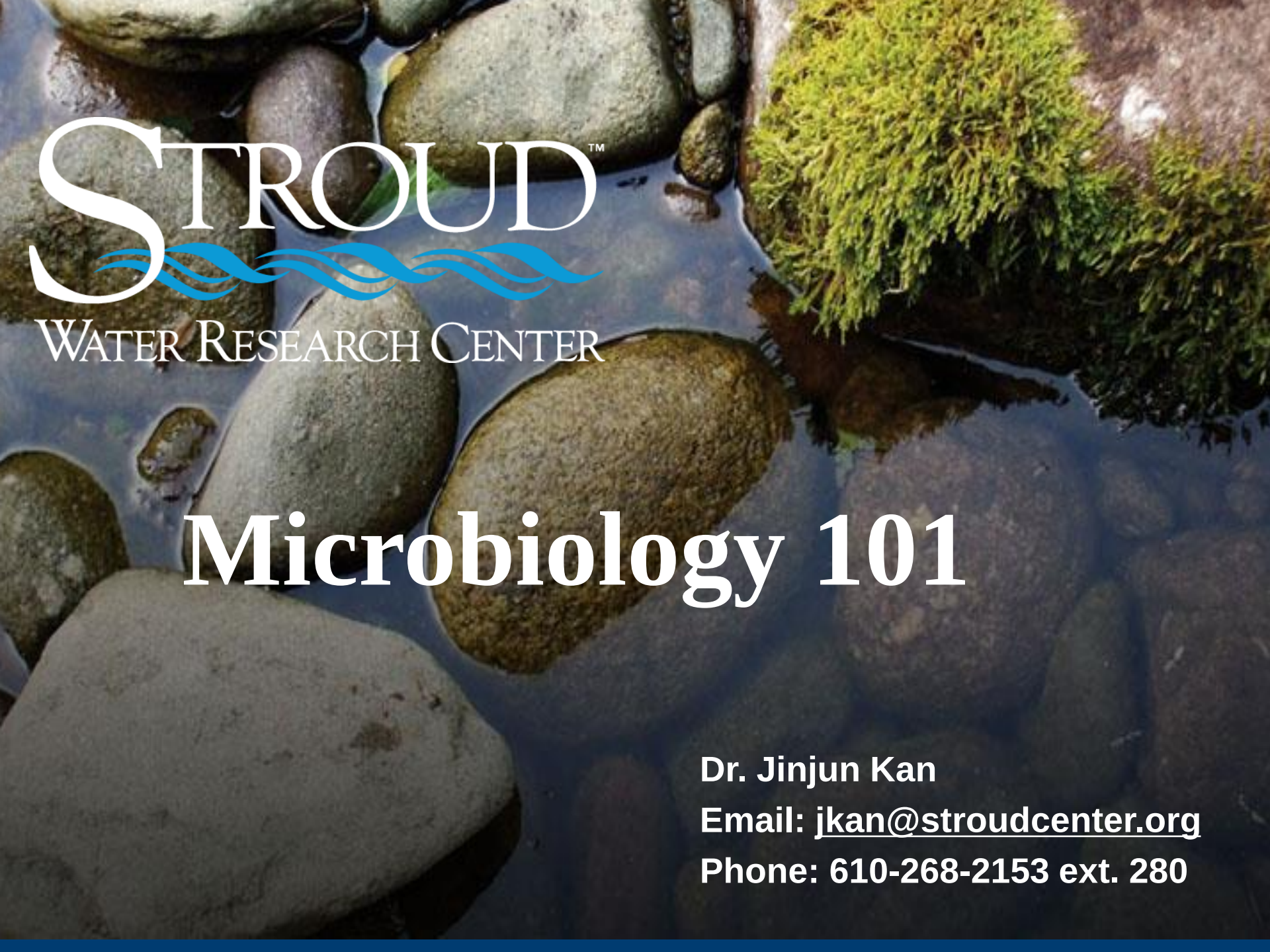

Microbiology 101

Dr. Jinjun Kan
Email: jkan@stroudcenter.org
Phone: 610-268-2153 ext. 280

Learning Objectives

- Understand what microorganisms (mainly bacteria) are and their abundance, diversity and distribution in streams
- Understand why we need to study/monitor these microorganisms: (1) ecosystem function and material/nutrient cycling, and (2) public health concerns
- Understand how scientists study microorganisms in freshwaters: research projects at Stroud Water Research Center
- Introduce pathogen/fecal indicator bacteria (FIB) monitoring: variety of approaches and molecular source tracking

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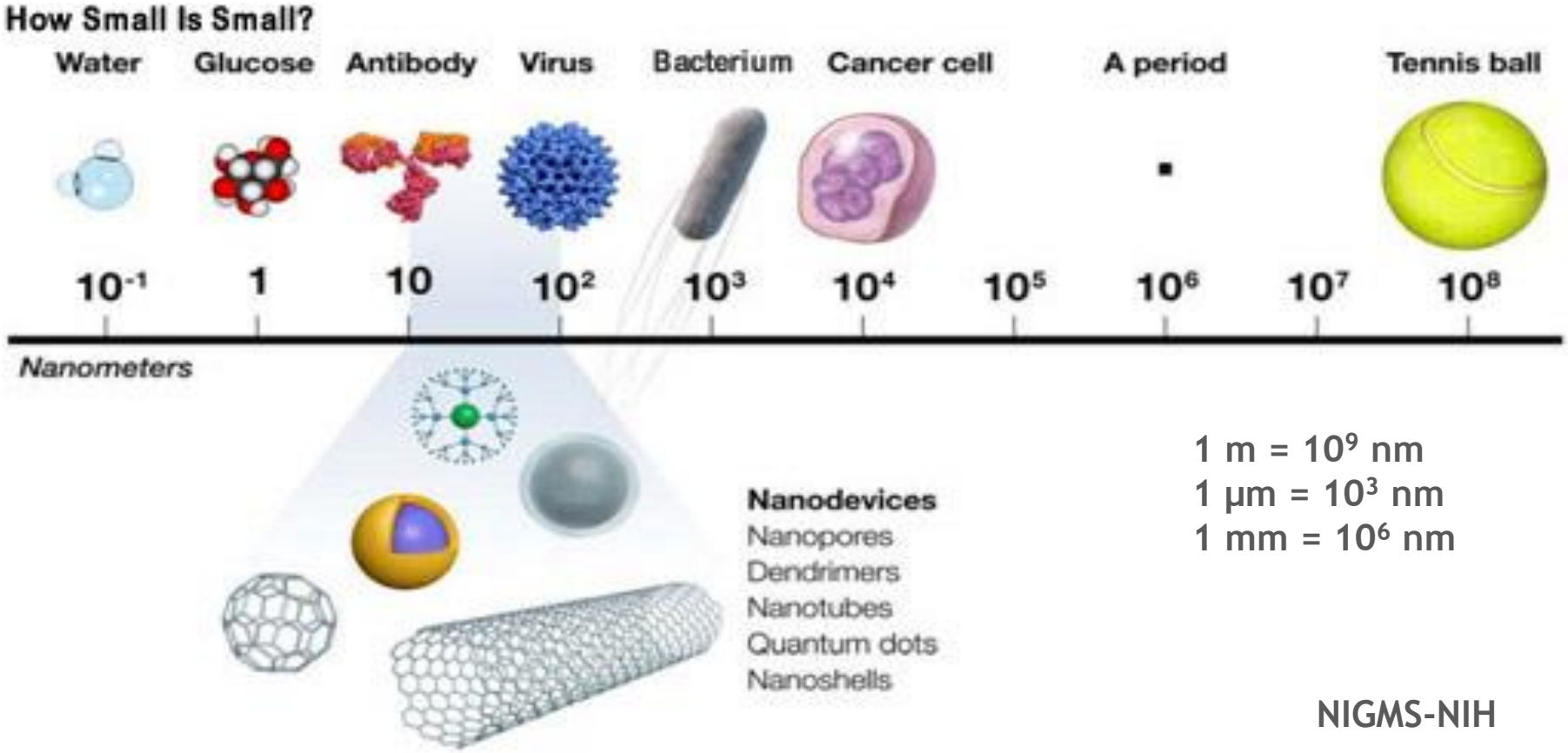
I. Introduction

Micro- + **organism**

Microscopic; Too small to be seen by unaided eyes

Living organisms, independent



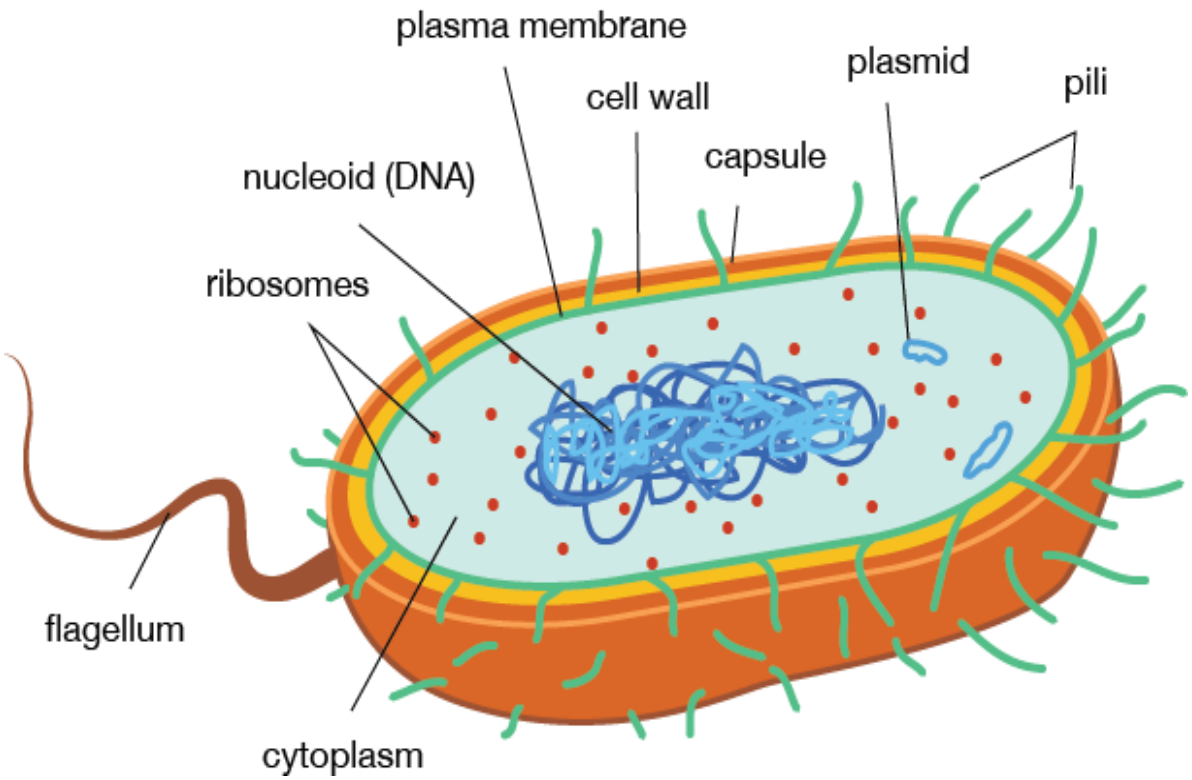
$1\ m = 10^9\ nm$

$1\ \mu m = 10^3\ nm$

$1\ mm = 10^6\ nm$

NIGMS-NIH

Structure of microbes



Use bacteria cells as an example: Single cell with cell wall; no membrane-enclosed nucleus or organelles; sensitive to antibiotics; heterotrophic or autotrophic

