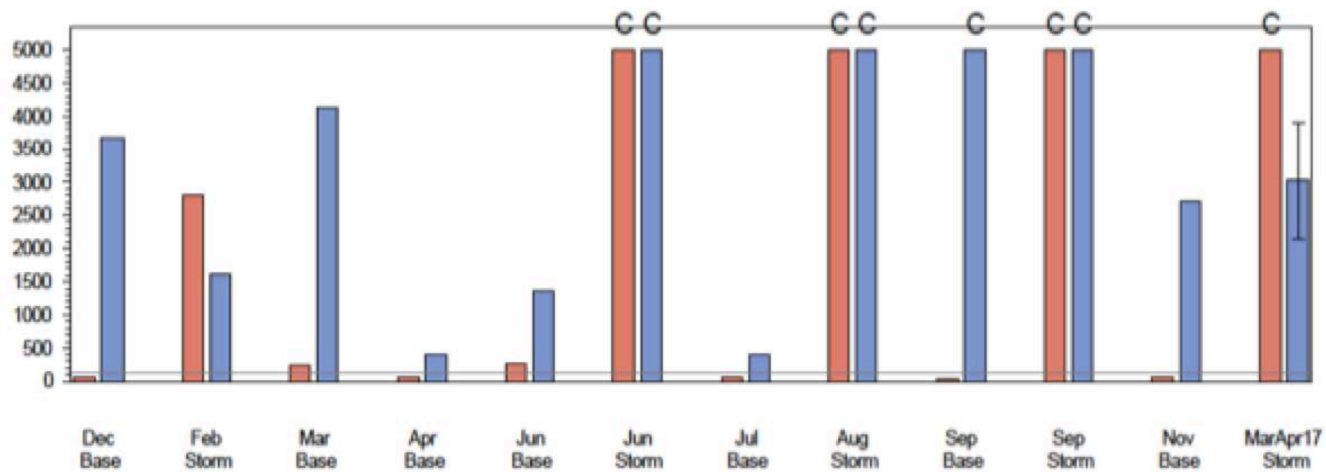


Reference site

Keep livestock out of the streams!



West London Grove & Lloyd Rd



Keep livestock out of the streams!

Fencing livestock out of streams is a highly effective method of reducing the amount of bacteria in surface waters

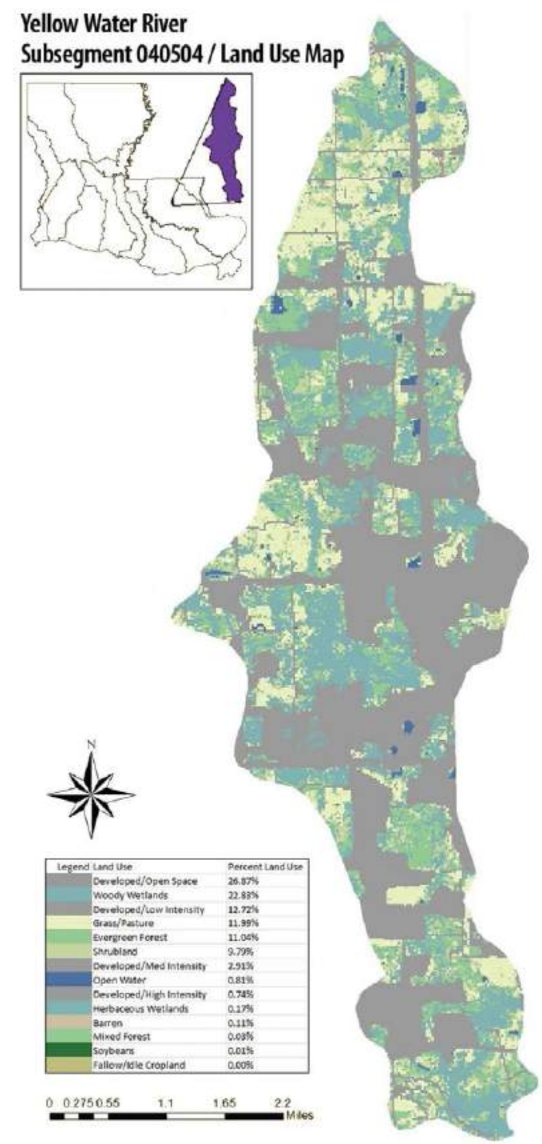
Case study: Yellow Water River

Yellow Water River in Louisiana

- Poor installation/maintenance on on-site treatment systems (septic system etc.)

