Pathogens in freshwater systems: are the *E. coli* whispering to us ?

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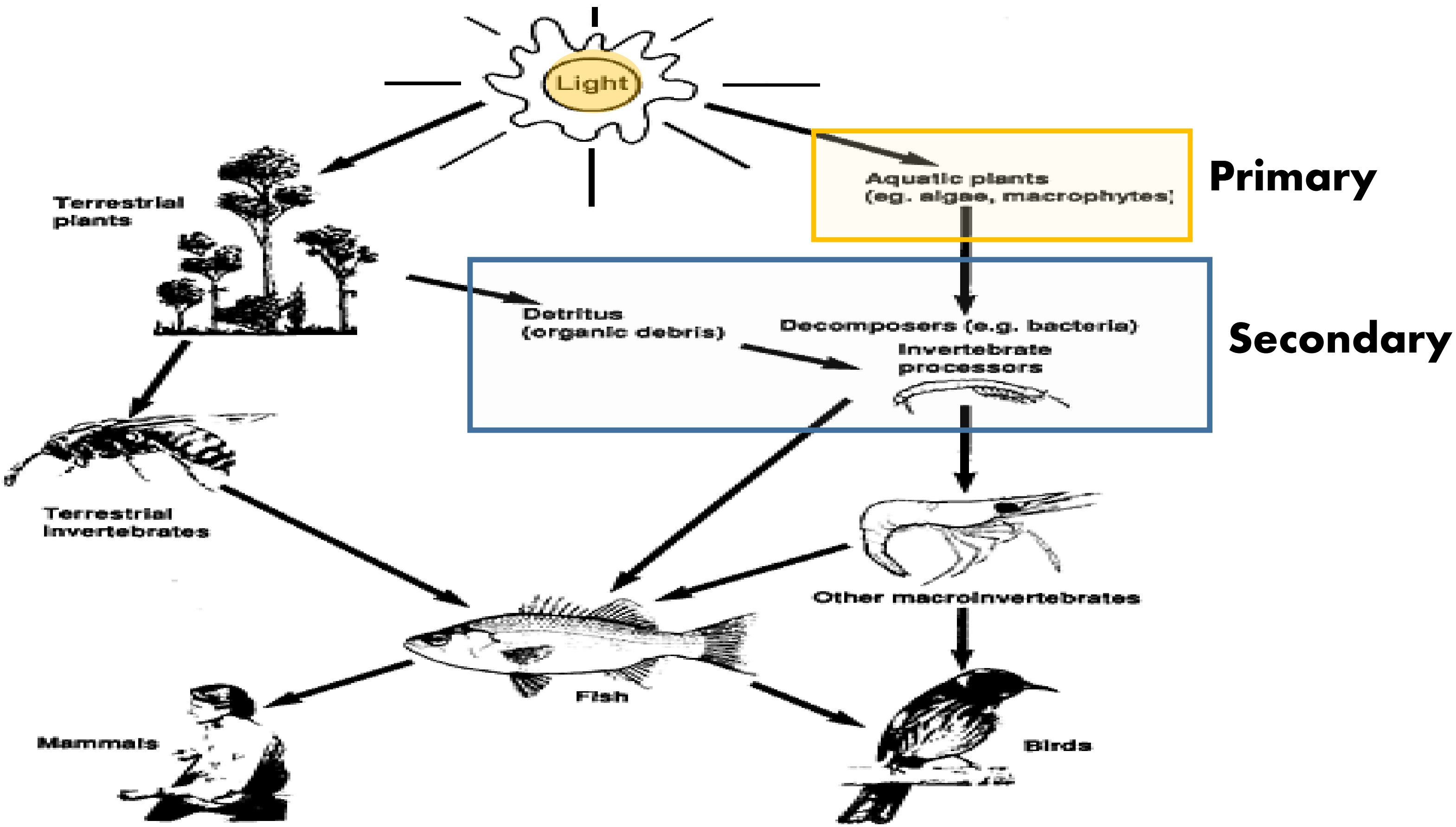
Outline -

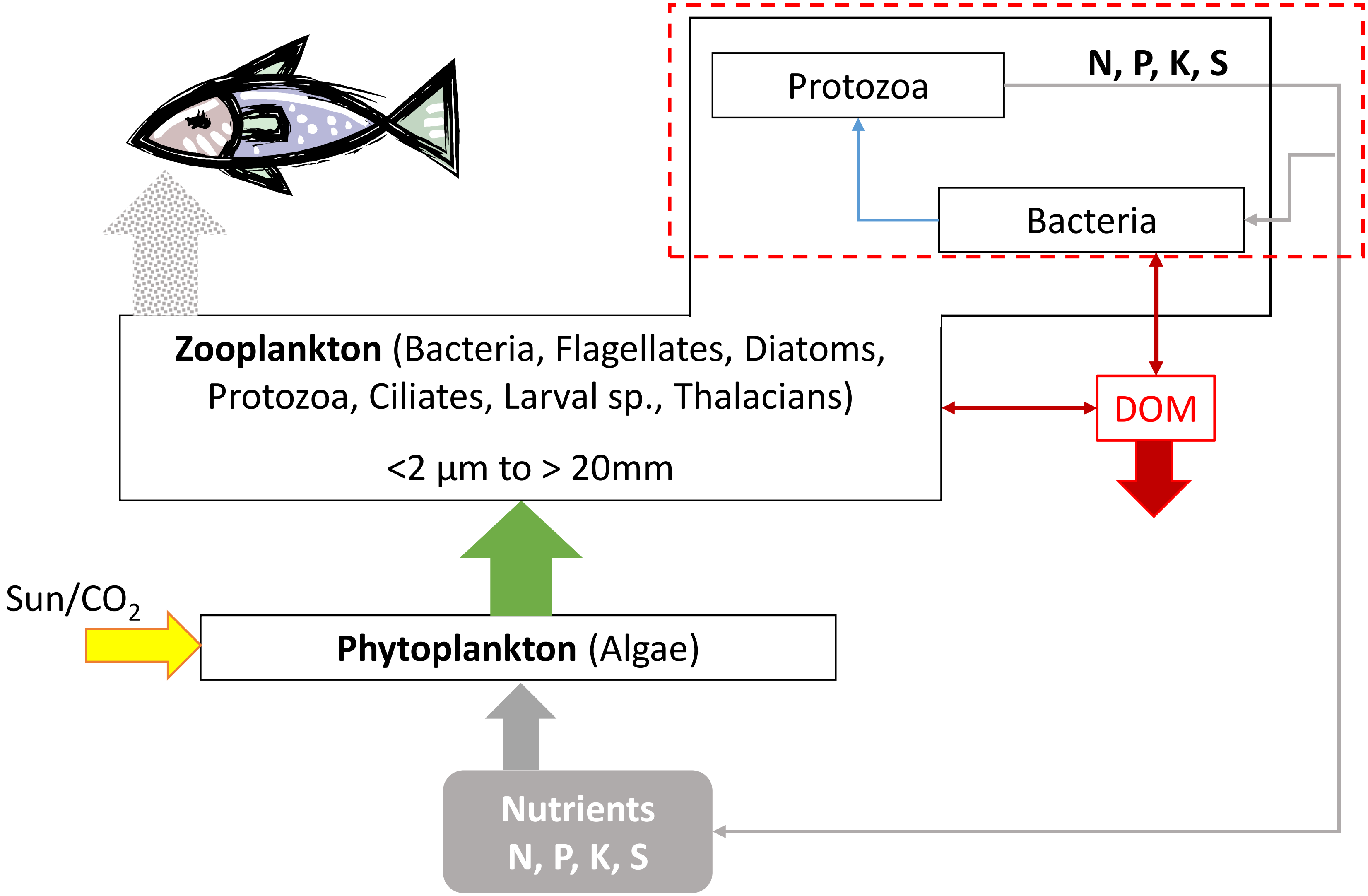
- Foodwebs of the water environment
- What are Microorganisms
  - Forms and functions
- Environmental behavior of microorganisms
- What are pathogens
- What are indicators
- Water and Bacteria in Indiana

Foodweb of Water



Decomposers of fixed organic materials (detritus)
Fix nitrogen (bacterial process only)
Food for higher level organisms

The real roles of bacteria


N, P, K, S
Protozoa
Bacteria
DOM
Zooplankton (Bacteria, Flagellates, Diatoms, Protozoa, Ciliates, Larval sp., Thalacians)
<2 µm to > 20mm
Sun/CO_2
Phytoplankton (Algae)
Nutrients
N, P, K, S

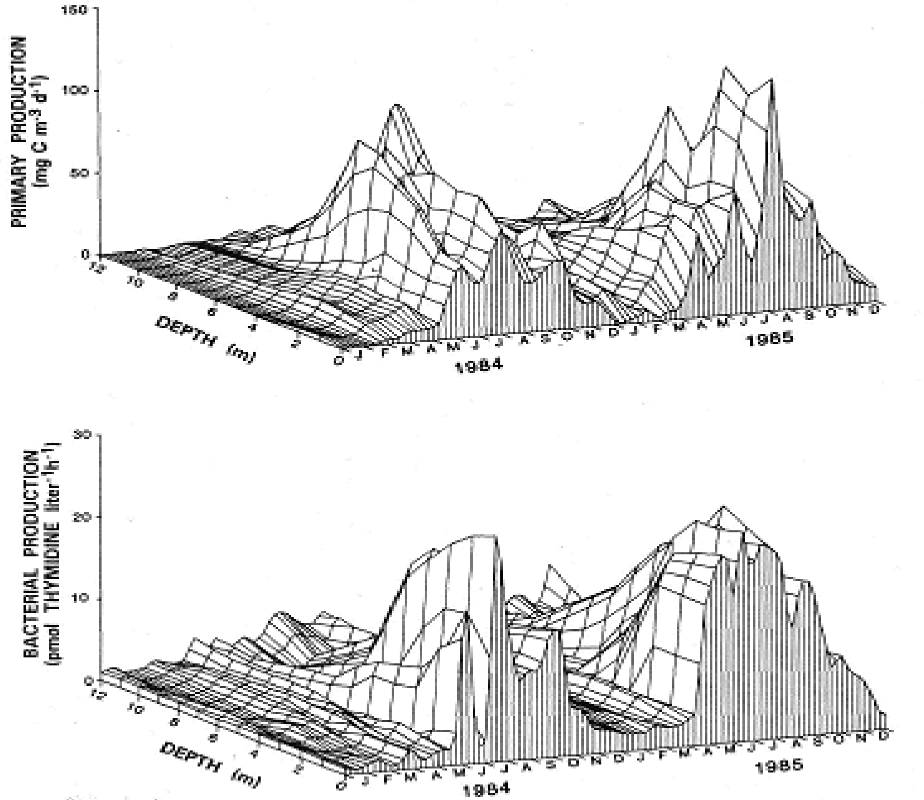


FIGURE 17-2 Comparison of simultaneous measurements of production of phytoplankton (*upper*) and bacterioplankton (*lower*) in oligotrophic–mesotrophic Lawrence Lake, southern Michigan, over a 2-yr period. Bacterial production was determined from *in situ* rates of conversion of radiolabeled thymidine to DNA of the bacteria. (From Coveney and Wetzel, 1995.)

<u>Primary Production</u> /
CO_2 and Light

<u>Secondary Activity</u> /
Bacterial Metabolism (up to 60%)
Cell Production

At the basic level, water is a microbial system
Dissolved Organic Matter (DOM) drives most of the activity in streams and rivers
Secondary nutrients can be limiting (N,P)

Mineral Nutrients

Elemental makeup of microbial dry matter

	%%%
Carbon	50
Oxygen	20
Nitrogen	14
Hydrogen	8
Phosphorus	3
Sulfur and Potassium	2
Calcium and Magnesium	0.5

Mass of one E. coli: 1×10^{-12} g

1,600,000,000 cells of *Escherichia coli* would weigh a gram.

http://hypertextbook.com/facts/2003/LouisSiu.shtml

What do bacteria need to grow?