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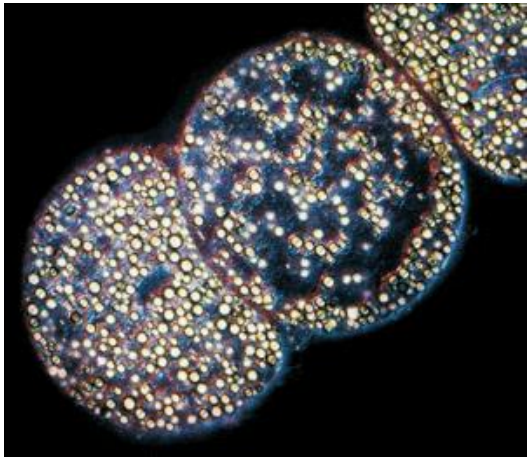
Size

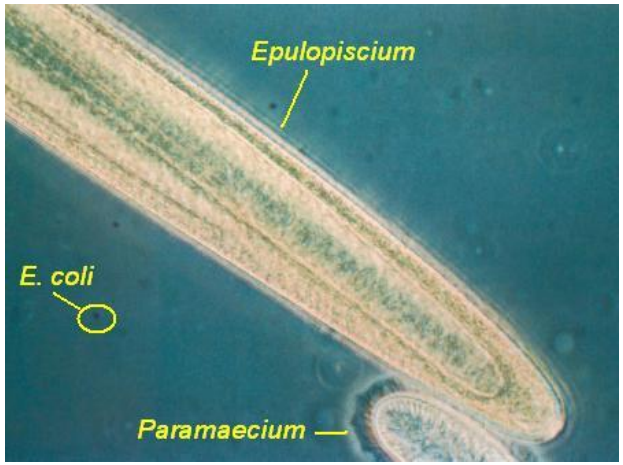
Individual cells: Cocci-most 0.1-0.2 µm in diameter

***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

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Abundance

Earth is a microbial planet

Population size:

Human: 7.5×10^9 (estimated April 2017)

Microbes: 5.5×10^{31} (including in both water and soils)

~ 7.3×10^{21} microbes for every human

Biomass

Humans (@150 lbs) = 4.97×10^{14} g

= 8.93×10^{13} g C

Microbes = ~ 4.5×10^{17} g C

Microbes "outweigh" humans ~ > 5,000 to 1

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

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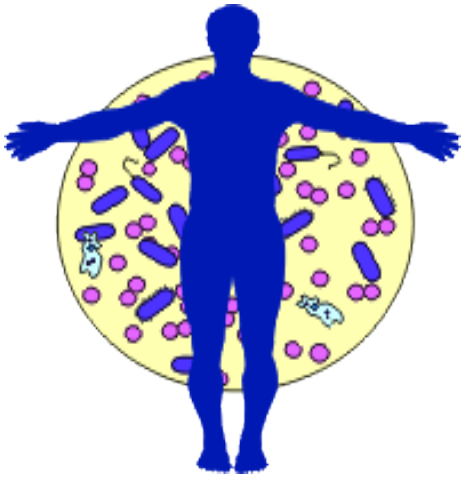
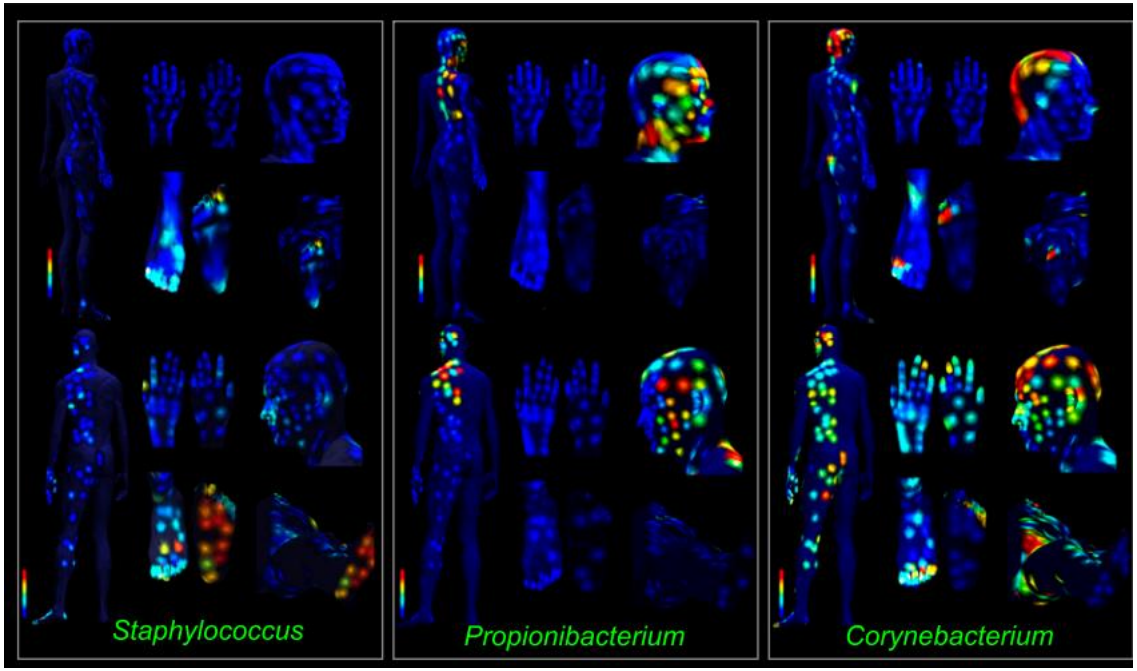
Whitman et al. 1998. PNAS. 95:6578-83

Human Microbiome

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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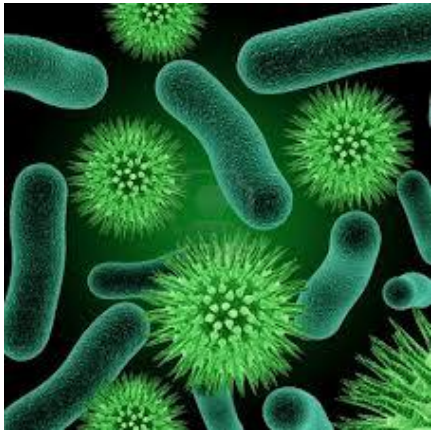
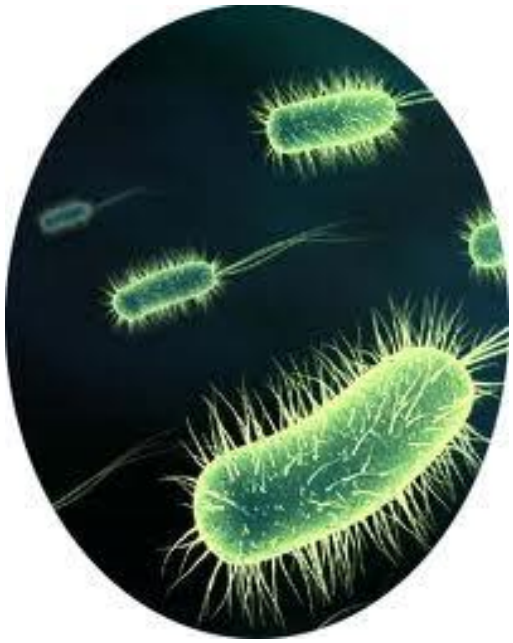
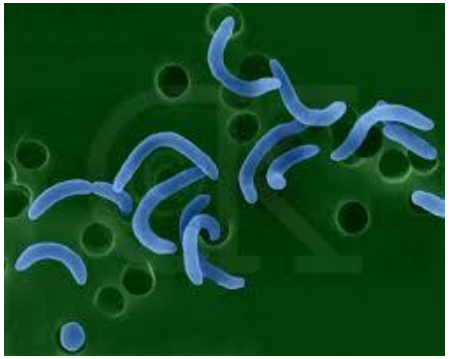
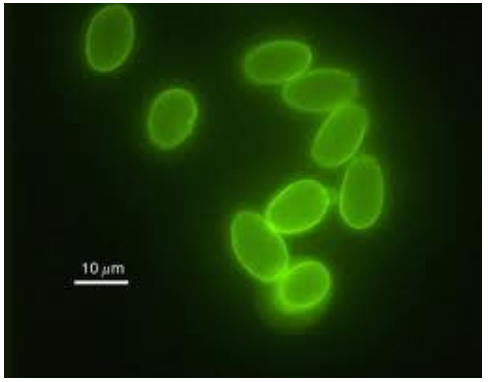
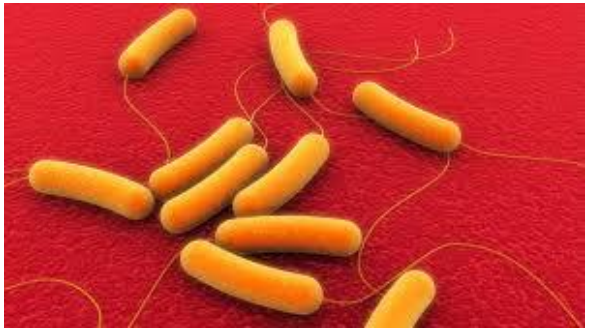
Diversity