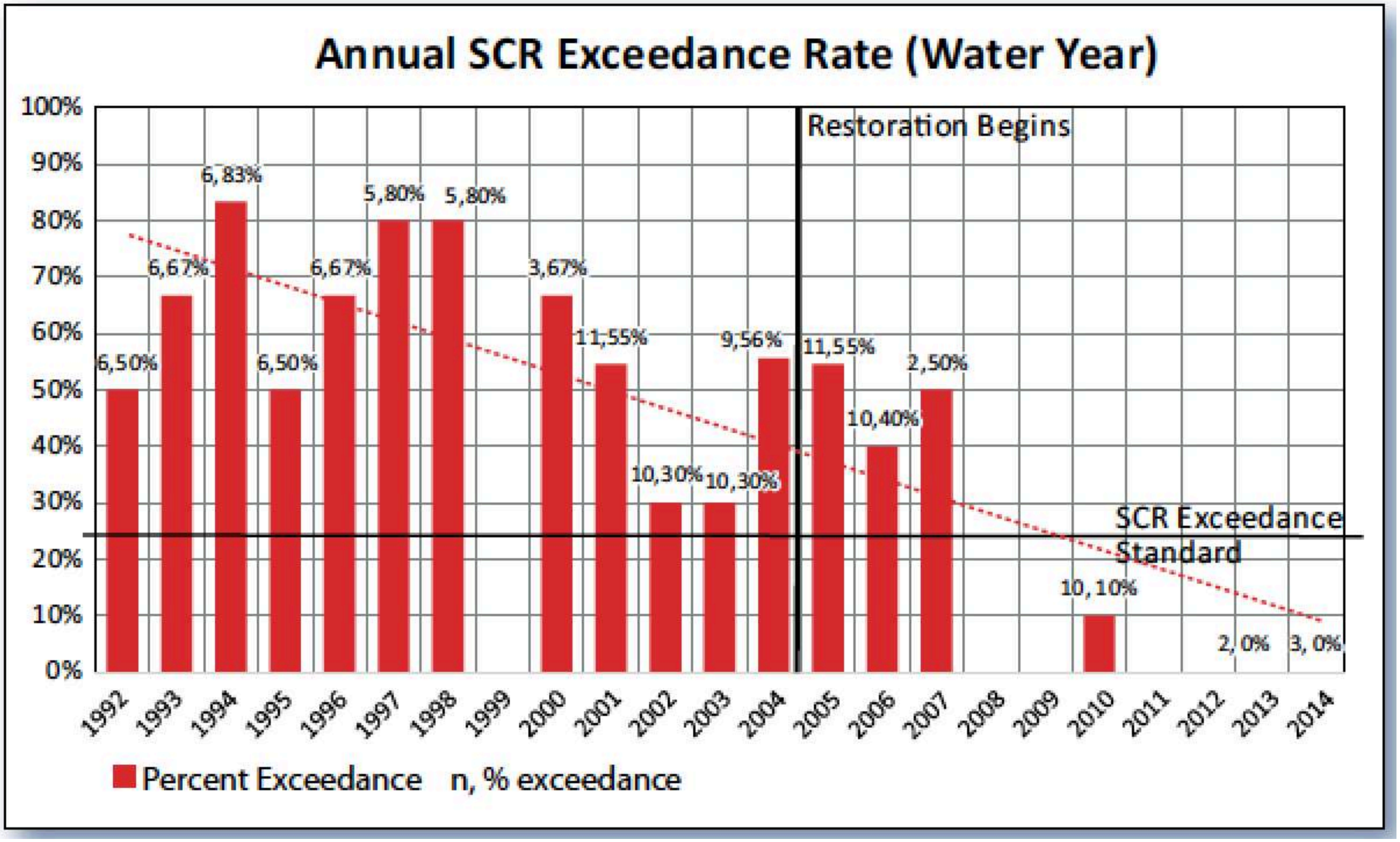
Approaches

- Thorough inspections on waste water treatment plants and home waste systems
- Additional restoration activities including educational outreach, sewage inspections, and water quality monitoring etc.