- Water (good osmatic conditions)
- Mineral nutrients (N,P,K)
- Energy sources (Carbon, Sunlight, Rocks)
- Carbon sources (CO_2 or Organic C)
- Electron donors (C, Light, Minerals)
- Electron acceptors (O_2, NO_3, Fe^{+3}, Oxidize C)

Micronutrients

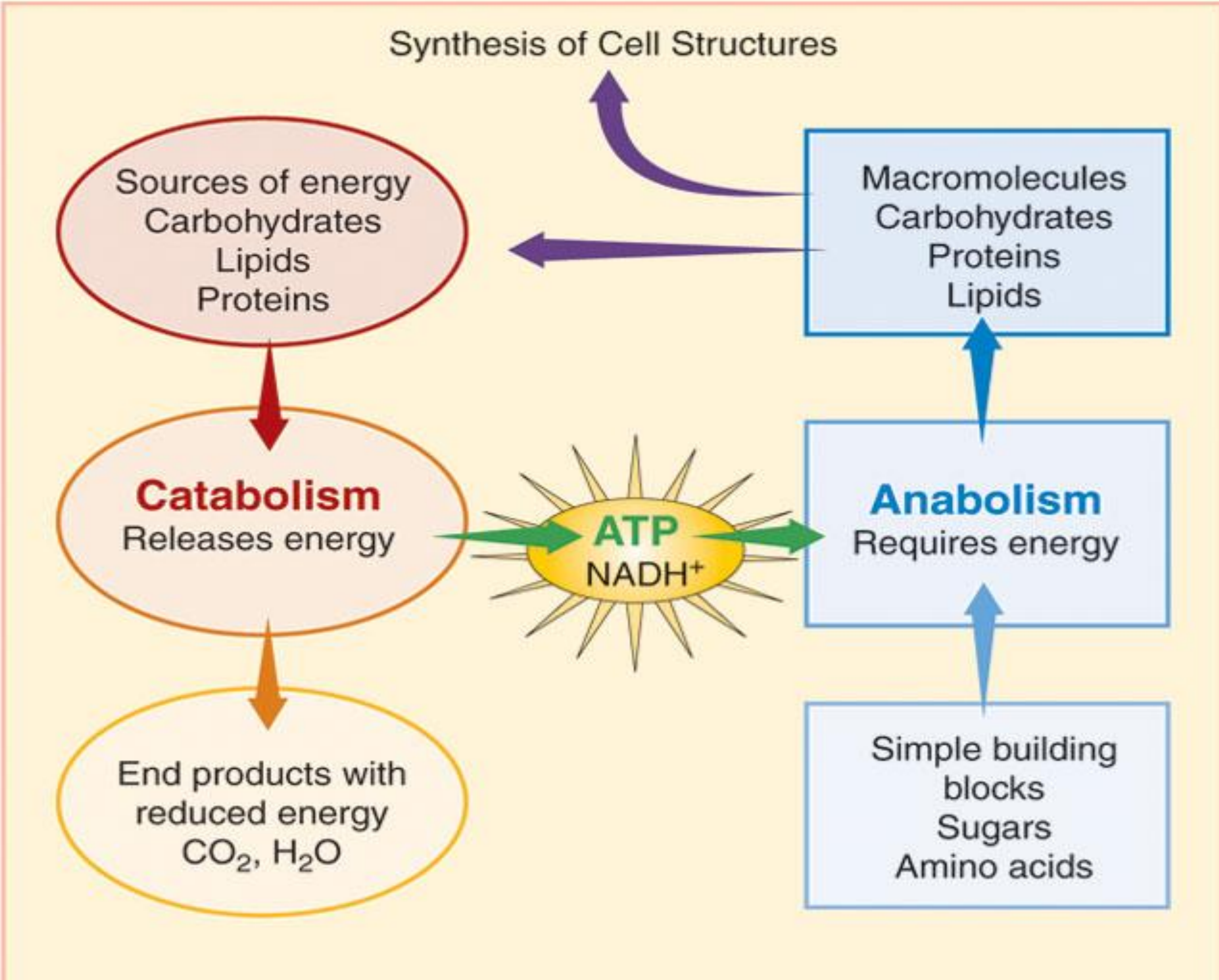
- Metal cofactors required by the microbes

  Cofactor is a non-protein compound required to make enzymes and coenzymes

    - Fe – cytochromes in ET
    - Mn – dismutates and photosynthesis
    - Zn – DNA polymerase
    - Cu – reductases
    - Co – nitrogenase
    - Mo – nitrate reductase
    - Ni – urease
    - Others – Va, Cl, Na, B, and Se

Carbon and Energy Sources

- Phototrophs – light and CO_2
- Chemotrophs – organisms that obtain energy by the oxidation of electron donating materials found in their environment
  - Lithotrophs (mineral)
    - Oxidation of Iron
  - Organotrophs (organo)
    - Oxidation of dead plant materials

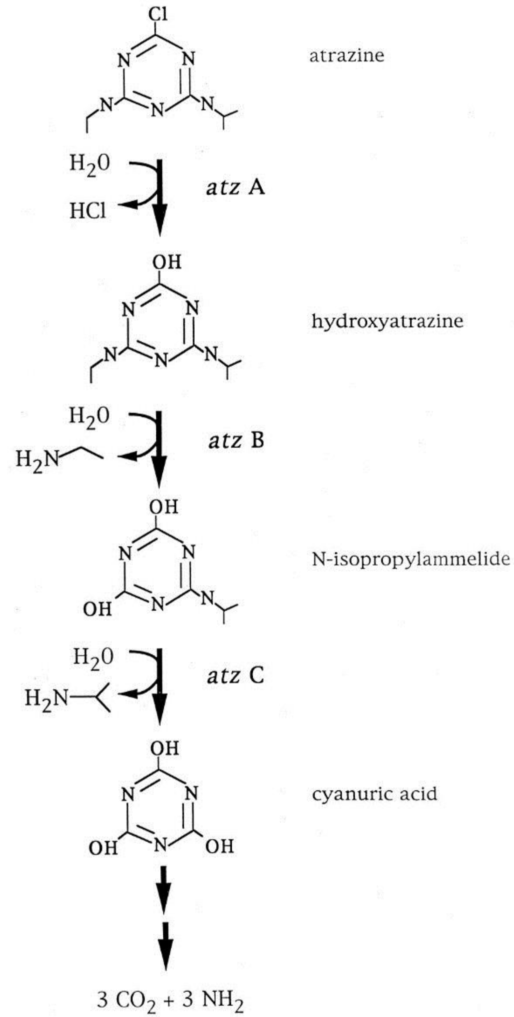

Synthesis of Cell Structures
Sources of energy
Carbohydrates
Lipids
Proteins
Macromolecules
Carbohydrates
Proteins
Lipids
Catabolism
Releases energy
ATP
NADH+
Anabolism
Requires energy
End products with
reduced energy
CO_2, H_2O
Simple building
blocks
Sugars
Amino acids

Enzymes – are the power!

- The organic materials (pollutants) may be degraded as a substrate (i.e. a good food source)
  - Some enzymes are specific to a particular chemical and do not degrade or transform other molecules

- Or they may be degraded via cooxidiation (i.e. the bacteria generally prefer something else but have the enzymes to partially degrade the pollutant.)
  - Other enzymes are non-specific and result in the “random” transformation of pollutants - this is the explanation for cooxidation.
  - Maybe membrane bound or free

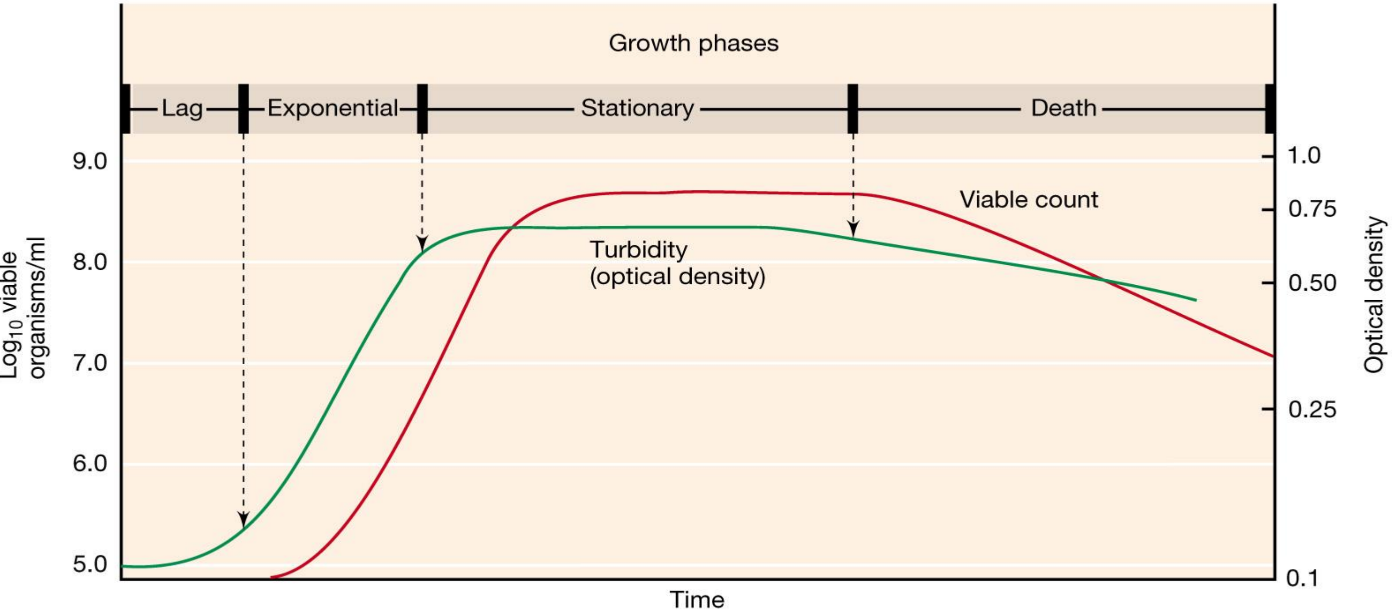
Atrazine catabolic pathway identified inPseudomonas sp. strain ADP, showing the first three enzymatic reactions encoded by atzABC (7, 17,32).



de Souza M L et al. Appl. Environ. Microbiol. 1998;64:178-184

Oxygen and Nitrate

- The solubility of O_2 in water is 0.028 mL O_2 mL^{-1} H_2O atm^{-1} or as the more common expression 8 mg O_2 L^{-1}.
- The diffusion rate of O_2 in water is about 1 x 10^{-4} of the diffusion of O_2 in air.
- The O_2 diffusion coefficient is 2.5 x 10^{-5} cm^2 sec^{-1} in water versus 0.189 cm^2 sec^{-1} for air.
- Nitrate solubility is infinite (almost).