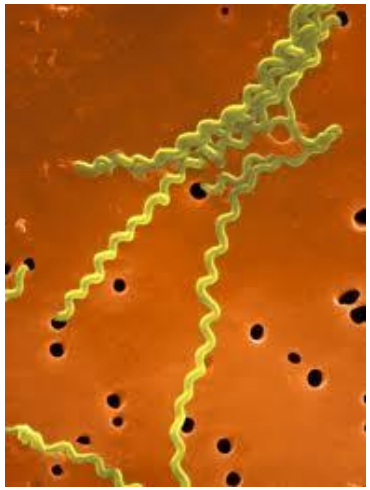












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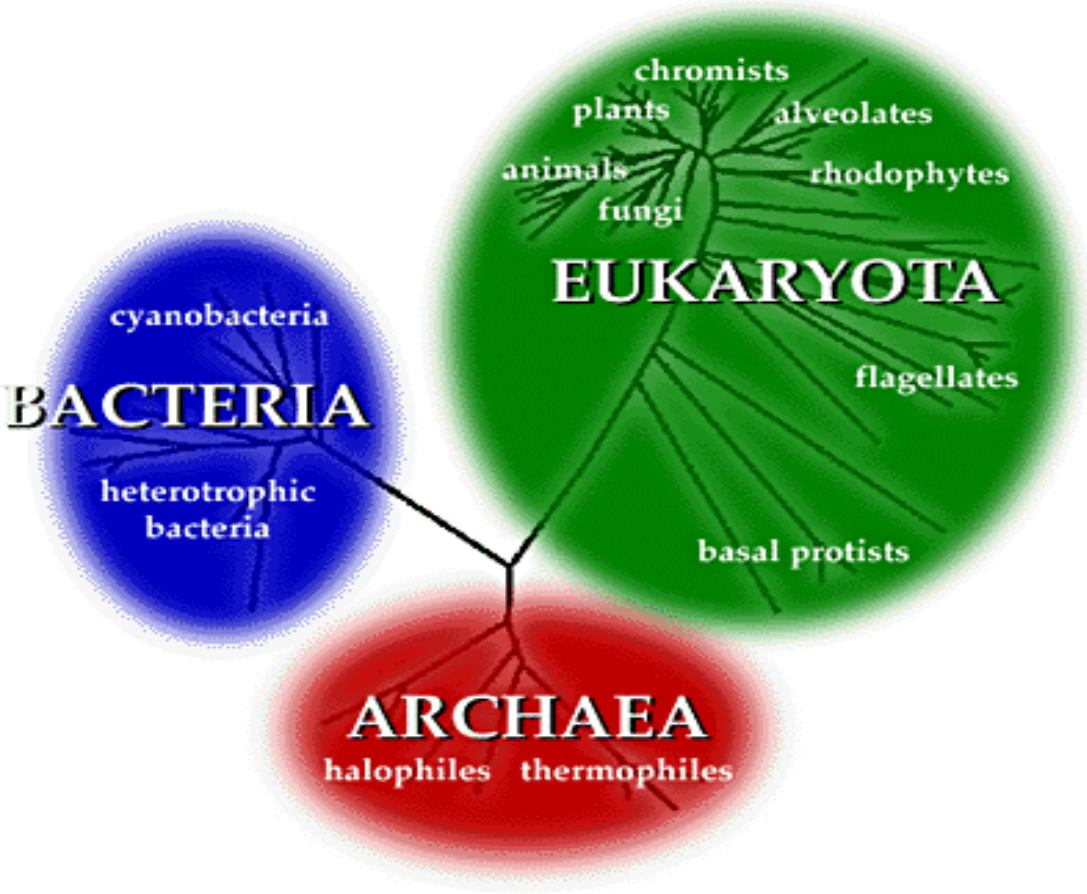
Diversity is Microbial



Bacteria + Archaea: 90 million

vs. Eukaryota: ~8.7 ± 1.3 million

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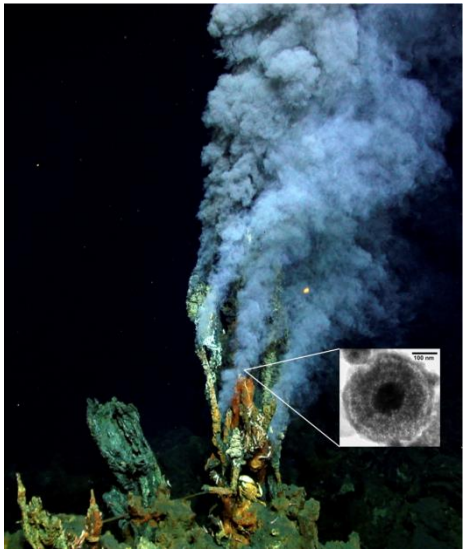
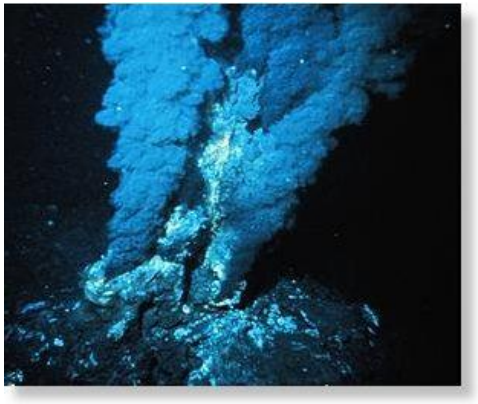
Woese and Fox, 1977; Pace, 1990; Woese et al., 1990; Mora et al, 2011

Ubiquitous Distribution





Black/white smokers
(high pressure, high temperature)

Badwater Basin, Death Valley (over-saturated salinity)

Acidic mine drainage

(low pH 3.6)

Ice (low temperature)

Mono Lake (extreme pH ~12)

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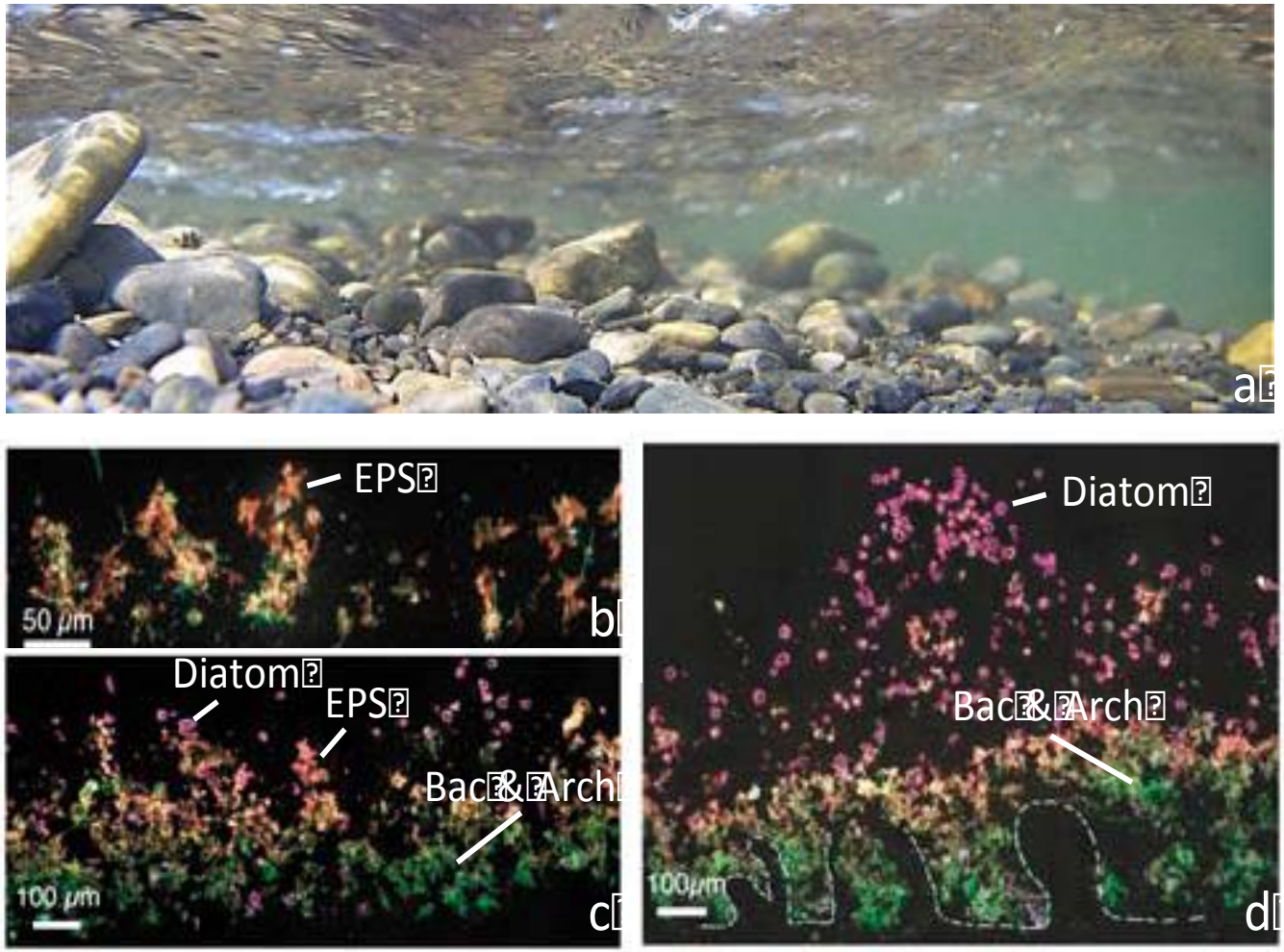
Microbes living in freshwater



Planktonic (free-floating) *vs.* Benthic (biofilm)