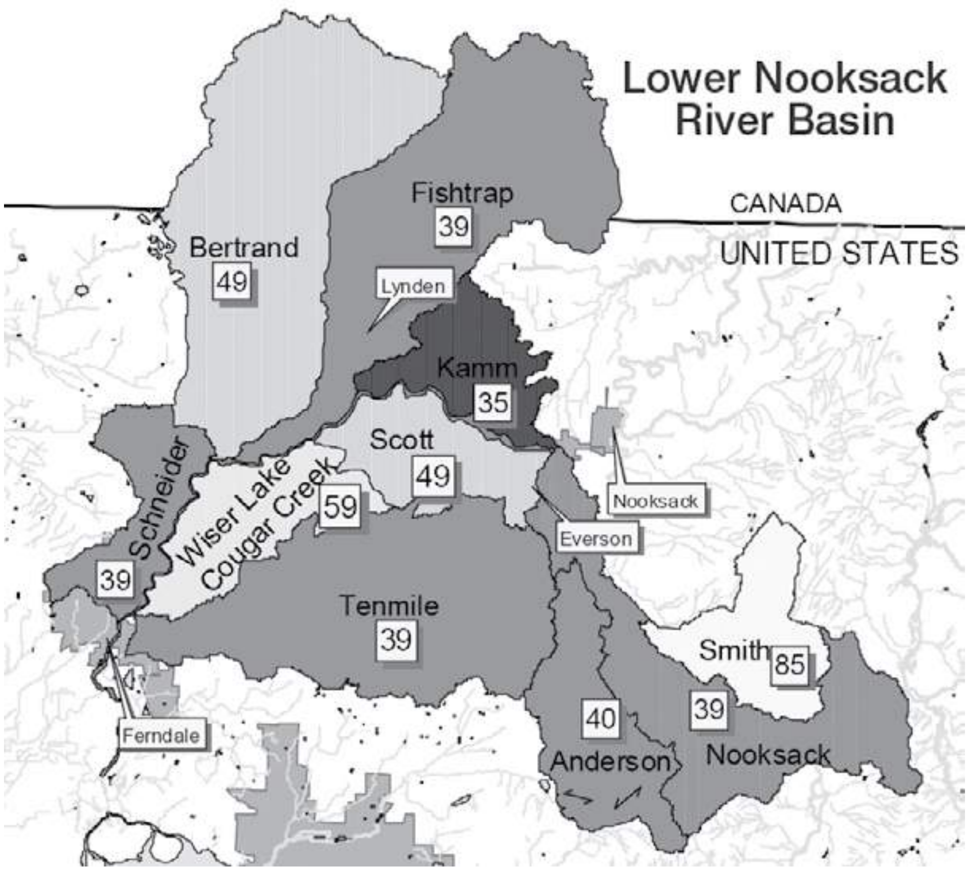
US EPA

Case study: Yellow Water River



US EPA

Case study: Upper Fishtrap Creek



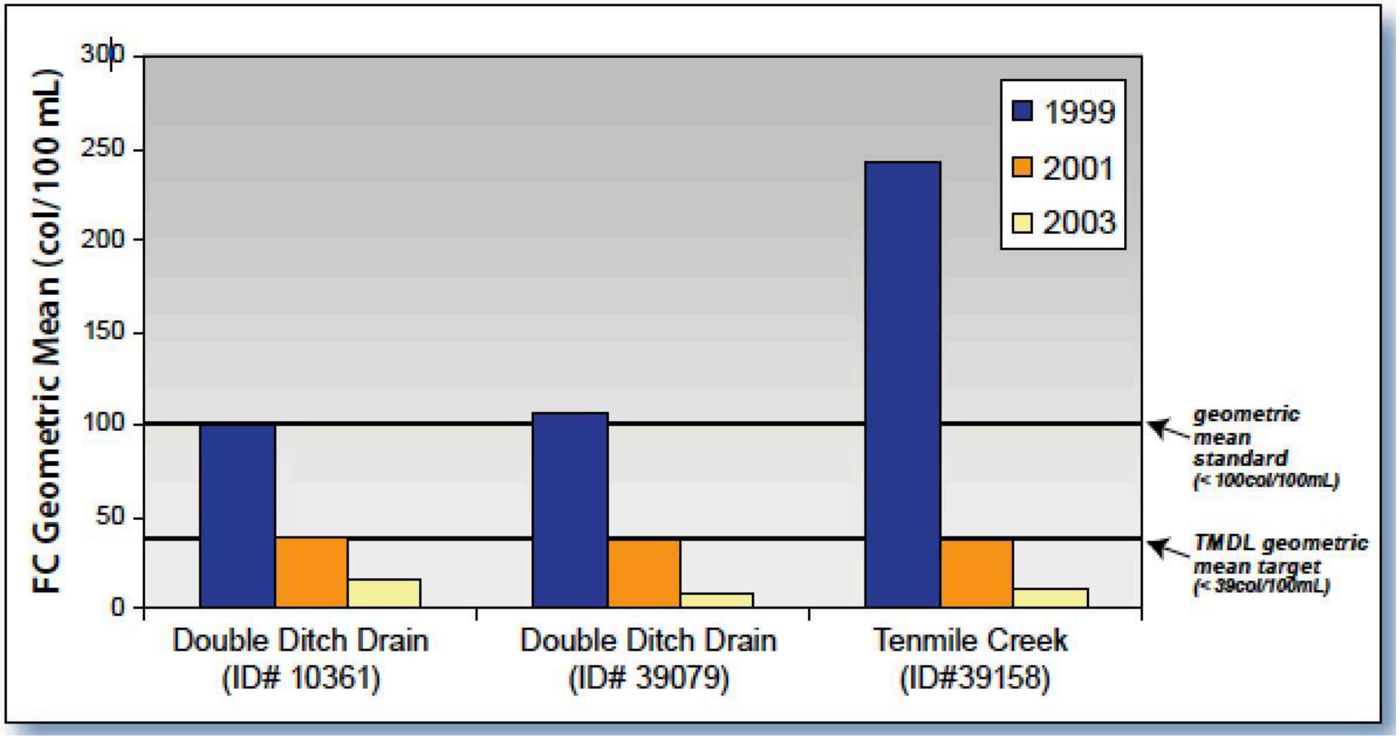
Lower Nooksack River in Washington

- Exceed 100 CFU/100ml
- FC impairments: state's CWA section 303(d) list

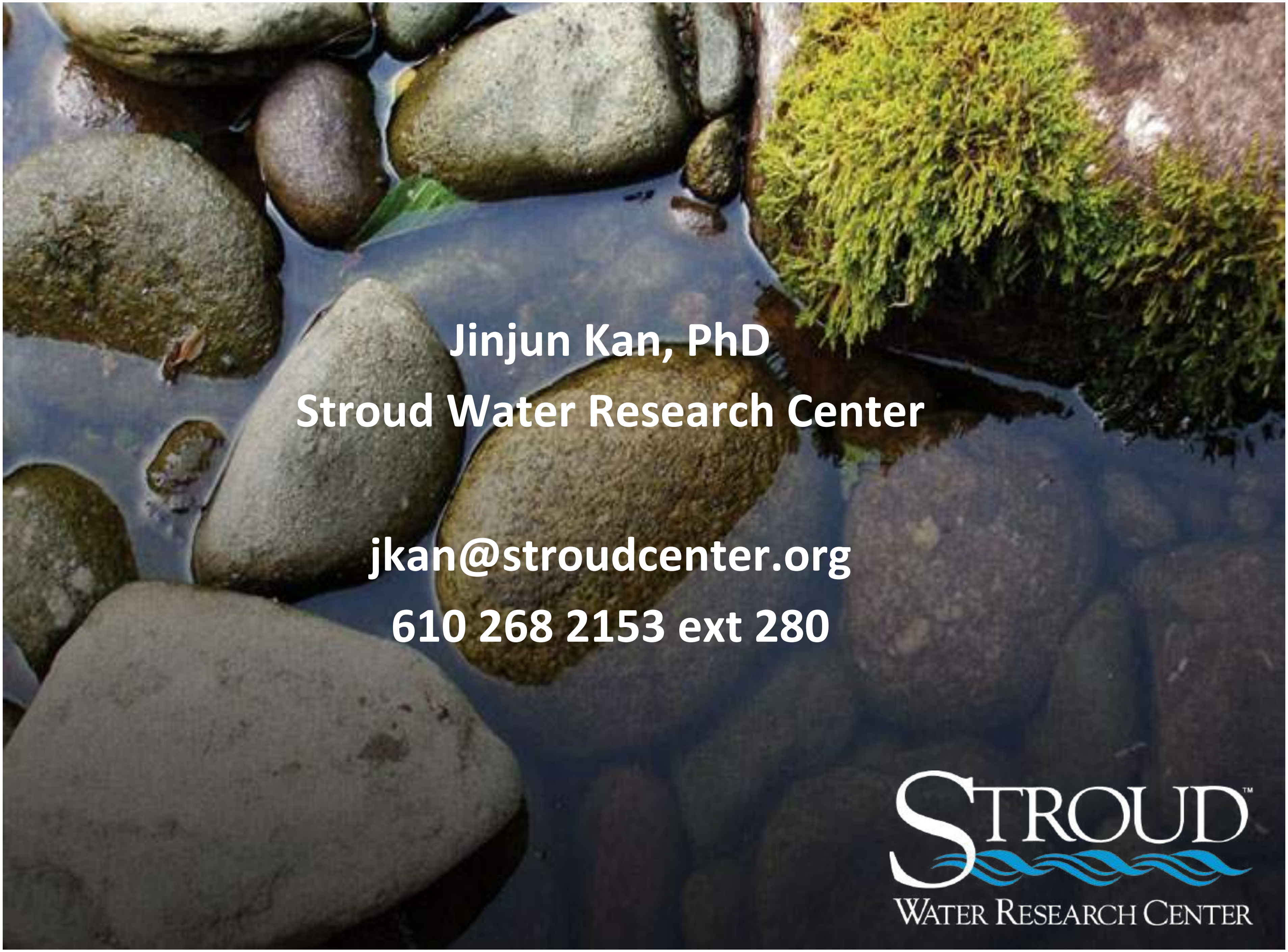
US EPA

Case study: Upper Fishtrap Creek

- Nutrient management plans for all dairies
- Fence animals out
- Install hedgerows and filter srips
- On-site inspection and improvement on septic systems
- Farmers growing trees program



US EPA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2012 EPA RWQC

A 30-day period geometric mean

Table 4. Recommended 2012 RWQC.

Criteria Elements	**Estimated Illness Rate (NGI): 36 per 1,000 primary contact recreators**			**Estimated Illness Rate (NGI): 32 per 1,000 primary contact recreators**	
	Magnitude			**Magnitude**	
Indicator	GM (cfu/100 mL)[a]	STV (cfu/100 mL)[a]	OR	GM (cfu/100 mL)[a]	STV (cfu/100 mL)[a]
Enterococci – marine and fresh	35	130		30	110
OR					
E. coli – fresh	126	410		100	320
Duration and Frequency: The waterbody GM should not be greater than the selected GM magnitude in any 30-day interval. There should not be greater than a ten percent excursion frequency of the selected STV magnitude in the same 30-day interval.					