Time (hours)	Number of cells
0	1
0.5	2
1	4
1.5	8
2	16
2.5	32
3	64
3.5	128
4	256
4.5	512
5	1024
5.5	2048
6	4096
.	.
.	.
10	1,048,576

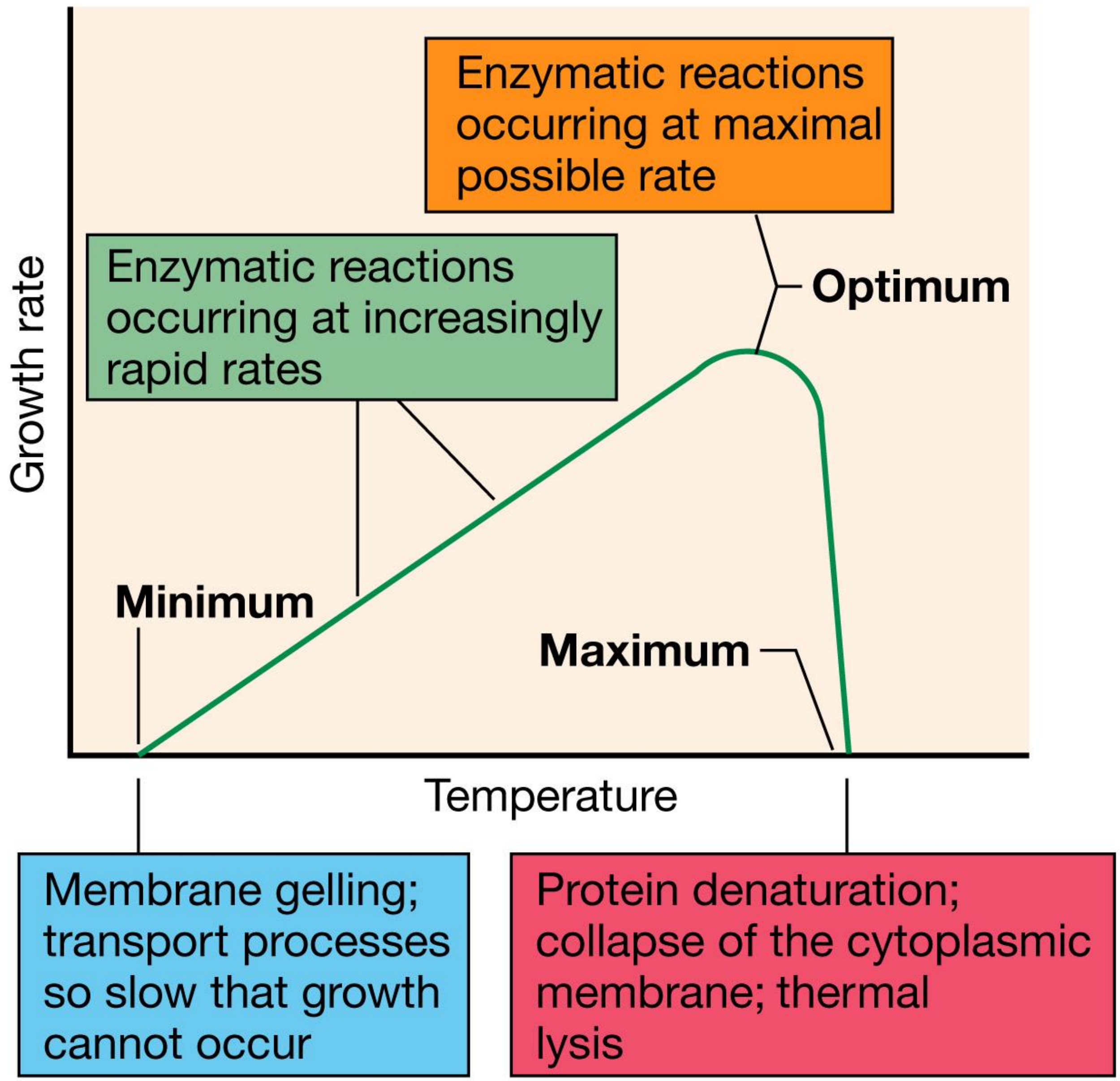
Population size is:

$x = x_o 2^n$

x_0 = initial population (1)

x = final population (after time 10 hrs) 1,048,576 cells

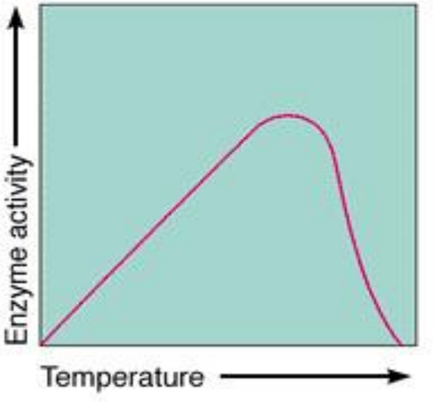
n = no. of generations (20 generations)


Enzymatic reactions
occurring at maximal
possible rate
Optimum
Enzymatic reactions
occurring at increasingly
rapid rates
Growth rate
Minimum
Maximum
Temperature
Membrane gelling;
transport processes
so slow that growth
cannot occur
Protein denaturation;
collapse of the cytoplasmic
membrane; thermal
lysis

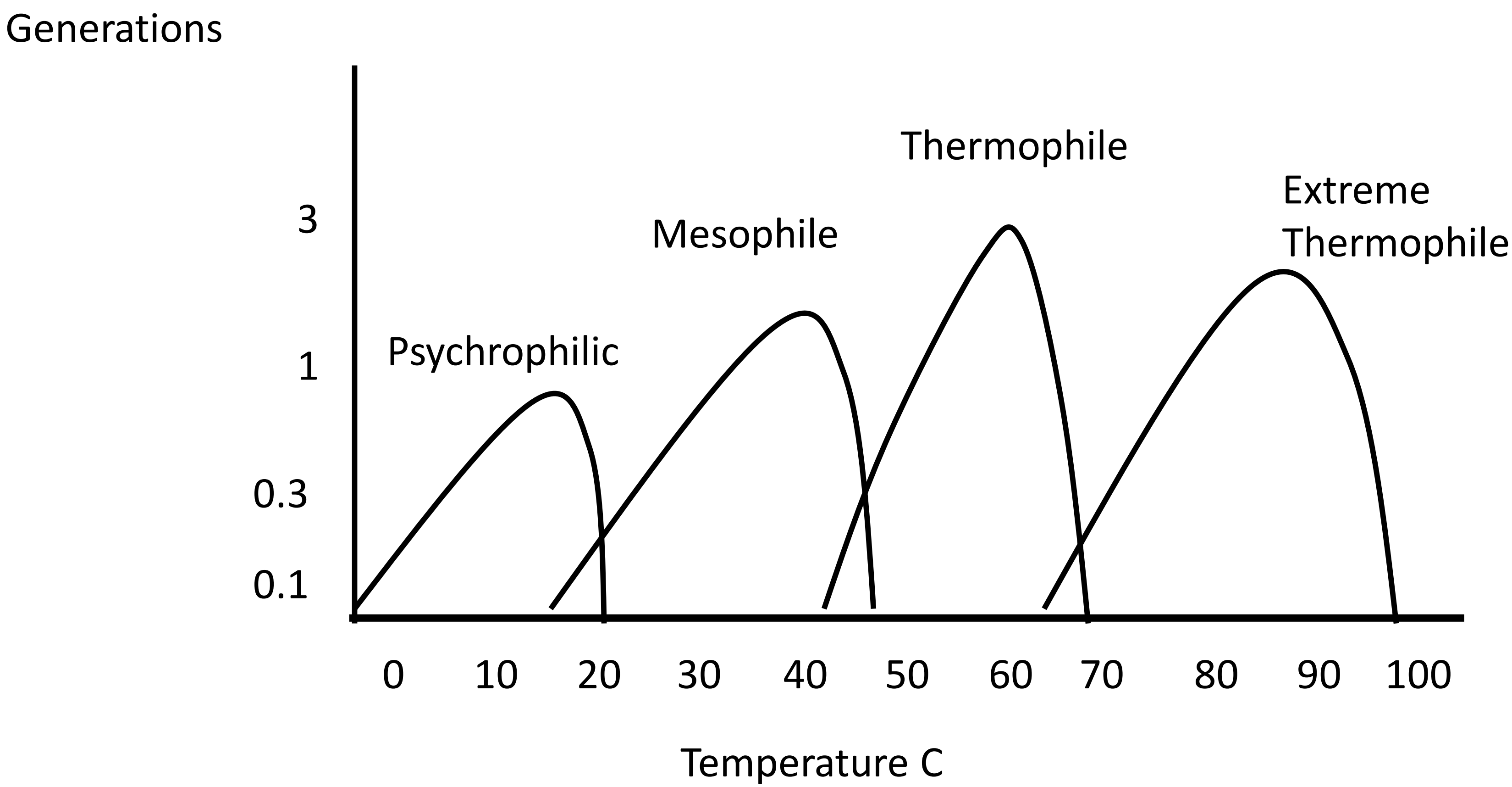
From Brock

Temperature
- Factors affecting activity

- Enzyme activity increases with increasing temperature.
- Activity drops when heat denatures enzyme.
- Activity drops with cold / alters membrane function



A Population Response



pH

The internal pH reflects the type of microbe:

Acidophiles: 6.5
Neutralphiles 7.8
Alkalophiles 9.5

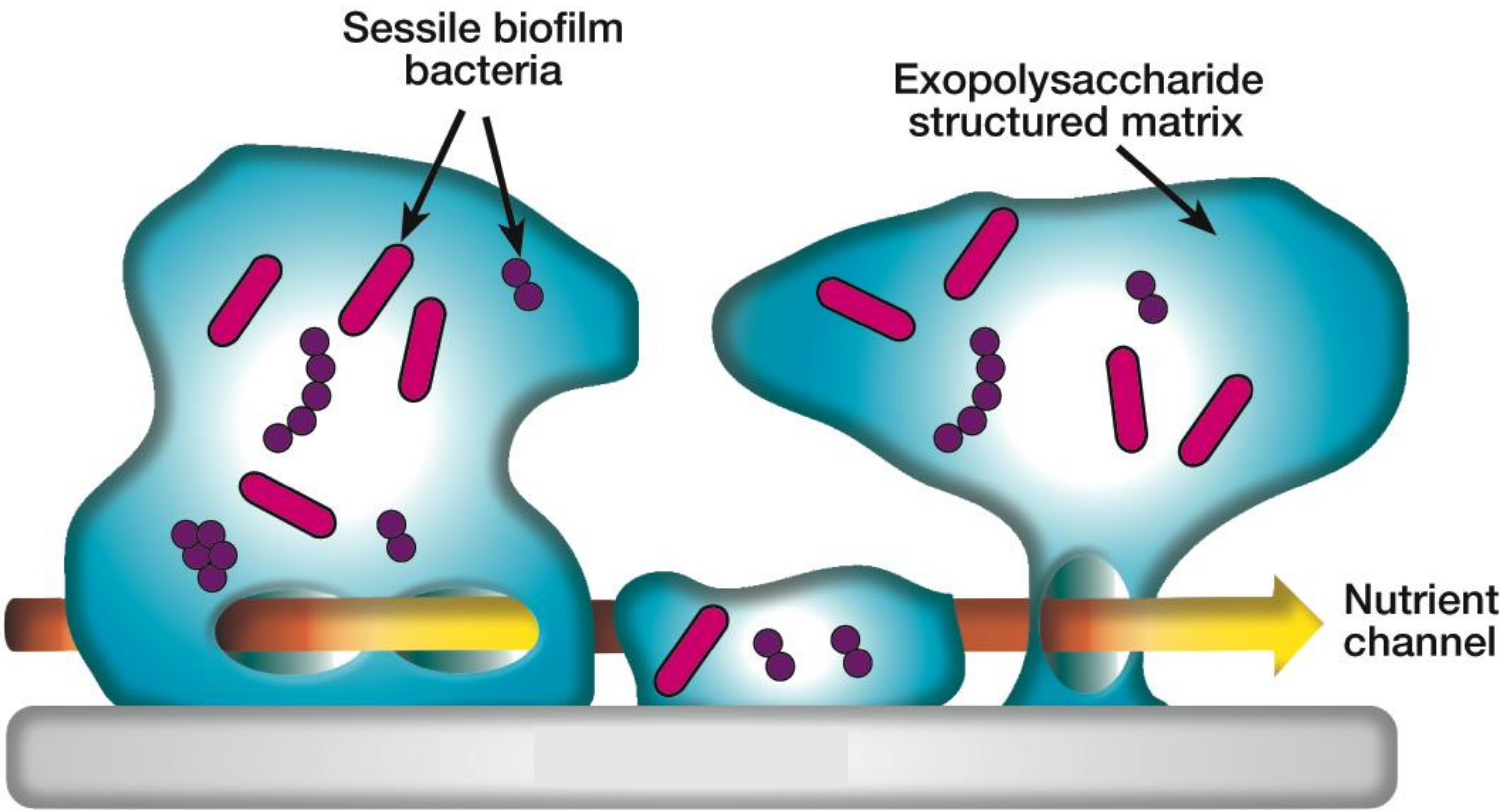
The optimal values for bacteria vary but the minimum tends to be about 4.4 and the max is some where near 9.