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Battin et al. 2003

Questionnaire

- **How many bacteria cells in one ml (milliliter) freshwater?**

- **The first thing pop to your head when you think of bacteria in general?**

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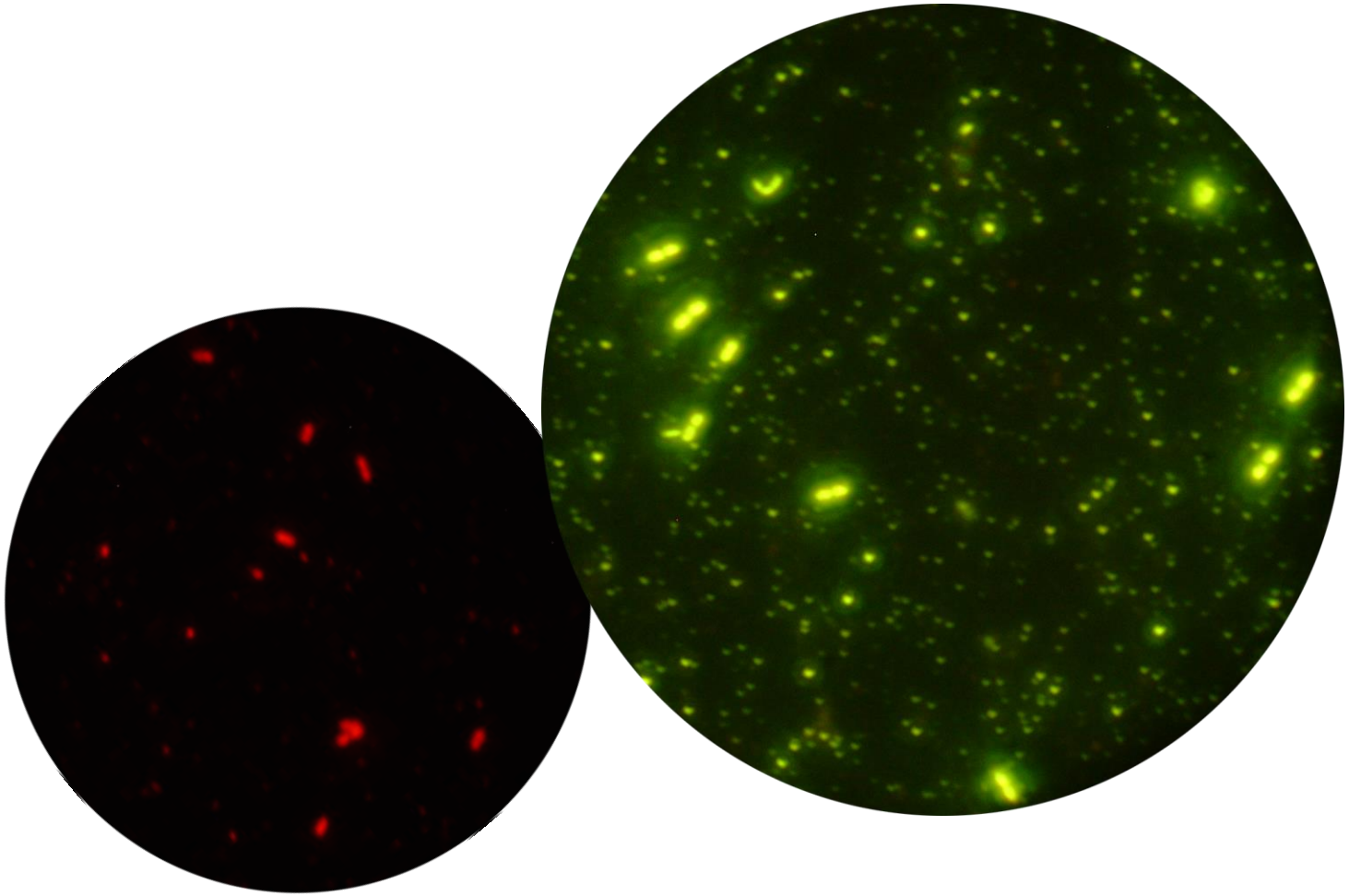
Under Microscope



Photosynthetic pigments

Epifluorescence (DNA staining)

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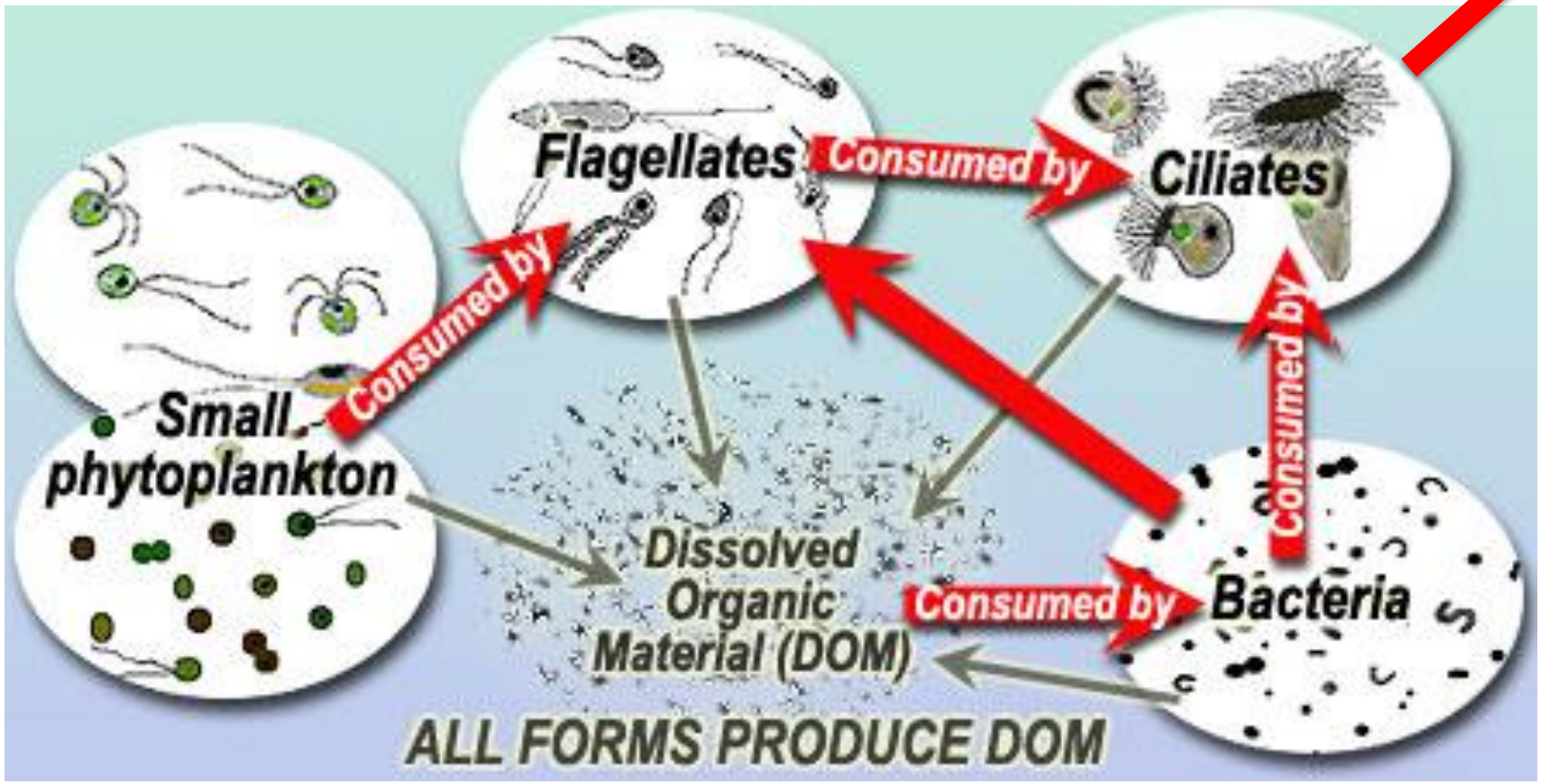
II. Significance



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

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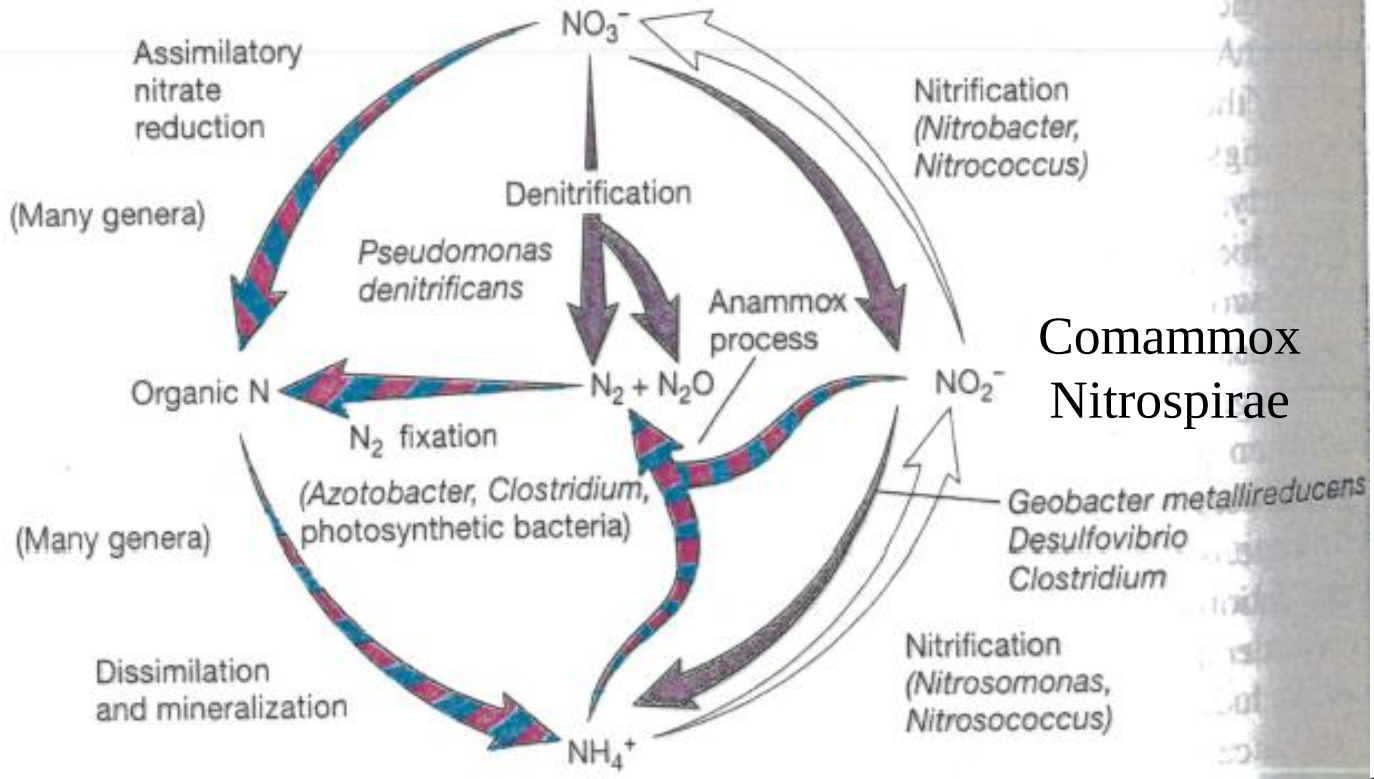
http://www.bigelow.org/foodweb/dom.jpg

Nutrient Cycling (e.g. N)

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



Comammox
Nitrospirae

Concerns/Issues

Nuisance growths

Sphaerotilus: periphyton filamentous bacterium-member of “sewage fungus association”;

Beggiatoa: another filamentous bacterium in rivers under high organic loads or pollution

- ❖ Coats surfaces-destroys macroinvertebrate habitats, and smothers organisms and eggs
- ❖ In sewage treatment plants-causes bulking of sludge so it floats and doesn’t settle.