[a] EPA recommends using EPA Method 1600 (U.S. EPA, 2002a) to measure culturable enterococci, or another equivalent method that measures culturable enterococci and using EPA Method 1603 (U.S. EPA, 2002b) to measure culturable *E. coli*, or any other equivalent method that measures culturable *E. coli*.

Beach Action Values

Table 5. Beach Action Values (BAVs).

Indicator	Estimated Illness Rate (NGI): 36 per 1,000 primary contact recreators BAV (Units per 100 mL)		Estimated Illness Rate (NGI): 32 per 1,000 primary contact recreators BAV (Units per 100 mL)
Enterococci – culturable (fresh and marine)[a]	70 cfu	OR	60 cfu
E. coli – culturable (fresh)[b]	235 cfu		190 cfu
Enterococcus spp. – qPCR (fresh and marine)[c]	1,000 cce		640 cce

[a] Enterococci measured using EPA Method 1600 (U.S. EPA, 2002a), or another equivalent method that measures culturable enterococci.

[b] *E. coli* measured using EPA Method 1603 (U.S. EPA, 2002b), or any other equivalent method that measures culturable *E. coli*.

[c] EPA *Enterococcus* spp. Method 1611 for qPCR (U.S. EPA, 2012b). See section 5.2.

Pennsylvania

TABLE 3

Parameter	*Symbol*	*Criteria*	*Critical Use**
		********	
Bacteria	Bac_1	**[(Fecal coliforms/ 100 ml)]** ***Escherichia coli*/100 ml)** —During the swimming season (May 1 through September 30), the maximum **[fecal coliform]** ***E. coli*** level shall be a geometric mean of **[200] 