As pH goes up, metals become unavailable as pH goes down metals become very available and possible toxic

How do the residents live ?

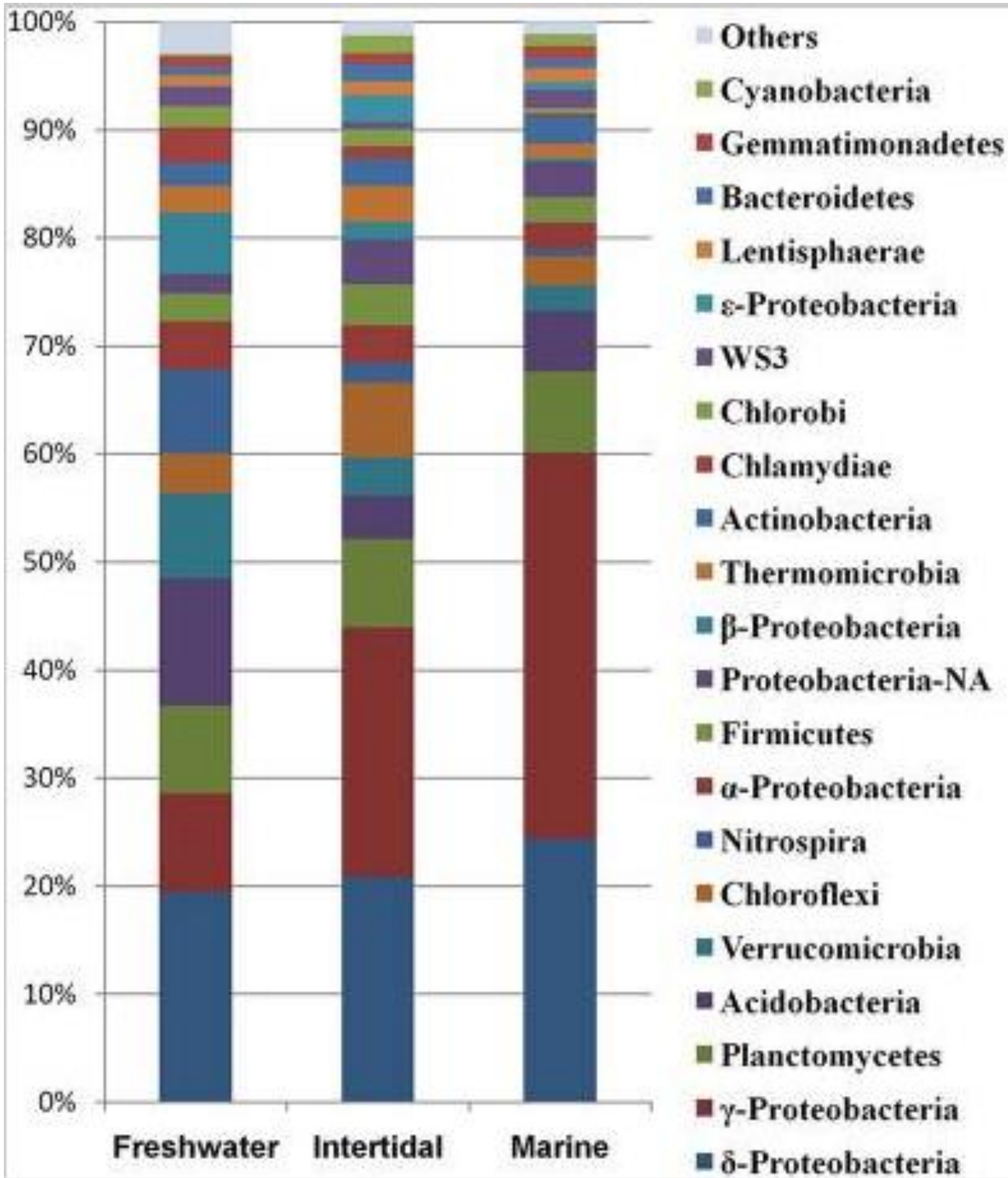
- The particle surface is a better location than the solution as the surfaces will collect and concentrate nutrients
  - This is true for cation, anions and dissolved organic materials as well.
- Enhances opportunity for genetic exchange
- Allows concentrated deposition of enzymes onto a common target
- Survival of the species in the event of colony death (a few members inside of the colony tend to stay alive).

Structure of a polymicrobial biofilm on tooth/rocks/soil surface
(or why do I need to floss?)



Resident Population is attached to surfaces
Offers protection from the Microbial Loop

CSH PRESS
NCBI
Essentials of Glycobiology
Second Edition

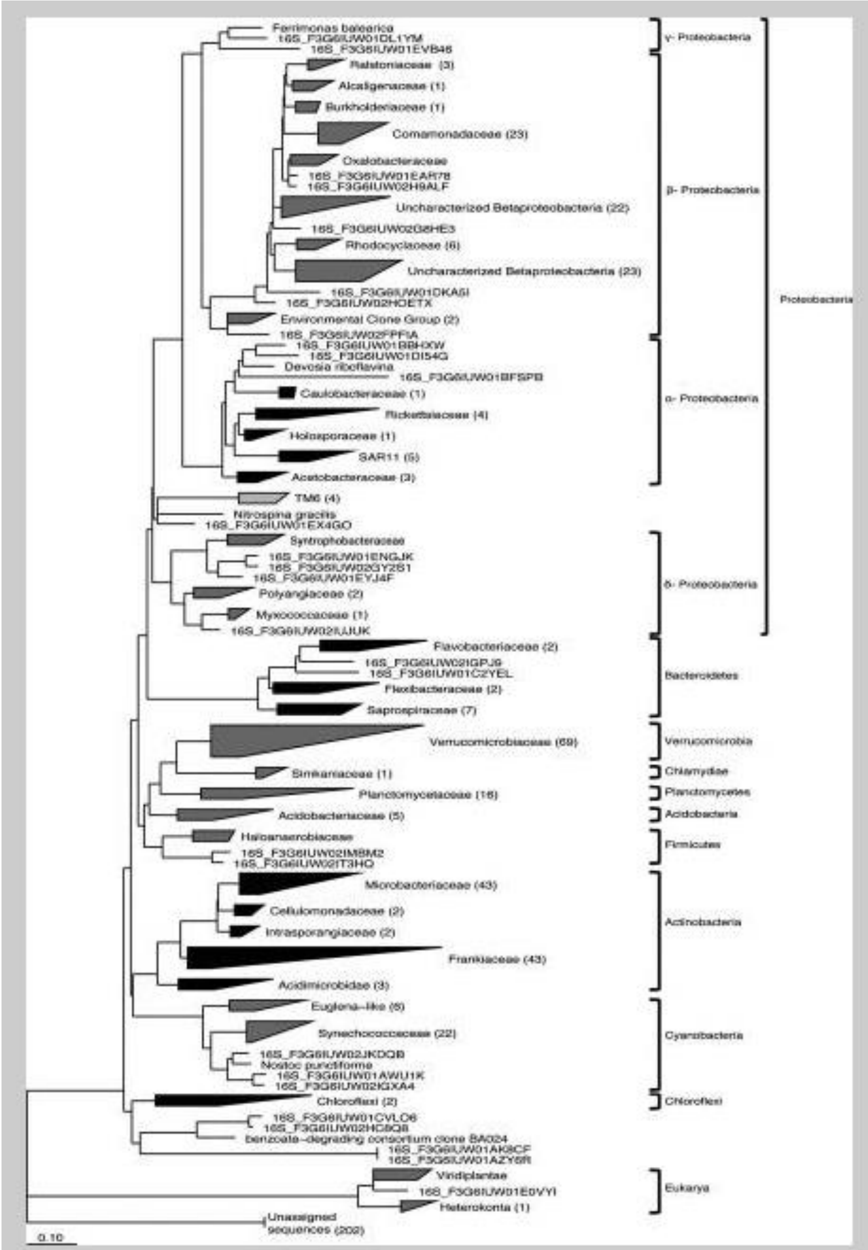


Illumina reads to
Phylum level in sediments

Diversity in 3 water types

[Appl Environ Microbiol. Dec 2012; 78(23): 8264–8271.]

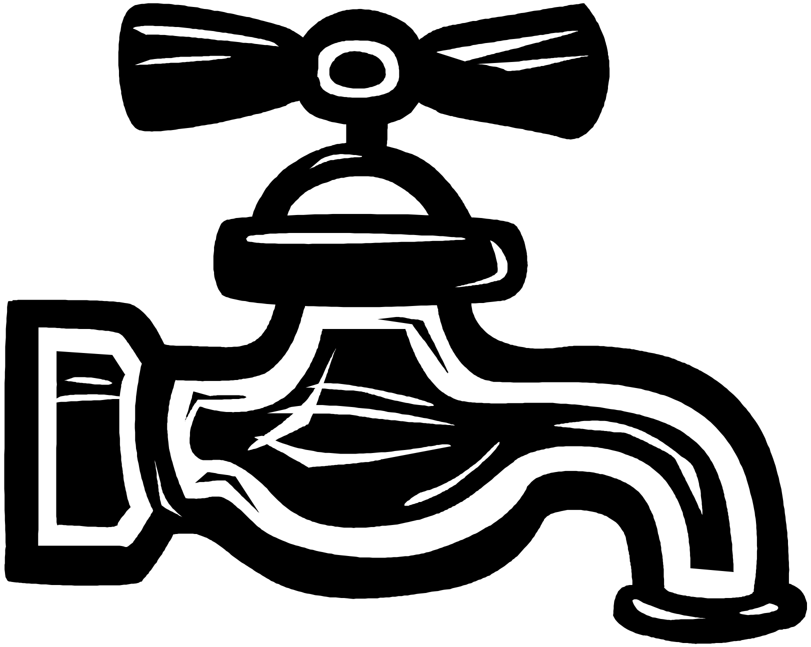
Lake Lanier, a Temperate Freshwater Ecosystem

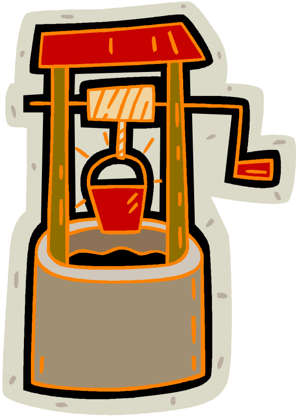


Illumina reads to
Phylum level in water

Appl Environ Microbiol. Sep 2011; 77(17): 6000–6011.