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Public Health

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

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III. How do we monitor them? Microbial Research at SWRC

Characterizing microbes in headwaters

- *Benthic biofilms on rocks and sediments, water column as well*
- *Diversity, population structure, distribution, and function*
- *Interaction with environmental gradients (including physical, chemical and other biological features)*

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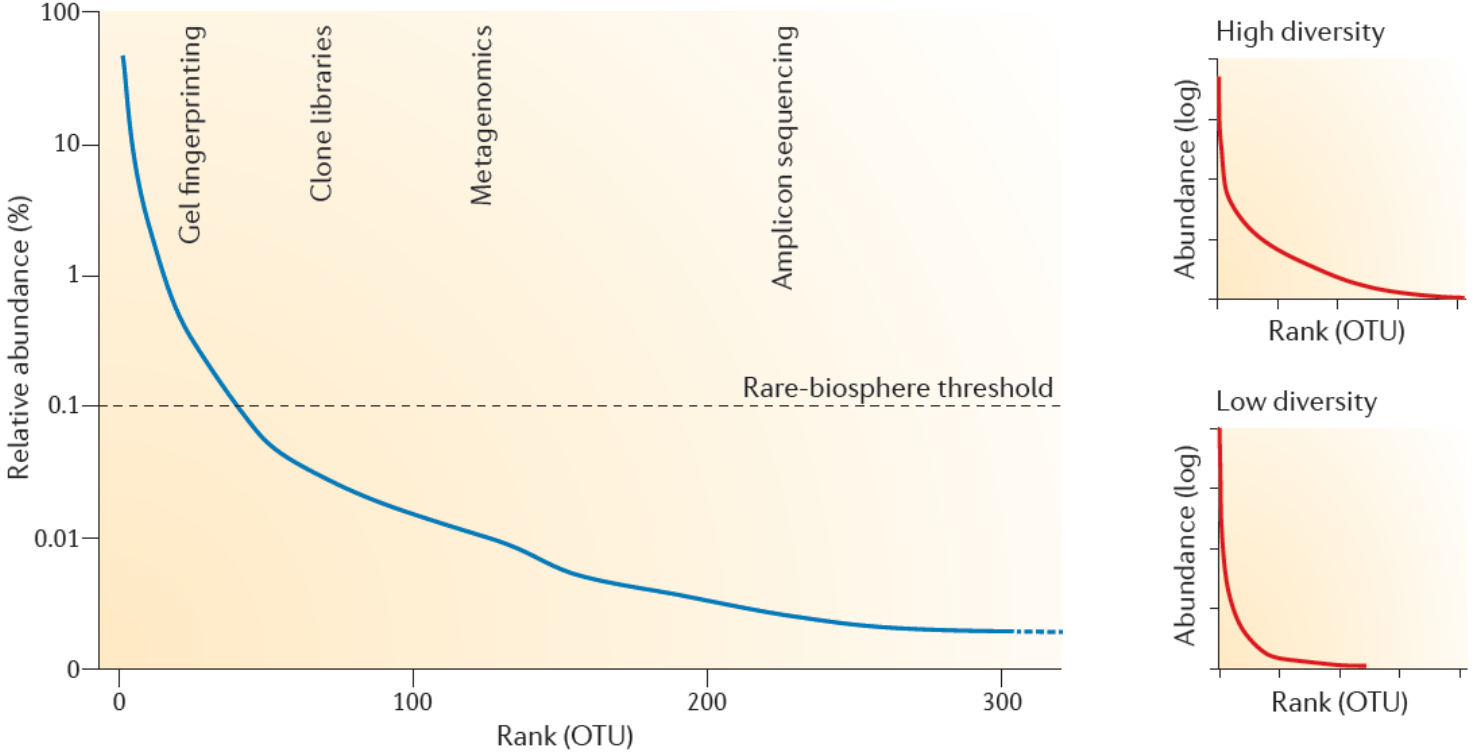
Microbial Research at SWRC

Microbial diversity and rare biosphere



- Very small portion (<1%) can be easily cultured in the lab
- Rely on molecular approaches to characterize them including the rare species
- High throughput sequencing technology (many sequences)

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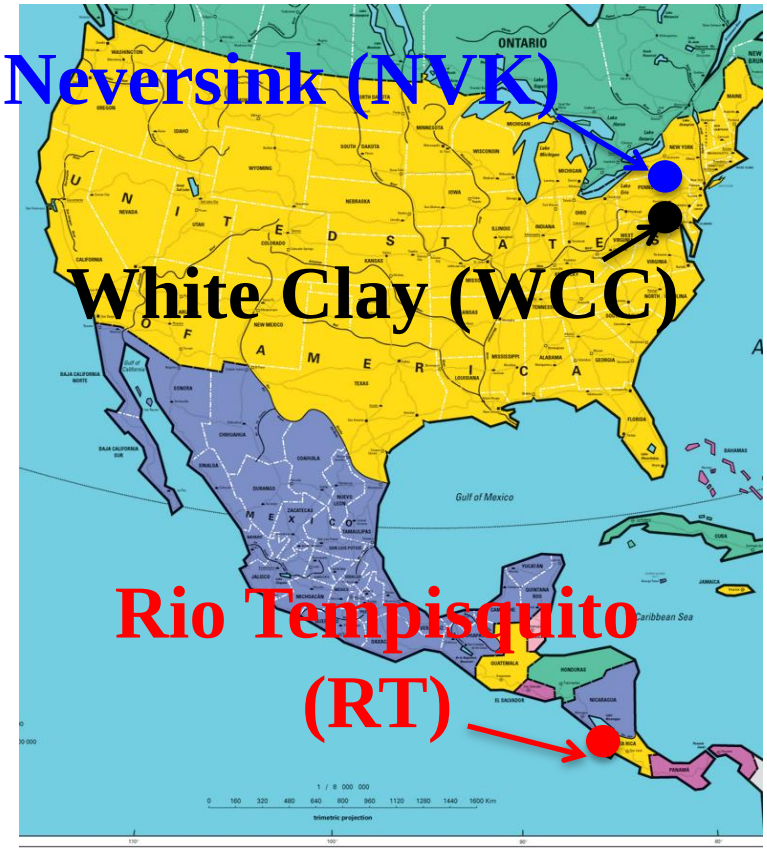
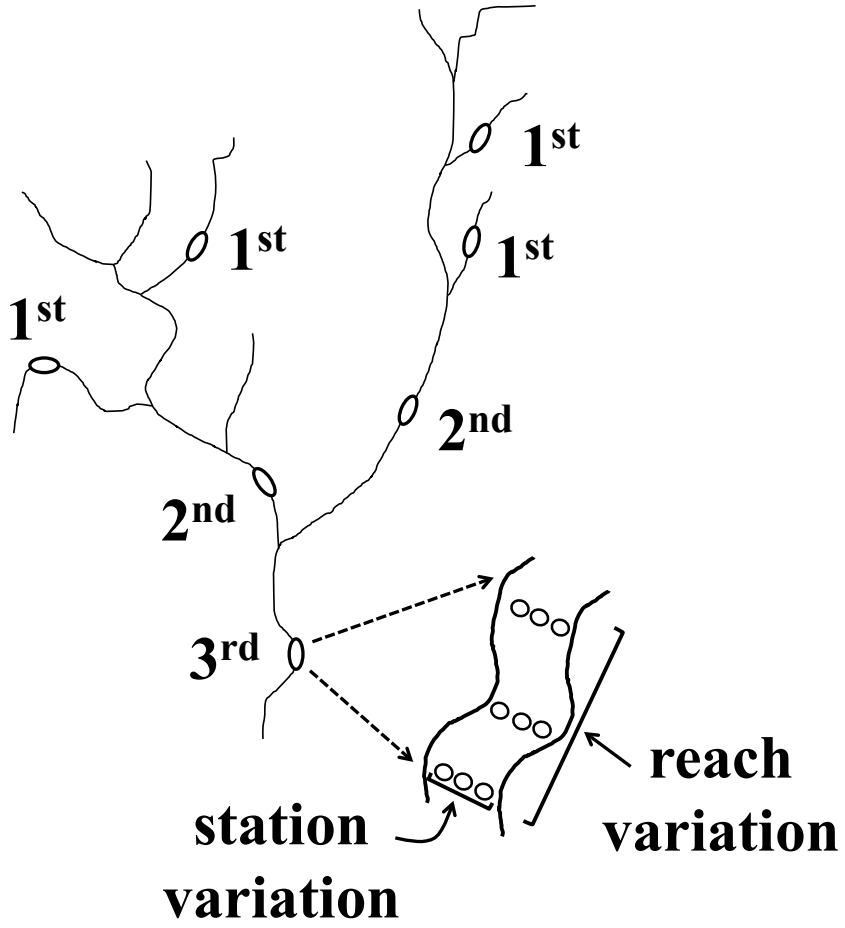
Lynch and Neufeld 2015