126** per 100 milliliters (ml) based on **[a minimum of five]** consecutive samples, each sample collected on different days, during a 30-day period. No more than 10% of the total samples taken during a 30-day period may exceed **[400] 410** per 100 ml. **(Fecal coliforms/ 100 ml)—For the remainder of the year, the maximum fecal coliform level shall be a geometric mean of 2,000 per 100 milliliters (ml) based on a minimum of five consecutive samples collected on different days during a 30-day period.]**	WC
	[Bac_2	**(Coliforms/100 ml)—Maximum of 5,000/100 ml as a monthly average value, no more than this number in more than 20% of the samples collected during a month, nor more than 20,000/100 ml in more than 5% of the samples.**	**PWS]**
		********	

Delaware

4.5.7 Bacterial Water Quality Criteria

4.5.7.1 Primary and Secondary Contact Recreation Waters:

The following criteria shall apply:

Waterbody Type	Single-Sample Value (Enterococcus Colonies/100ml)	Geometric Mean (Enterococcus Colonies/ 100ml)
Primary Contact Recreation Fresh Waters	185	100
Primary Contact Recreation Marine Waters	104	35
Secondary Contact Recreation Fresh Waters	925	500
Secondary Contact Recreation Marine Waters	520	175

New York

703.4 Water quality standards for coliforms.

Total and fecal coliform standards for specific classes are provided in this section.