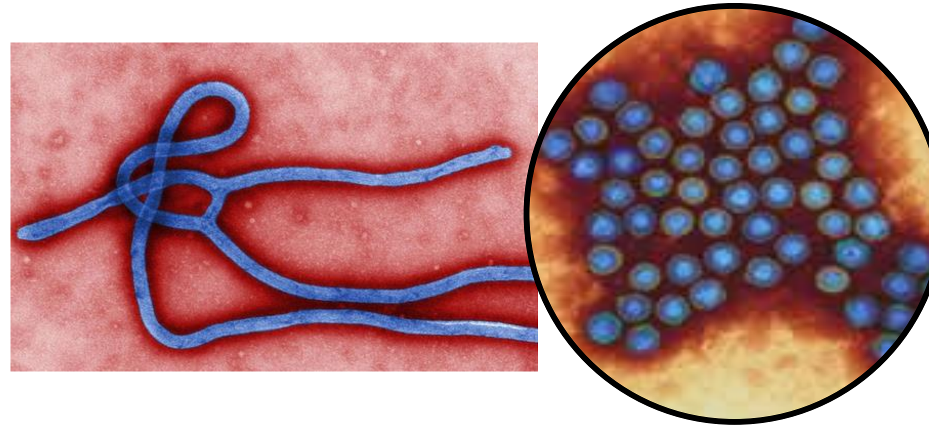
Drinking or *potable water* is water that is free from pathogens and chemicals that are dangerous to human health.





The Pathogens

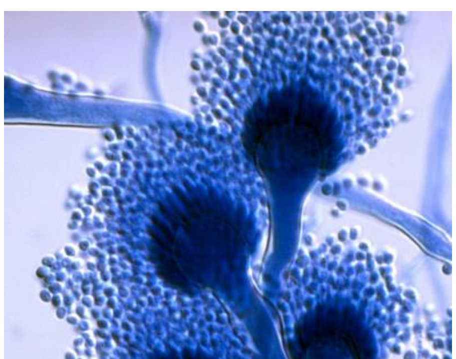
Bacteria



Viruses

Protozoa

Fungi



Prions

Pathogens

Derived from the Greek "birth of pain"

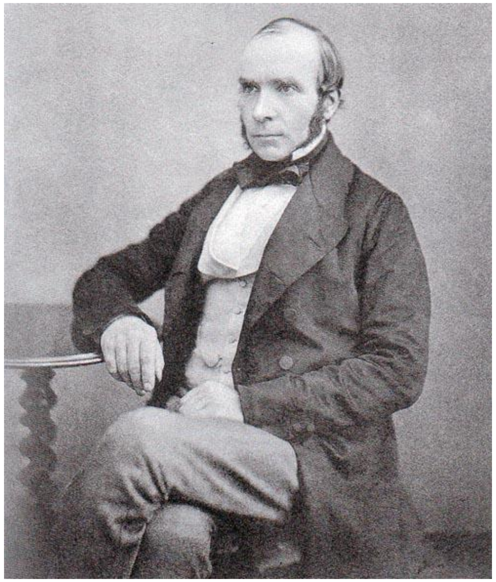
- **Pathogens:** are ***biological agents*** that cause a disease (i.e., illness) in a host.
- **Most Pathogens don't survive outside of their host**
- **Environmental Pathogens:** Microorganisms that normally spend a substantial part of their lifecycle outside human hosts, but when introduced to humans cause disease with a measurable frequency.
  - Soil and Water can be a reservoir of infection.

Pathogens -- problem long recognized

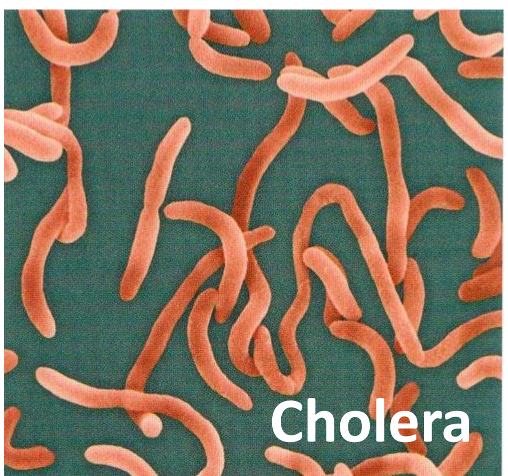
- In ancient Rome (6oo B.C.), aqueducts were built to supply water.
- The community employed a "Water Commissioner" to oversee to the safety of the public water supply.
- Contamination of the water system was punishable by ***death***.

Cholera outbreak in London, 1854
Vibrio cholera

- Cholera was found to occurred in the Soho district of London, but not in all districts
  - No connection to bacteria at that time
- Over 600 people died from cholera in a 10-day period
- John Snow, used locational data and maps to show the **Broad Street well** was the source of contamination
- He showed an underground cesspool located only a few feet from the well was the source.
- For surface water this is occurring in Africa and other places in the world.



John Snow
(1813–1858)



[Andrew Hill](http://www.guardian.co.uk/news/datablog/interactive/2013/mar/15/cholera-map-john-snow-recreated)

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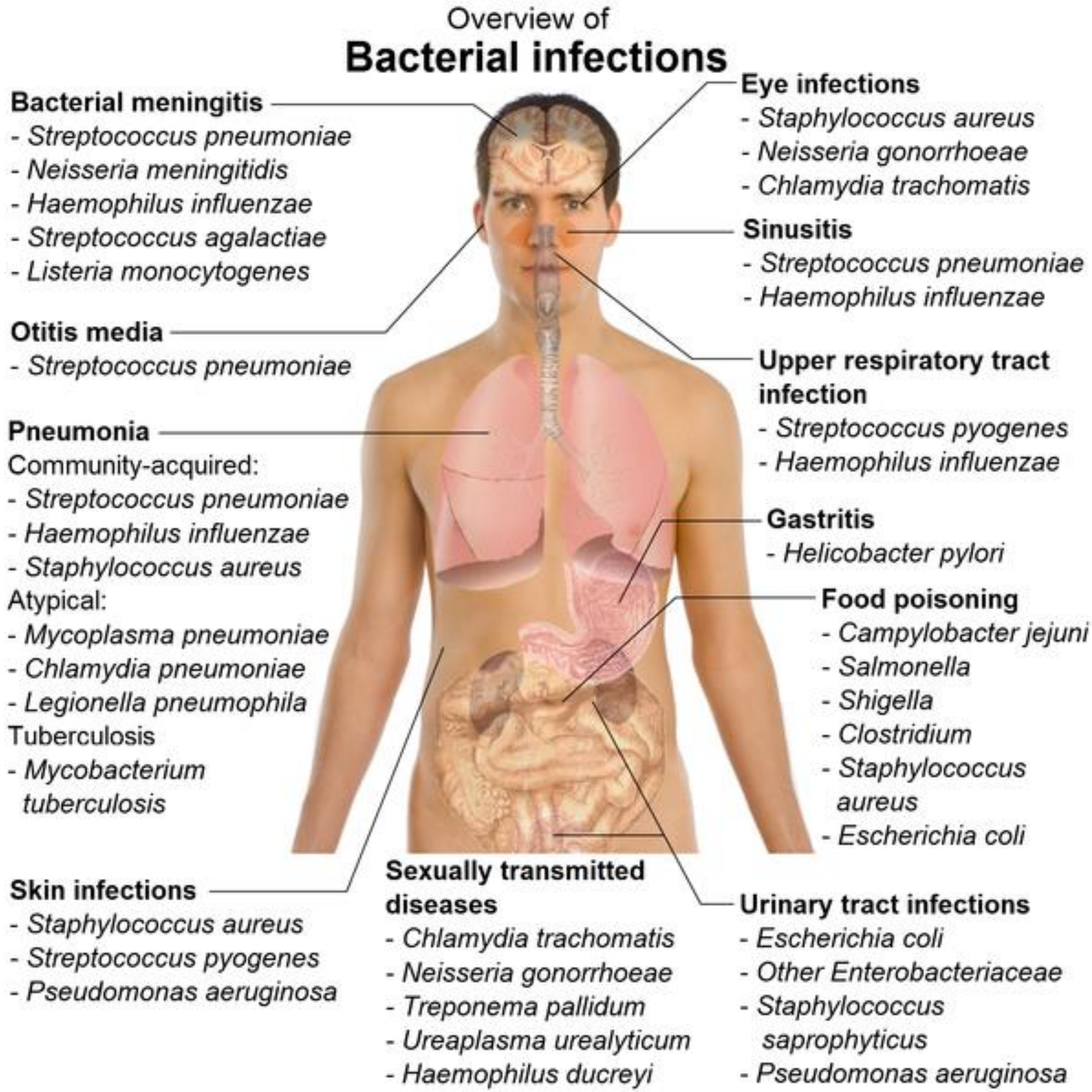
How we interact with the Microbial World

	Organism 1	Organism 2	Example
Mutualism	Benefits	Benefits	Bacteria in human colon
Commensalism	Benefits	Neither benefits nor is harmed	*Staphylococcus* on skin
Parasitism	Benefits	Is harmed	Tuberculosis bacteria in human lung
		YOU	


Overview of
Bacterial infections
Bacterial meningitis
- Streptococcus pneumoniae
- Neisseria meningitidis
- Haemophilus influenzae
- Streptococcus agalactiae
- Listeria monocytogenes
Otitis media
- Streptococcus pneumoniae
Pneumonia
Community-acquired:
- Streptococcus pneumoniae
- Haemophilus influenzae
- Staphylococcus aureus
Atypical:
- Mycoplasma pneumoniae
- Chlamydia pneumoniae
- Legionella pneumophila
Tuberculosis
- Mycobacterium tuberculosis
Skin infections
- Staphylococcus aureus
- Streptococcus pyogenes
- Pseudomonas aeruginosa
Eye infections
- Staphylococcus aureus
- Neisseria gonorrhoeae
- Chlamydia trachomatis
Sinusitis
- Streptococcus pneumoniae
- Haemophilus influenzae
Upper respiratory tract infection
- Streptococcus pyogenes
- Haemophilus influenzae
Gastritis
- Helicobacter pylori
Food poisoning
- Campylobacter jejuni
- Salmonella
- Shigella
- Clostridium
- Staphylococcus aureus
- Escherichia coli
Sexually transmitted diseases
- Chlamydia trachomatis
- Neisseria gonorrhoeae
- Treponema pallidum
- Ureaplasma urealyticum
- Haemophilus ducreyi
Urinary tract infections
- Escherichia coli
- Other Enterobacteriaceae
- Staphylococcus saprophyticus
- Pseudomonas aeruginosa