Microbial Research at SWRC

MetaEcosystem project





3 watersheds, **2** substrates, **7** longitudinal reaches, composite samples (**9**), different seasons and years

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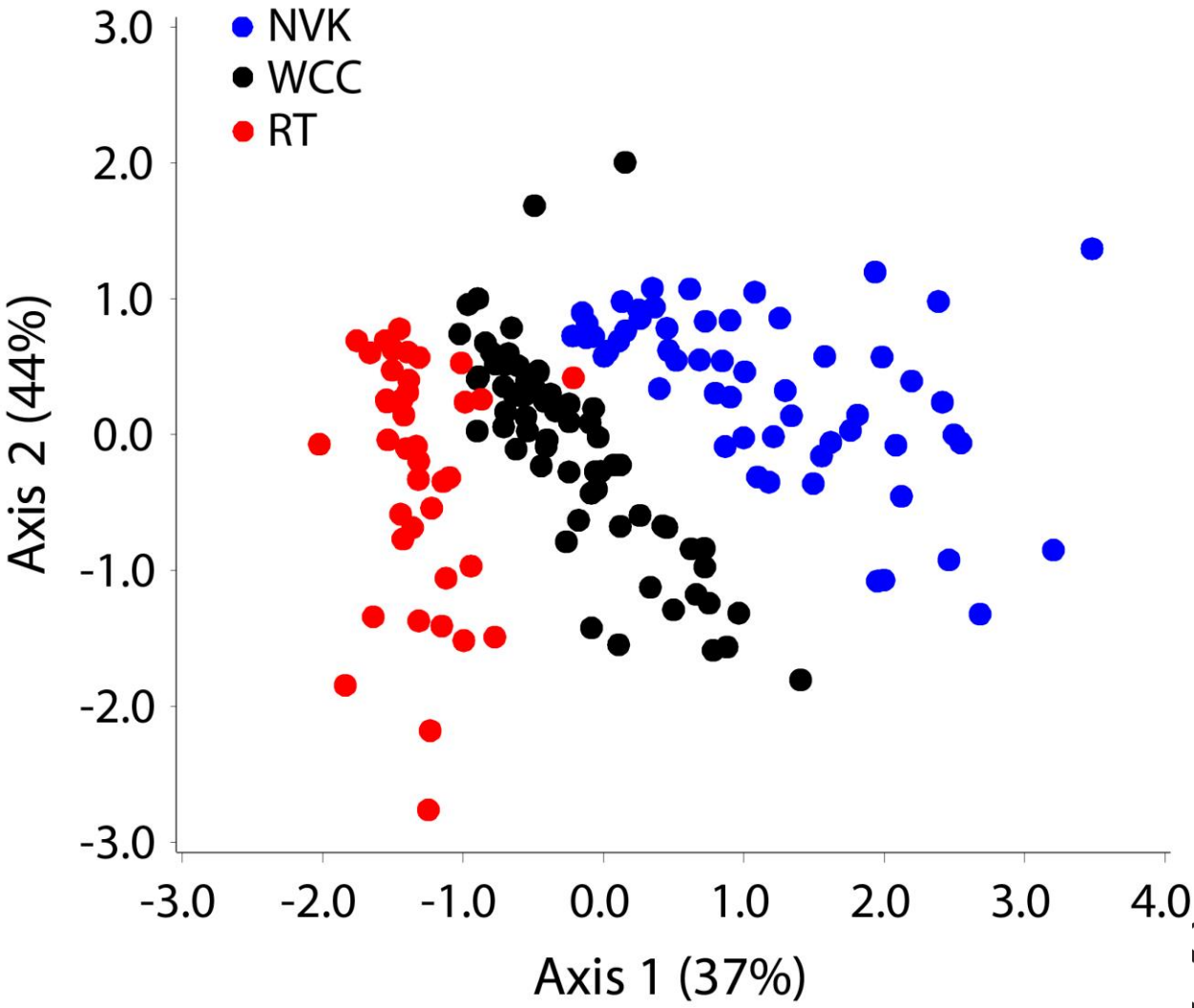
Microbial Research at SWRC



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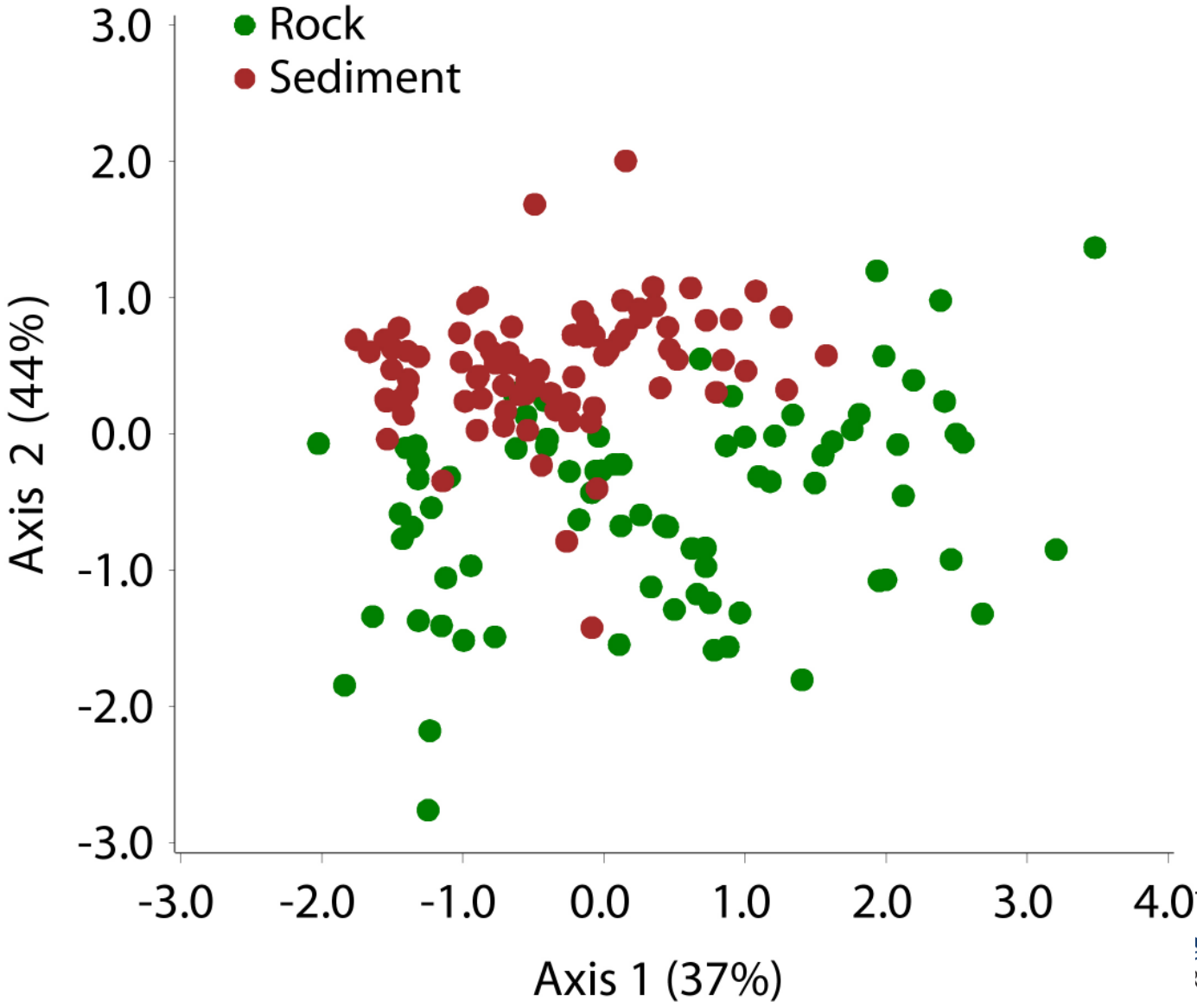
Microbial Research at SWRC



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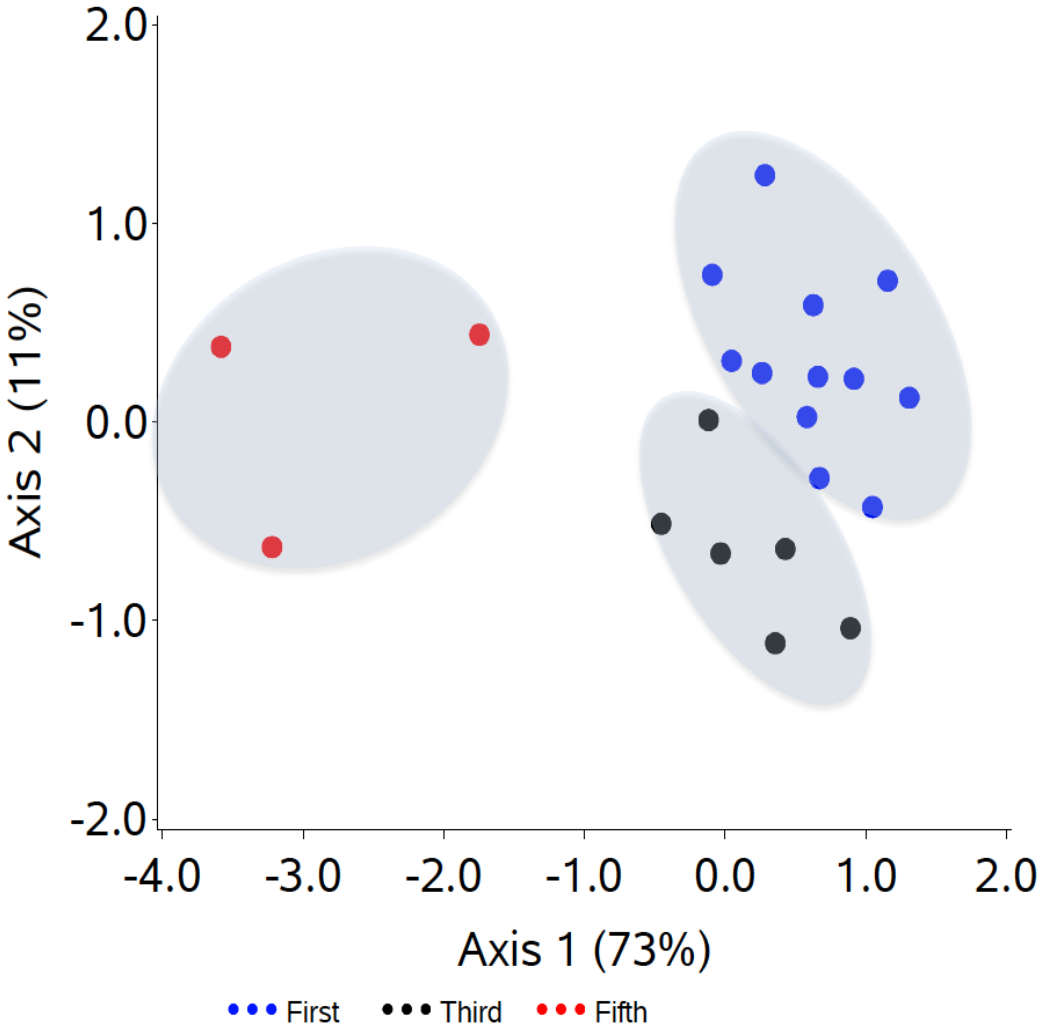
Microbial Research at SWRC