(a) Total coliforms (number per 100 ml).

Classes	*Standard*
AA	The monthly median value and more than 20 percent of the samples, from a minimum of five examinations, shall not exceed 50 and 240, respectively.
A, B, C, D, SB, SC, I, SD	The monthly median value and more than 20 percent of the samples, from a minimum of five examinations, shall not exceed 2,400 and 5,000, respectively.
SA	The median most probable number (MPN) value in any series of representative samples shall not be in excess of 70.
A-Special	The geometric mean, of not less than five samples, taken over not more than a 30-day period shall not exceed 1,000.
GA	The maximum allowable limit is 50.

(b) Fecal coliforms (number per 100 ml).

A, B, C, D, SB, SC, I, SD	The monthly geometric mean, from a minimum of five examinations, shall not exceed 200.
A-Special	The geometric mean, of not less than five samples, taken over not more than a 30-day period shall not exceed 200.

(c) The total and fecal coliform standards for classes B, C, D, SB, SC and I shall be met during all periods:

(1) when disinfection is required for SPDES permitted discharges directly into, or affecting the best usage of, the water; or

(2) when the department determines it necessary to protect human health.

6 CRR-NY 703.4
Current through September 15, 2016

Maryland

A. Criteria for Class I Waters — Water Contact Recreation and Protection of Nontidal Warmwater Aquatic Life.

(1) Bacteriological.

(a) Table 1. Bacteria Indicator Criteria for Frequency of Use.

Steady State Geometric Mean Indicator Density		Single Sample Maximum Allowable Density			
Indicator	All Areas	Frequent Full Body Contact Recreation (Upper 75% CL)	Moderately Frequent Full Body Contact Recreation (Upper 82% CL)	Occasional Full Body Contact Recreation (Upper 90% CL)	Infrequent Full Body Contact Recreation (Upper 95% CL)
Freshwater (Either apply)					
Enterococci	33	61	78	107	151
E. coli	126	235	298	410	576
Marine water					
Enterococci	35	104	158	275	500

CL = confidence level

All numbers are counts per 100 milliliters

(b) In freshwater for E. coli, the following formula is used to calculate the upper 75 percent confidence interval for single sample maximum allowable density: antilog[(log 126) + 0.675 * log(SD)].

(c) In freshwater for enterococci, the following formula is used to calculate the upper 75 percent confidence interval for single sample maximum allowable density: antilog[(log 33) + 0.675 * log(SD)], where log(SD) is the standard deviation of the log transformed E. coli or enterococci data. If the site data are insufficient to establish a log standard deviation, then 0.4 is used as the log standard deviation for both indicators. At the default log standard deviation, the values are 235 for E. coli and 61 for enterococci.

(d) In saltwater, for enterococci, the following formula is used to calculate the upper 75 percent confidence interval for single sample maximum allowable density: antilog[(log 35) + 0.675 * log(SD)], where log(SD) is the standard deviation of the log transformed enterococci data. If the site data are insufficient to establish a log standard deviation, then 0.7 is used as the log standard deviation. At the default log standard deviation, the value is 104.

New Jersey

1. ml)	Bacterial quality (Counts/100	i.	Shellfish Harvesting: Bacterial Indicators shall not exceed, in all shellfish waters, the standard for approved shellfish waters as established by the National Shellfish Sanitation Program as set forth in its current manual of operations.	Shellfish Waters
		ii.	Primary Contact Recreation:	
			(1) Enterococci levels shall not exceed a geometric mean of 35/100 ml, or a single sample maximum of 104/100 ml.	SE1 and SC
			(2) E. Coli levels shall not exceed a geometric mean of 126/100 ml or a single sample maximum of 235/100 ml.	All FW2
		iii.	Secondary Contact Recreation:	
			(1) Fecal coliform levels shall not exceed a geometric mean of 770/100 ml.	SE2
			(2) Fecal coliform levels shall not exceed a geometric mean of 1500/100ml.	SE3