Bacteria and Fungi

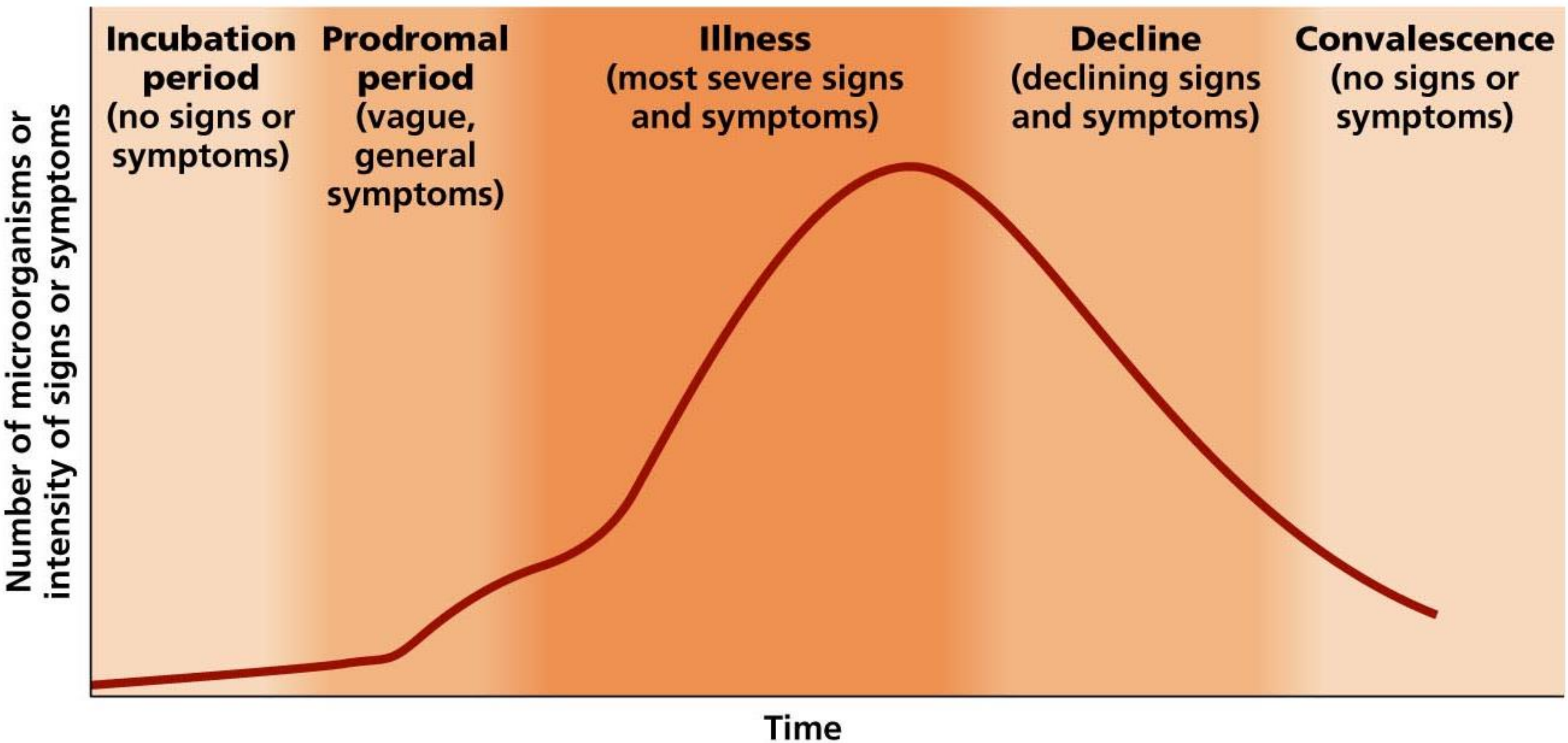
Enzymes secreted by the pathogen, dissolve structural chemicals in the body
Help pathogen maintain infection, invade further, and avoid body defenses

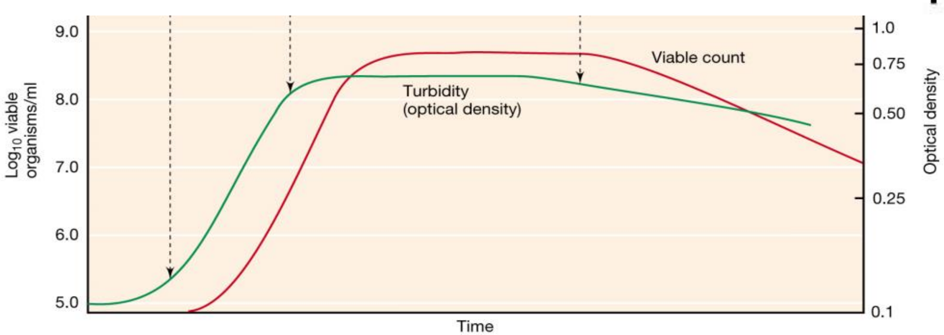
Virus

The virus reproduces by hijacking the cells of another organism (host) and getting the host cell to reproduce more viruses. This causes damage and cellular breakdown.

Protozoa

Typically water borne and the interactions occur in the hosts gut.

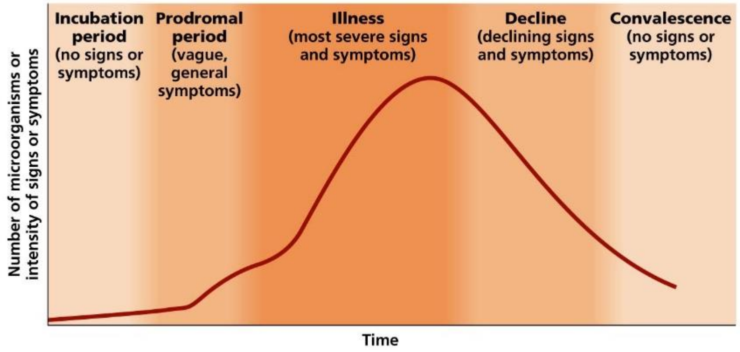




TABLE

Incubation Periods of Selected Infectious Diseases

Disease	Incubation Period
Staphylococcus foodborne infection	<1 day
Influenza	About 1 day
Cholera	2 to 3 days
Genital herpes	About 5 days
Tetanus	5 to 15 days
Syphilis	10 to 21 days
Hepatitis B	70 to 100 days
AIDS	1 to >8 years
Leprosy	10 to >30 years



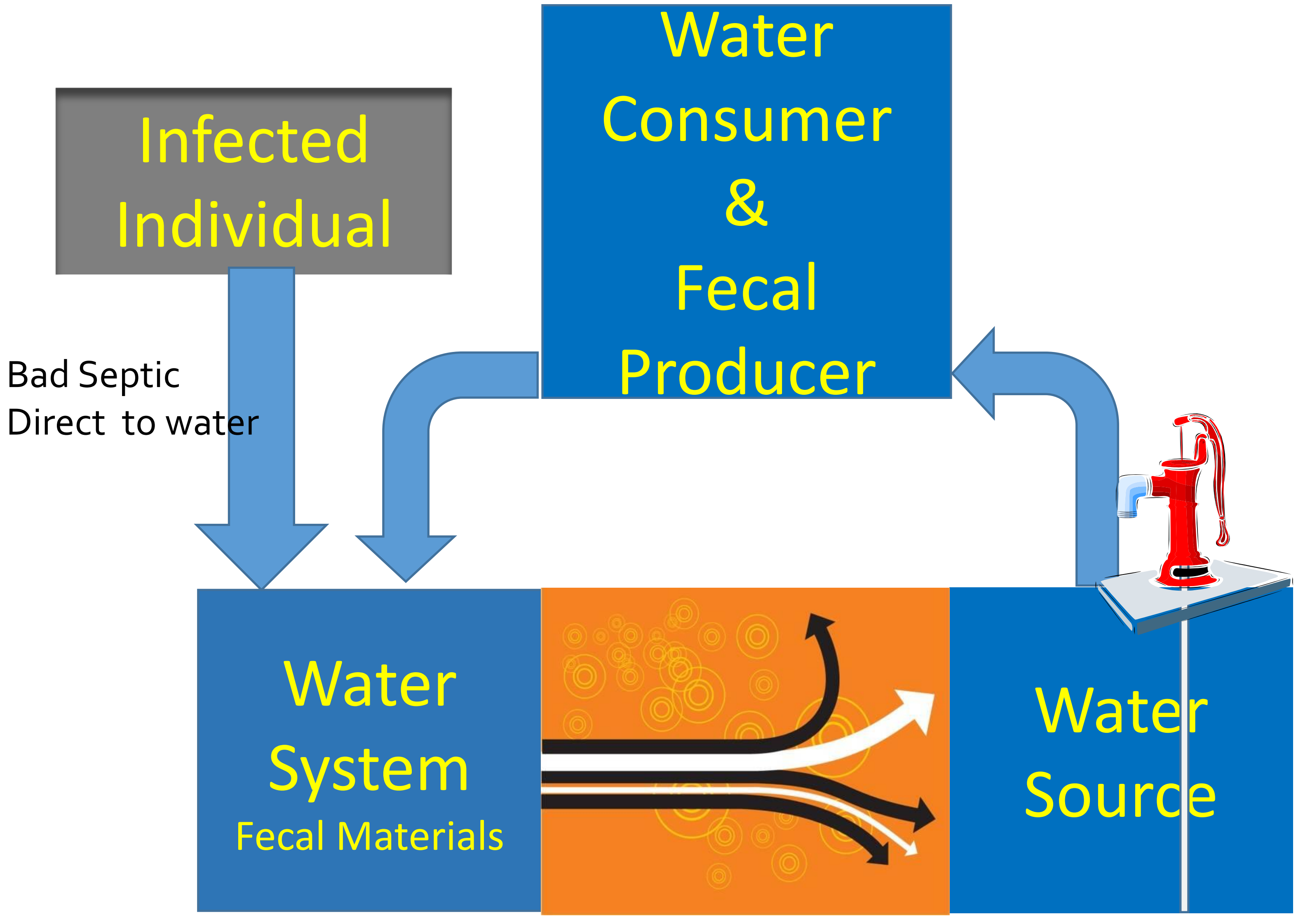
Pathogen spreads from the first host

- Blood
- Urine
- Feces
- Silva
- Tears
- Skin Flakes

Direct Transmission

Pass into "Living Reservoir"

Pass into a "Non Living Reservoir"



Water, soil and food can be reservoirs / vehicle of infection

In Flood Water!!

"Concentrations of Escherichia coli, intestinal enterococci and Campylobacter were measured in samples from 3 sewer flooding incidents. **The results indicate fecal contamination: fecal indicator organism concentrations were similar to those found in crude sewage under high-flow conditions and Campylobacter was detected in all samples**."

Water Res. 2010 May;44(9):2910-8. Epub 2010 Feb 23.
Microbial risks associated with exposure to pathogens in contaminated urban flood water. ten Veldhuis JA, Clemens FH, Sterk G, Berends BR.

Waterborne microbial disease

- Caused by ingesting water contaminated with pathogens.
- World-wide: 4 billion episodes of diarrhea result in about 2 million deaths each year, mostly children.
  - Waterborne bacterial infections may account for half of these episodes and deaths.
- In the US: Residents of periurban and remote rural areas with poor water treatment and delivery systems also are at risk.

Microbial Pathogens in Water

- **Disease** caused by water borne microbial pathogens have a rapid onset (matter of days.)
- It may only **1-10 individual organisms** of some microbial pathogens to cause disease.
- Intestinal cramping, nausea, vomiting and/or fever are common symptoms.
- Most bacteria make some sort of TOXIN
- The TOXIN causes the infected host to help the bacteria spread.. Vomiting, Diarrhea ect

Examples of bacteria pathogens

Pathogen	**Disease**
Salmonella typhi	*Typhoid fever*
Shigella spp.	*Shigellosis: dysentery*
Escherichia coli O157:H7	*gastroenteritis, can lead to kidney failure*
Campylobacter spp.	*gastroenteritis*
Legionella pneumoniae	*fever, pneumonia*
Helicobacter pylori	*gastritis*
Vibrio cholerae and sp.	*cholera*
Yersinia	*Yersiniosis: diarrhea*
Aeromonas hydrophila	*pneumonia*

Water Related Diseases

- Treated waters in the U.S. are “usually” safe
  - Chlorine used from about 1908
  - Eliminated typhoid fever in the US
  - 98 percent of all U.S. water utilities

- Water related diseases are prevented by better hygiene, functional sanitation processes leading to <u>safer raw water</u> supply.

- Cleaner source area: cleaner water supply

What makes them pathogenic ?