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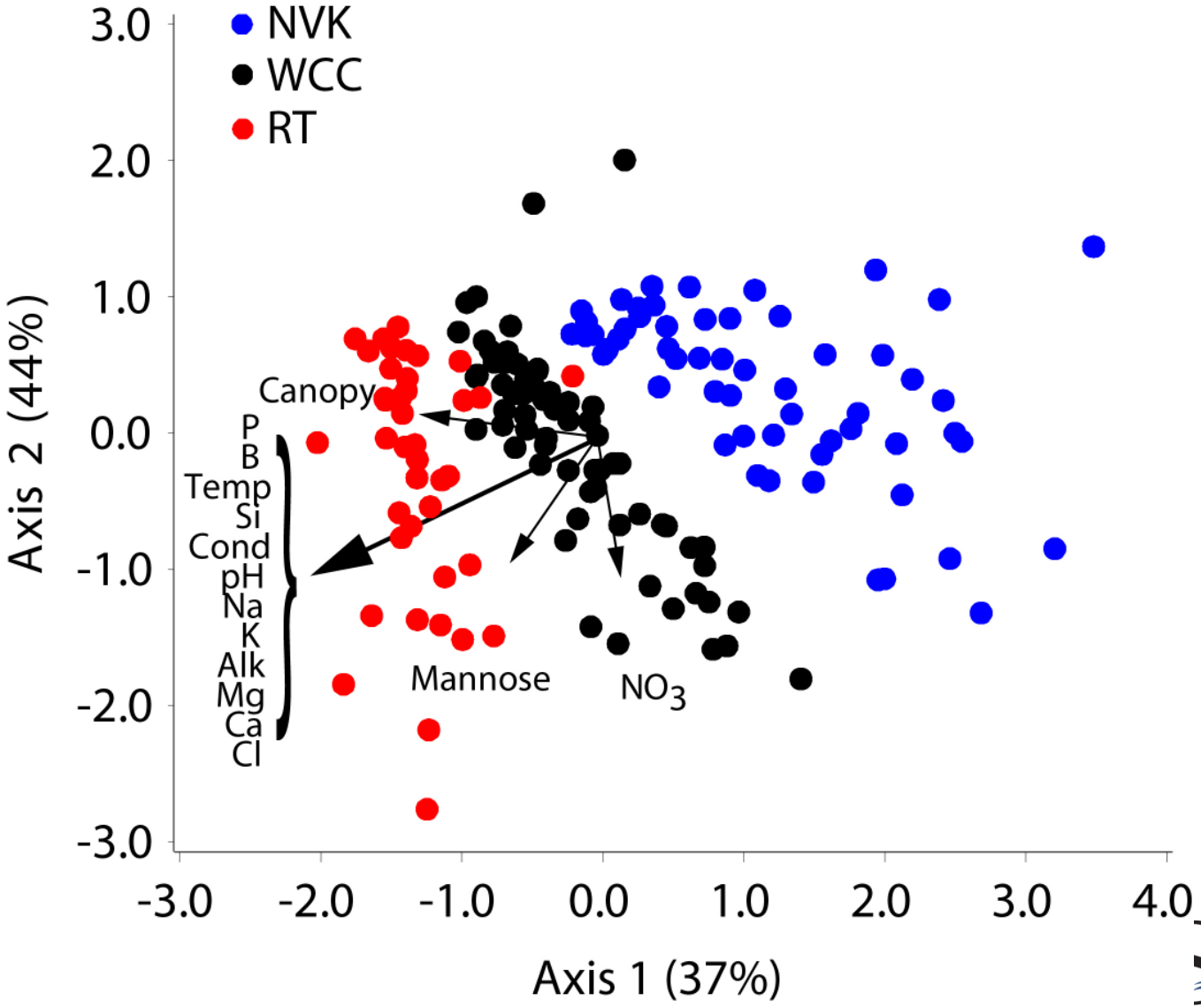
Microbial Research at SWRC



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IV 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)

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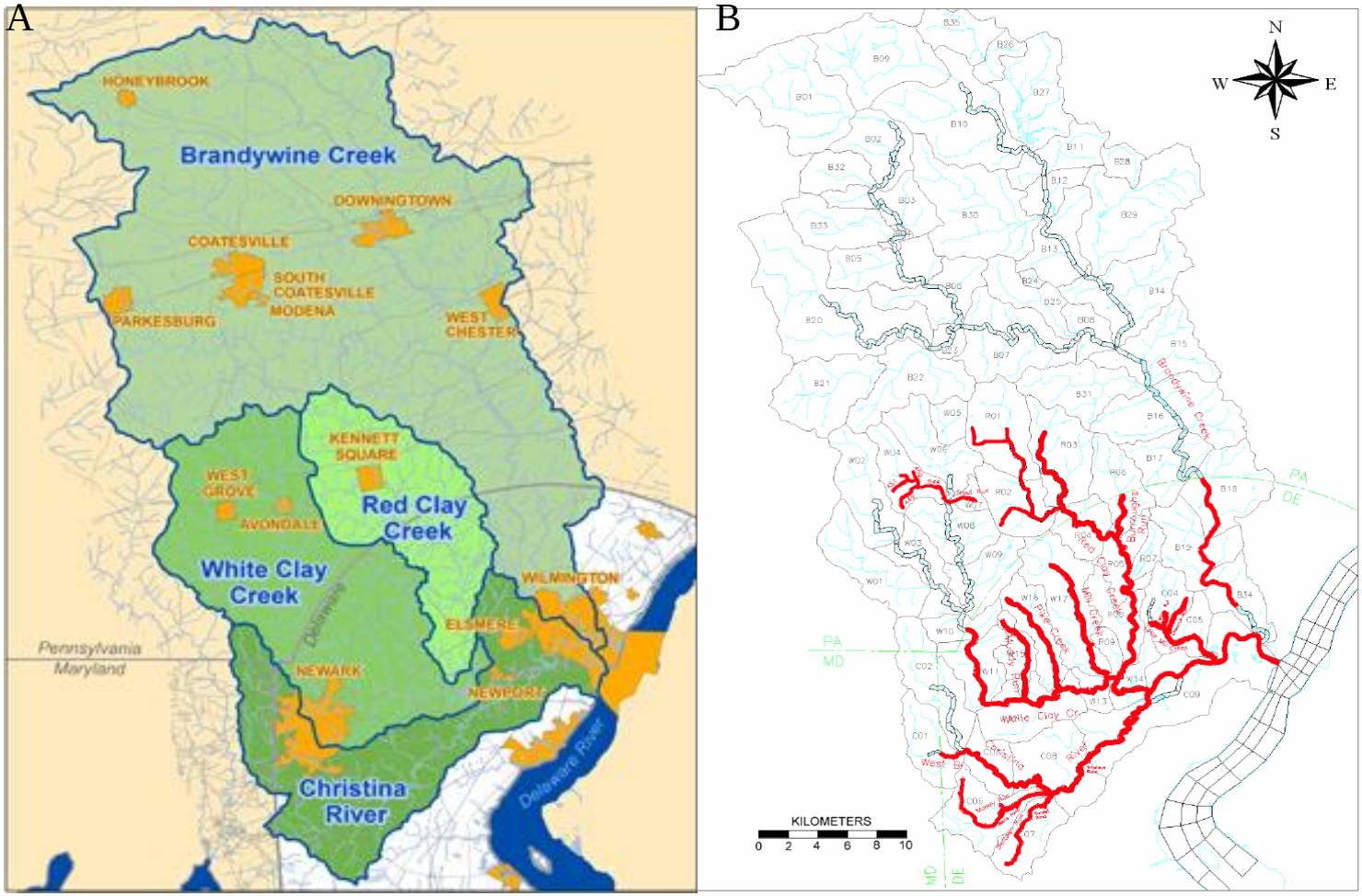
Brandywine-Christina Basin



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).

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FIB monitoring

- Pathogenic microbes are associated with fecal waste and can cause a variety of diseases, **BUT** they are very difficult to measure.