Pathogenicity – Or have the ability to cause disease

Less than 1% of the know bacteria can cause a diseases

Virulence factor or the degree of pathogenicity

1. Adhesion factors
2. Biofilms
3. Extracellular enzymes
4. Toxins (Bacteria reproduce rapidly and may give off toxins which damage body tissue)
5. Antiphagocytic factors

US Water – disease outbreaks

TABLE 5. Waterborne disease outbreaks associated with drinking water (n = 16), by state/jurisdiction — Waterborne Disease and Outbreak Surveillance System, United States, 2008

State/ Jurisdiction	Month	Class*	Etiology	Predominant illness†	No. of cases [deaths] (n = 1,672 [3]§)	Type of system¶	Deficiency**	Water source	Setting
Colorado	Mar	I	*Salmonella* Typhimurium	AGI	1,300 [1]	Com	4	Well	Community
Connecticut	Aug	I	*Providencia*††	AGI	55 —§§	Com	2	Well	Apartment complex
Georgia	Sep	III	*Legionella pneumophila* serogroup 1	ARI	6 —	Com	5A	Reservoir	Hospital/Health-care facility
Illinois	Jun	III	*L. pneumophila* serogroup 1	ARI	4 —	Com	5A	Well	Hospital/Health-care facility
Illinois	Oct	II	*Escherichia coli* O157:H7	AGI	6 —	Ind	2	Well	Farm
Illinois	Sep	I	*Shigella sonnei*, *Cryptosporidium*, *Giardia*	AGI	41 —	Com	6	Lake	Boat
New Jersey	Aug	III	*L. pneumophila* serogroup 1	ARI	9 —	Com	5A	Reservoir	Hospital/Health-care facility
New York	Jul	III	*L. pneumophila* serogroup 1	ARI	13 [1]	Com	5A	Well, river	Seniors housing complex
New York	Aug	IV	*L. pneumophila* serogroup 1	ARI	19 —	Com	5A	Lake	Assisted living facility
New York	Sep	III	*L. pneumophila* serogroup 1	ARI	3 [1]	Com	5A	Lake	Nursing home
Oklahoma	Jun	I	Norovirus genogroup 1.4	AGI	62 —	Com	3,4	Well	Neighborhood/Subdivision
Puerto Rico	Apr	II	*Cyclospora cayetanensis*	AGI	82 —	Com	99A	River	Community/Municipality
Tennessee	Mar	I	Hepatitis A virus	Hep	9 —	Ind	2	Well	Community/Municipality
Tennessee	Aug	I	*Salmonella* serotype I 4,5,12:i:-	AGI	5 —	Ncom	2	Spring	Private residence
Utah	Jun	III	*Campylobacter*	AGI	50 —	Ncom	2	Spring	Camp/Cabin
West Virginia	May	I	*C. jejuni*	AGI	8 —	Ind	2	Well	Private residence

Abbreviations: AGI = acute gastrointestinal illness, ARI = acute respiratory illness; Hep = hepatitis; Com = community; Ncom = noncommunity; Ind = individual; Bottle = commercially bottled water.

Top 5 Causes of Waterborne Outbreaks from Private Wells

- Hepatitis A (virus)
- *Giardia intestinalis (protozoan)*
- *Shigella spp*. (bacteria)
- *E. coli 0157:H7* (bacteria)

Tied:

- *Campylobacter jejuni* (bacteria)
- *Salmonella serotype typhimurium* (bacteria)

From CDC

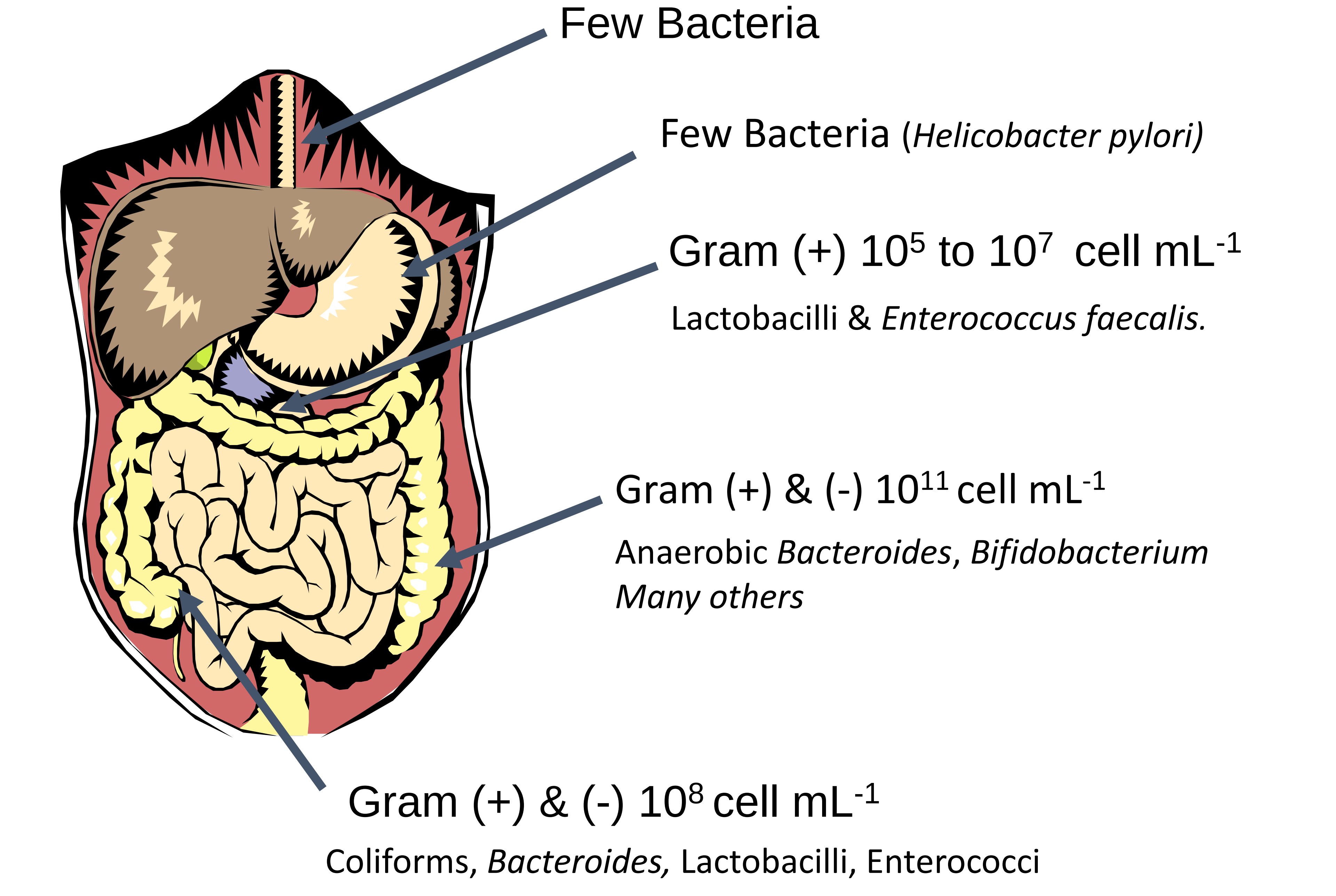
What are Indicators?

E. coli

- Traditionally indicators are use to predict the presence of pathogens
- There is a “weak” tie between indicator number and the occurrence of enteric pathogens
- **Indiana used fecal coliforms (still) and *E. coli* counts as the indicator for other bacteria**

Use of Indicators

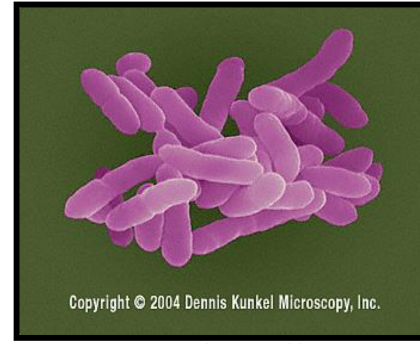
- General Microbial indicators – indicates the efficacy of a process (ex. Chlorine disinfection)
- Index and model organisms- indicates the occurrence and behavior of pathogens
- Indicators of Fecal material - indicates the presence of fecal contamination. They INFER the existence of pathogens


Few Bacteria
Few Bacteria (*Helicobacter pylori)*
Gram (+) 10^5 to 10^7 cell mL^{-1}
Lactobacilli & *Enterococcus faecalis.*
Gram (+) & (-) 10^{11} cell mL^{-1}
Anaerobic *Bacteroides*, *Bifidobacterium*
Many others
Gram (+) & (-) 10^8 cell mL^{-1}
Coliforms, *Bacteroides,* Lactobacilli, Enterococci

Normal Bacteria in Large Intestine and Feces is diverse

Bacteroides fragilis, Bacteroides melaninogenicus, Bacteroides oralis, Lactobacillus, Clostridium perfringens, Clostridium septicum, Clostridium tetani, Bifidobacterium bifidum, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella enteritidis, Salmonella typhi, Klebsiella sp., Enterobacter sp., Proteus mirabilis, Pseudomonas aeruginosa, Peptostreptococcus sp., Peptococcus sp., Methanogens

Coliforms



Klebsiella pneumonae

- Large bacterial group found in the environment and the feces of human and warm blooded animals. They are aerobic and facultative anaerobic, gram negative rod shape bacteria.

- Are comprised by two groups (procedural split):
  - Total Coliforms – they are commonly found in feces and also occur naturally in unpolluted soils and waters. This group includes the fecal coliforms and other species of the genera Citrobacter, Enterobacter, Escherichia and Klebsiella.
  - Fecal coliforms - are exclusively of fecal origin to be composed mainly of E. coli.

Representation of the Masses

- Fecal Coliforms:
  - Aerobic bacteria found in the colon and or in feces, used as indicators of fecal contamination.
- History:
  - U.S. Department of Treasury (1914) promulgated drinking water bacteriological standard, 2 coliforms per 100 mL (Interstate Waters only)
    - Lowered to 1 coliforms per 100 mL (1925)
  - 1942, the US Public Health Service takes over
  - 1957, membrane filter process for analysis
  - 1974, Safe Drinking Water Act

All the Bad Guys

Salmonella spp.
Clostridium botulinum
Staphylococcus aureus
Campylobacter jejuni
Yersinia enterocolitica & Yersinia pseudotuberculosis
Listeria monocytogenes
Vibrio cholerae O1
Vibrio cholerae non-O1
Streptococcus
Shigella spp.
Vibrio parahaemolyticus and other vibrios
Vibrio vulnificus
Clostridium perfringens
Bacillus cereus
Aeromonas hydrophila and other spp.
Plesiomonas shigelloides
Enterics:Klebsiella, Enterobacter, Proteus, Citrobacter, Aerobacter, Providencia, Serratia

← **E. coli**

Maybe?

Enterococcus

Tippecanoe County, Indiana Gage Sites

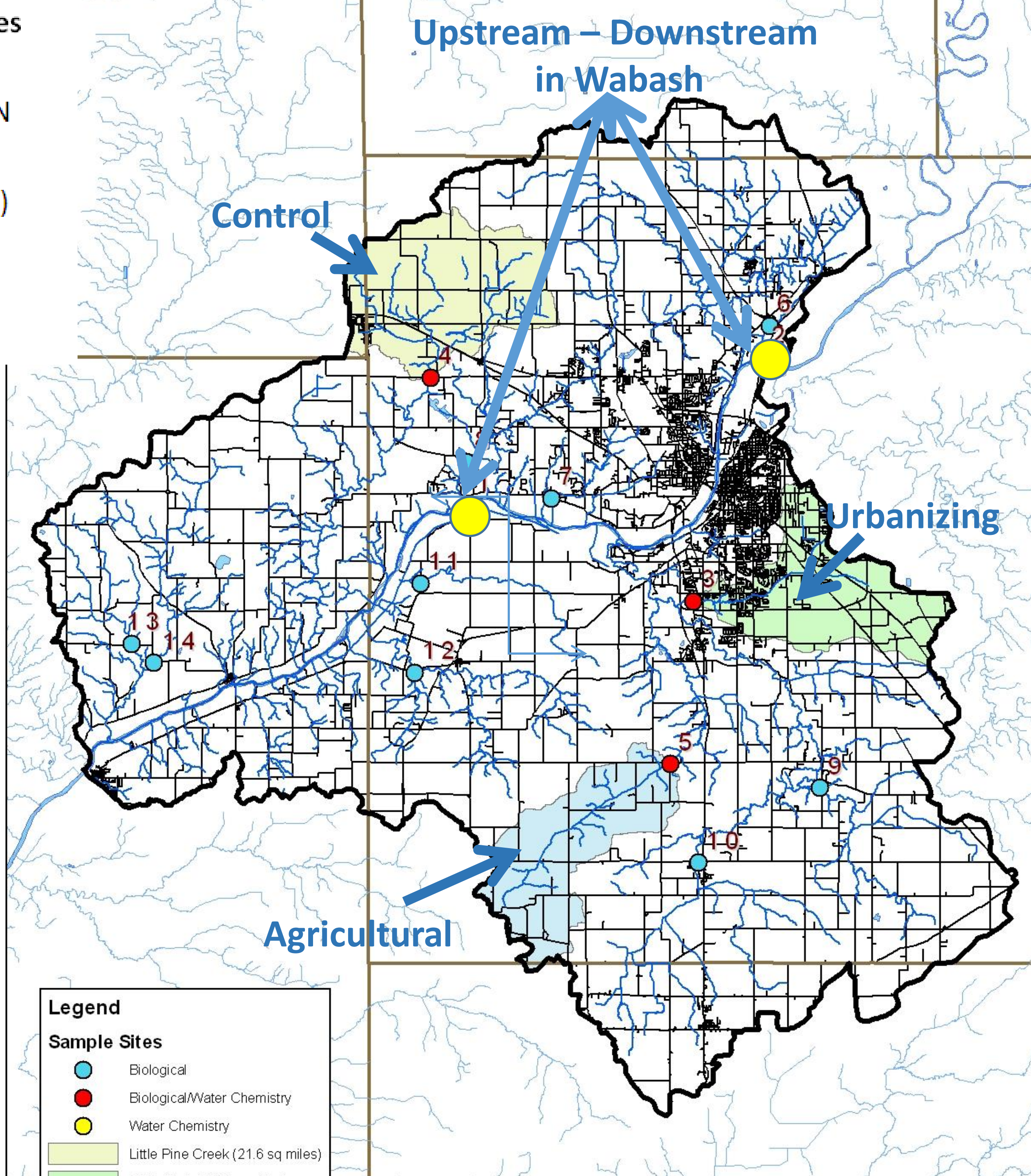
03335500 Wabash River – Lafayette IN

03335671 Elliott Ditch (lower section)

033356725 Elliott Ditch (upper section)

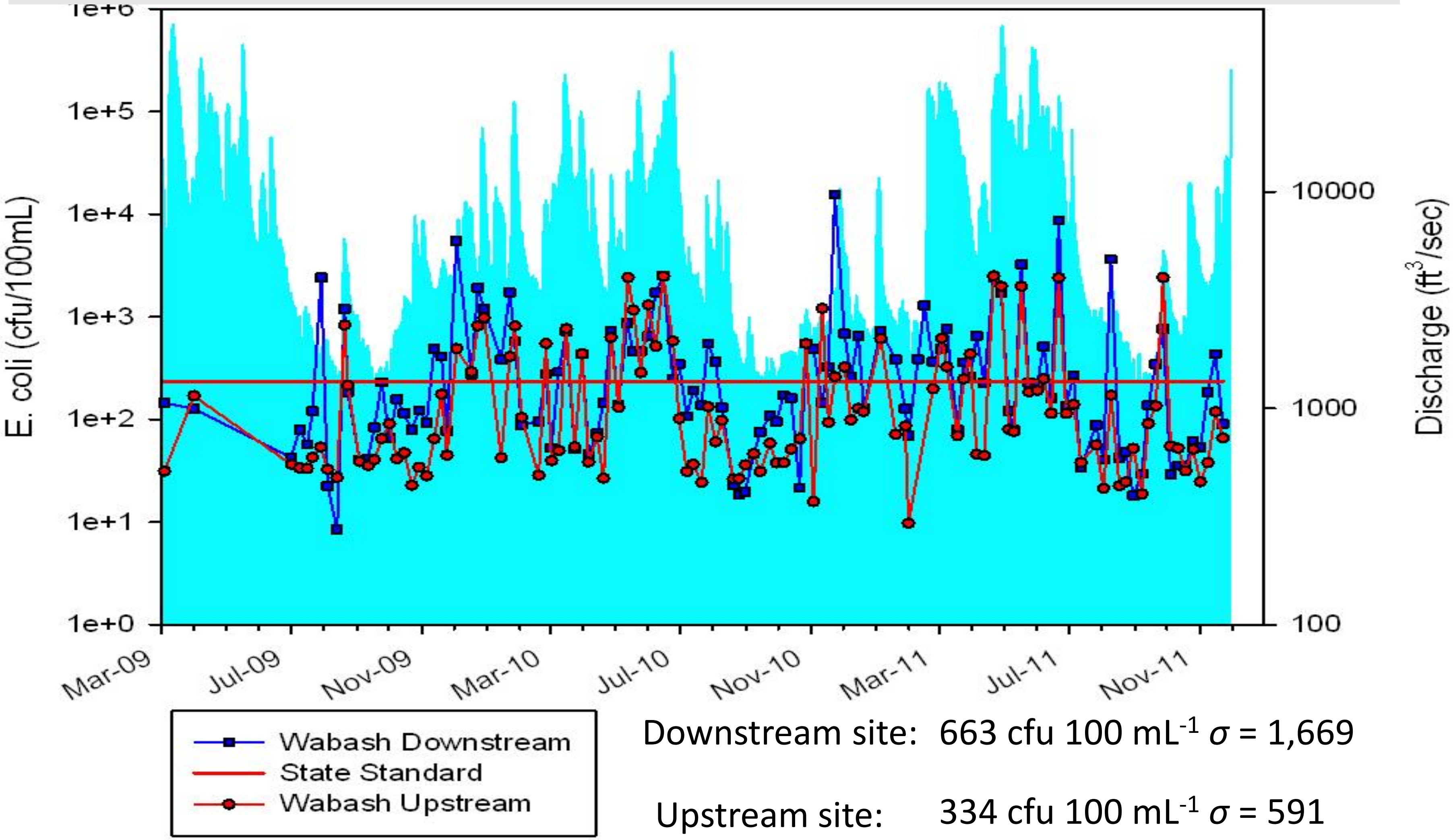
03335673 Little Wea Creek

033356786 Little Pine Creek



319 Grant
WREC/Purdue Univ
IWRRC money
USGS support
Graduate Students
Undergrads
Faculty

E. coli in the Wabash

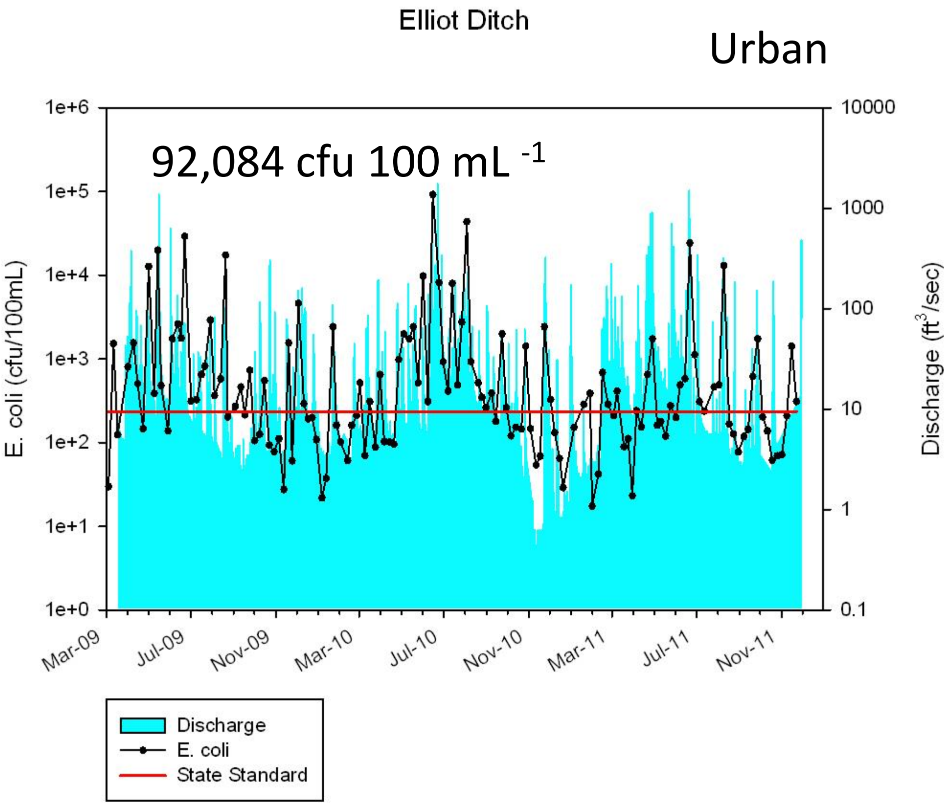


Downstream site: 663 cfu 100 mL^{-1} σ = 1,669

Upstream site: 334 cfu 100 mL^{-1} σ = 591

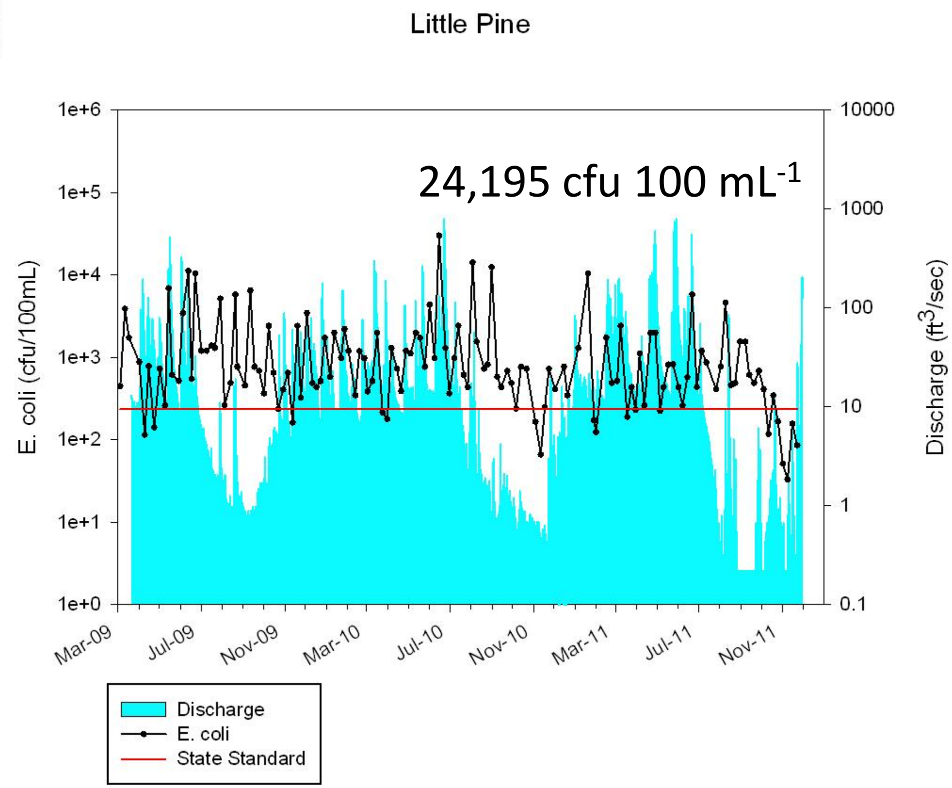
E. coli & Small Tributaries

Urban



X= 2,452 cfu 100 mL^{-1}

σ =9,444



X= 1,671 cfu 100 mL^{-1}

σ =1,237