- **Fecal indicator bacteria (FIB)**:

    a. *Be present when pathogens are present and vice versa*

    b. *More numerous than pathogen*

    c. *Easier to cultivate in lab than pathogen*

    d. *More resistant to die-off in field than pathogen*

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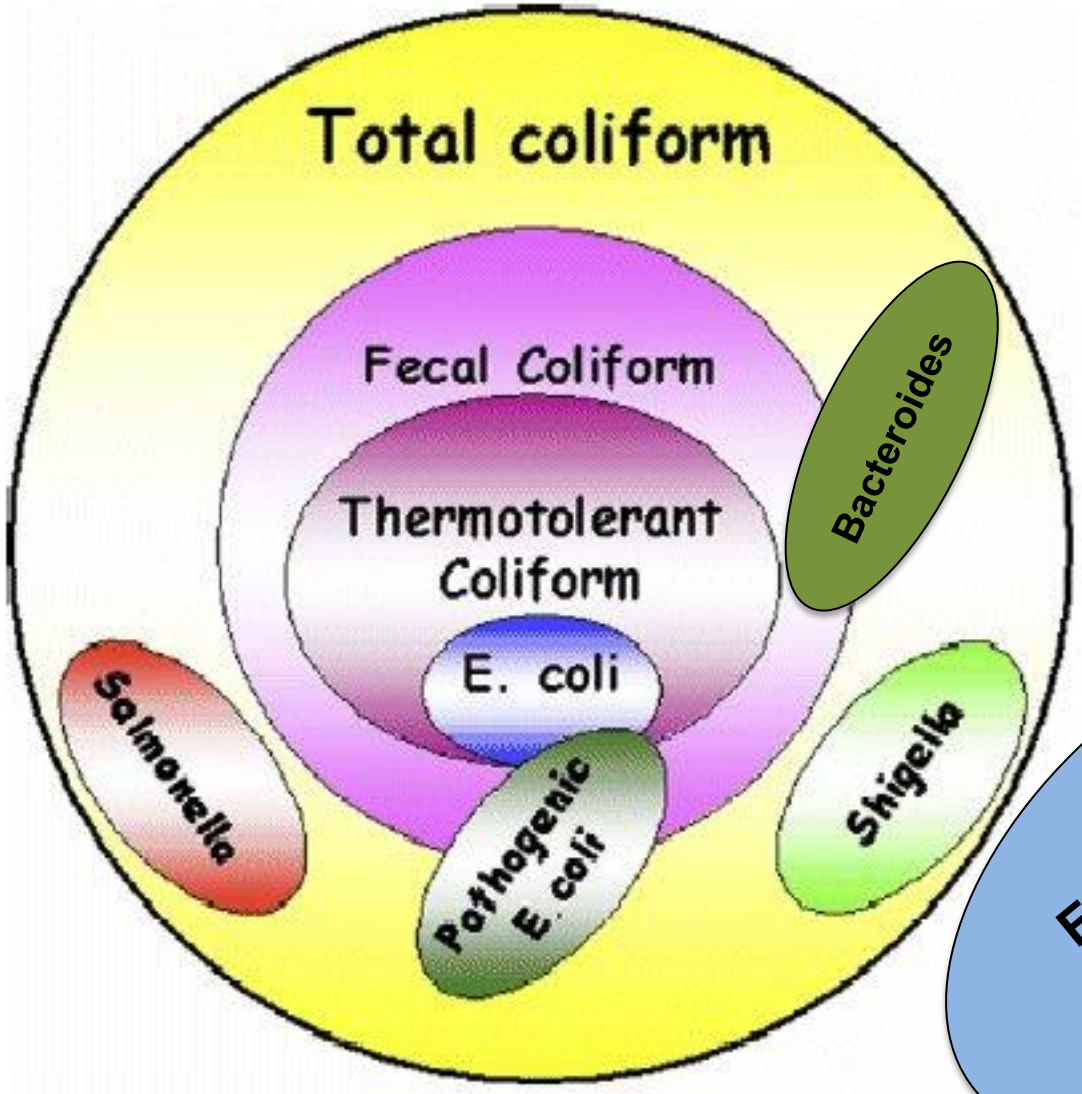
Classification and FIB



FIB:
Fecal
Indicator
Bacteria

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Coliforms and Total Coliform

- **Coliform bacteria** are a commonly used indicator of sanitary quality of foods and water. They are defined as rod-shaped Gram-negative non-spore forming and motile or non-motile bacteria which can ferment lactose with the production of acid and gas when incubated at 35–37° C. Coliforms can be found in the aquatic environment, in soil and on vegetation; they are universally present in large numbers in the feces of warm-blooded animals. While coliforms themselves are not normally causes of serious illness, they are easy to culture, and their presence is used to indicate that other pathogenic organisms of fecal origin may be present.

- Public health agencies have used **total coliforms** and **fecal coliforms** as indicators since the 1920's.

- For recreational waters, total coliforms are **no longer** recommended as an indicator. For **drinking water**, total coliforms are still the standard test because their presence indicates contamination of a water supply by an outside source.

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Fecal Coliform

Fecal coliforms, a subset of total coliform bacteria, are more fecal-specific in origin. However, even this group contains a genus, *Klebsiella*, with species that are not necessarily fecal in origin. *Klebsiella* are commonly associated with textile and pulp and paper mill wastes. Therefore, if these sources discharge to your stream, you might wish to consider monitoring more fecal and human-specific bacteria. For recreational waters, this group was the primary bacteria indicator **until relatively recently**, when EPA began recommending ***E. coli*** and ***Enterococci*** as better indicators of health risk from water contact.

Fecal coliforms are still being used in many states as the indicator bacteria.

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E.coli and *Enterococci*

- ***E. coli*** is a type of fecal coliform bacteria commonly found in the intestines of warm blooded animals and humans. E. coli is short for *Escherichia coli*. **The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination.** Sewage may contain many types of disease- causing organisms.

- ***Enterococci*** are a subgroup within the fecal streptococcus group. ***Enterococci*** are distinguished by their ability to survive in salt water, and in this respect they more closely mimic many pathogens than do the other indicators. Enterococci are typically more human-specific than the larger fecal streptococcus group. EPA recommends ***Enterococci*** as the best indicator of health risk in **salt water** used for recreation and as a useful indicator in **fresh water** as well.

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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.

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State Bacteria Standards

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

Portable 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*

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Monitoring Methods

- **Membrane filtration (MF)**
  - Well-established, counting colonies
  - mTEC or Coliscan
- **Pour plate/direct inoculation**
  - Similar to MF, counting colonies; sample water mixed with media
  - Coliscan Easygel or Petrifilm (3M)
- **Multiple tube fermentation (MTF)**
- **Most probable number**
  - IDEXX Quanti-tray
- **Chromogenic and fluorogenic substrate**
  - Colisure
  - IDEXX Quanti-tray

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Method selection

- Use stringent, EPA-approved methods if:
  a) Providing data to State or other regulatory body
  b) Providing scientific evidence of a problem
  c) Other reasons to provide defensible data

- Use simple methods if:
  a) New bacteria monitoring program without funds for big investments;
  b) Data for internal management decisions only
  c) Just need sense of baseline or magnitude of bacteria in your area of concern

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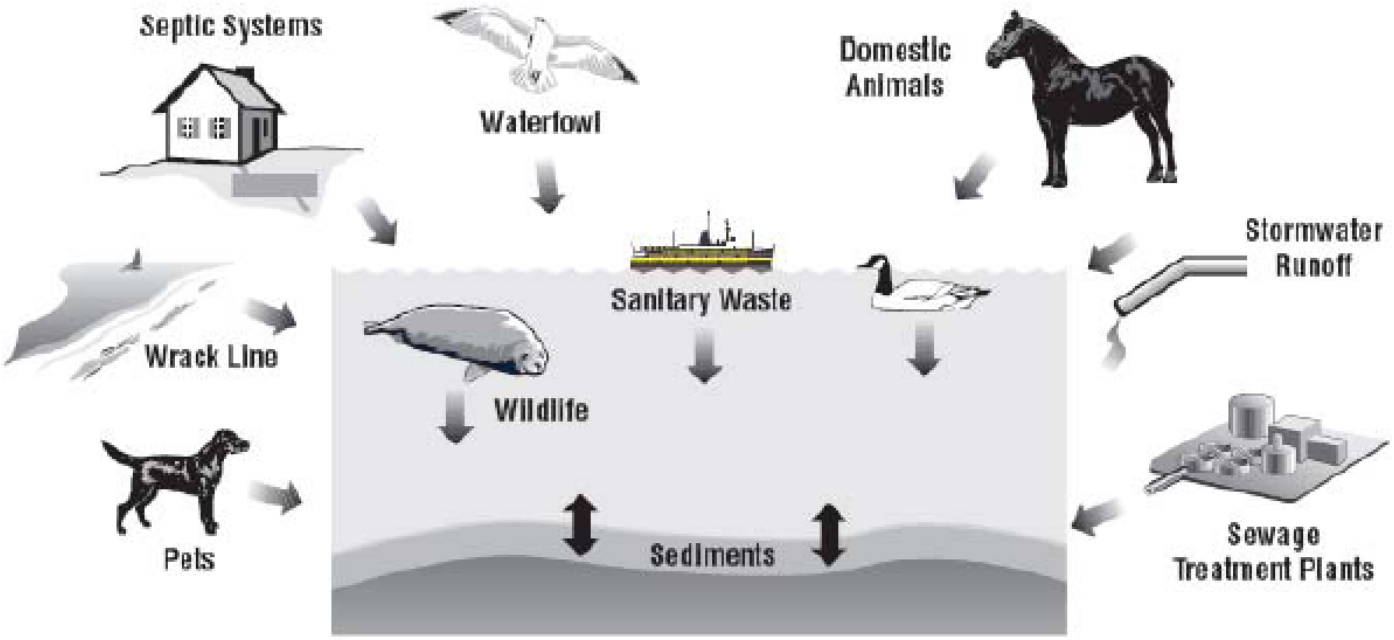
Source of bacterial contaminants



- Waste water treatment plants: sewer breaks, sewer overflows, and sewer misconnections
- On-site septic systems
- Human feces
- Livestock
- Pet waste and wildlife
- Storm runoff

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Source Tracking

- Cultivation vs. cultivation-independent
- Genotypic vs. phenotypic

- DNA-based host-specific PCR approach, or community fingerprinting/sequencing (requires comparing water samples vs. host reference library)
- Bacterial host-specific genes (e.g., *Bacteroides* sp. 16S rRNA gene sequences) qPCR approach

Summary/revisiting learning objectives

- Microorganisms (mainly bacteria) are small free-living organisms, and they are widely distributed in freshwater environments and very diverse.
- Bacteria are critical and beneficial to ecosystems because they are involved in most biogeochemical cycles; they can also cause unpleasant growth and public issues.
- Scientists study/monitor microorganisms in freshwaters at different time and space scales, and also try to understand how they interact ambient environments.
- Fecal indicator bacteria (FIB) monitoring is used as one important indicator for water quality and different approaches have been developed. Source tracking techniques can facilitate the identification of potential sources.

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Thank you!

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Stroud Water Research Center

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610 268 2153 ext 280

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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]**
		********	

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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.

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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.	Bacterial quality (Counts/100 ml)	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

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