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Knock-knock, Who's There?

Using NGS to Understand Your Environment

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Presented by: Melody Vachon
RemTech East
Niagara Falls Ontario
31-May-2023



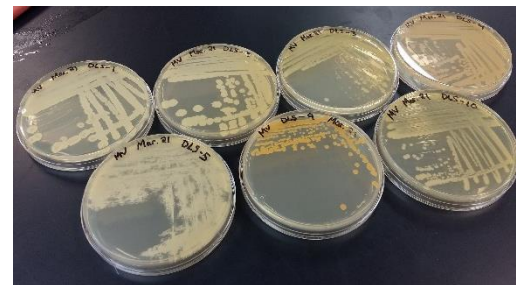
About Me

I am a:

- Microbiologist
- Molecular biologist
- Bioinformatician
- Big nerd

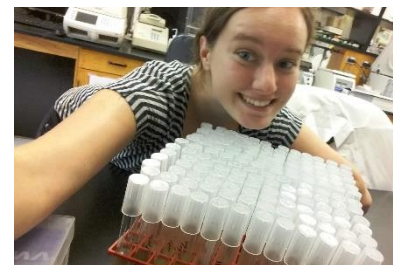


B.Sc. Microbiology



UNIVERSITY OF
WATERLOO

M.Sc. Environmental
Microbiology



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A Few Ways to Look at Your Bugs



Quantitative PCR

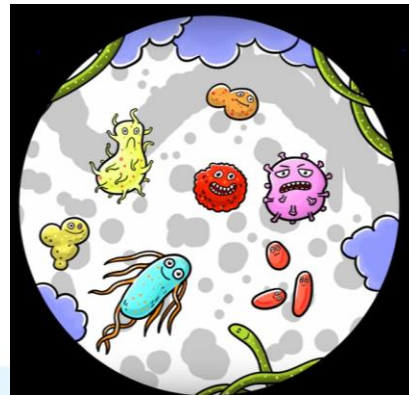
Quantify specific pre-selected targets:

- Microbial, e.g., *Dhc*, *Dhb*, *Dhg*
- Functional genes e.g., *tceA*, *bvcA*, *vcrA*



Next Generation Sequencing

Characterize the entire microbial community





If Microbes Were Cars in a Lot...

Quantitative PCR Tests



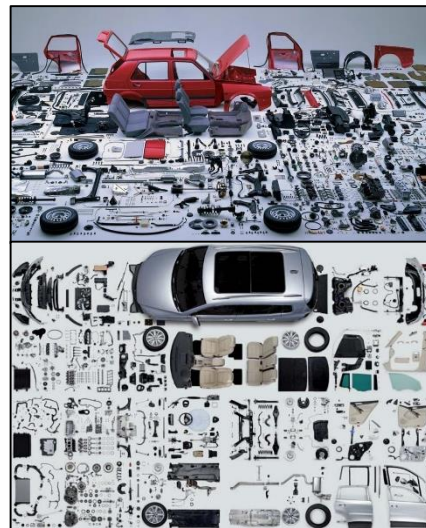
“There are 27 VWs”

Next Generation Sequencing (16S rRNA Amplicons)



“There are 27 VW, 14 Honda, 30 Toyota, 2 Ford, 6 Chevrolet...”

Metagenome Sequencing

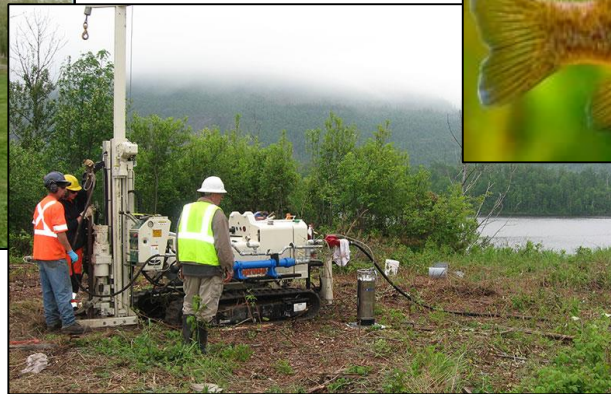


Learn every car make (microbe) and
all the individual parts of the cars
(genes)



Some uses of Molecular Biological Tools

- Enhanced *in situ* groundwater remediation
- Characterization of microbial cultures used in bioaugmentation
- Characterization of plant associated bacteria in phytoremediation
- Characterization of water associated biota using eDNA





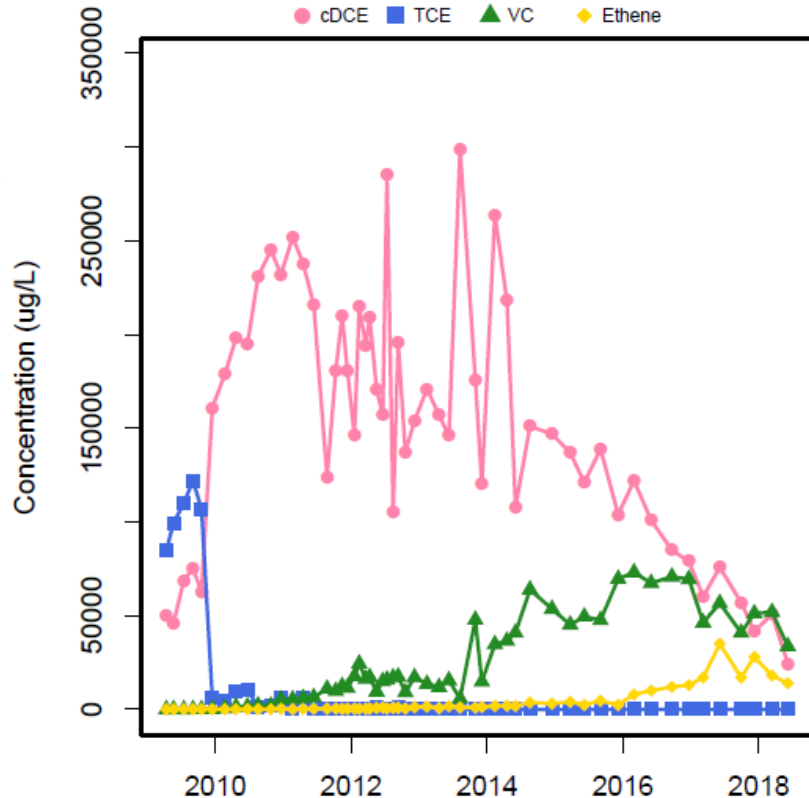
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Using NGS Over Time in Remediation Projects



Oregon Site Dechlorination



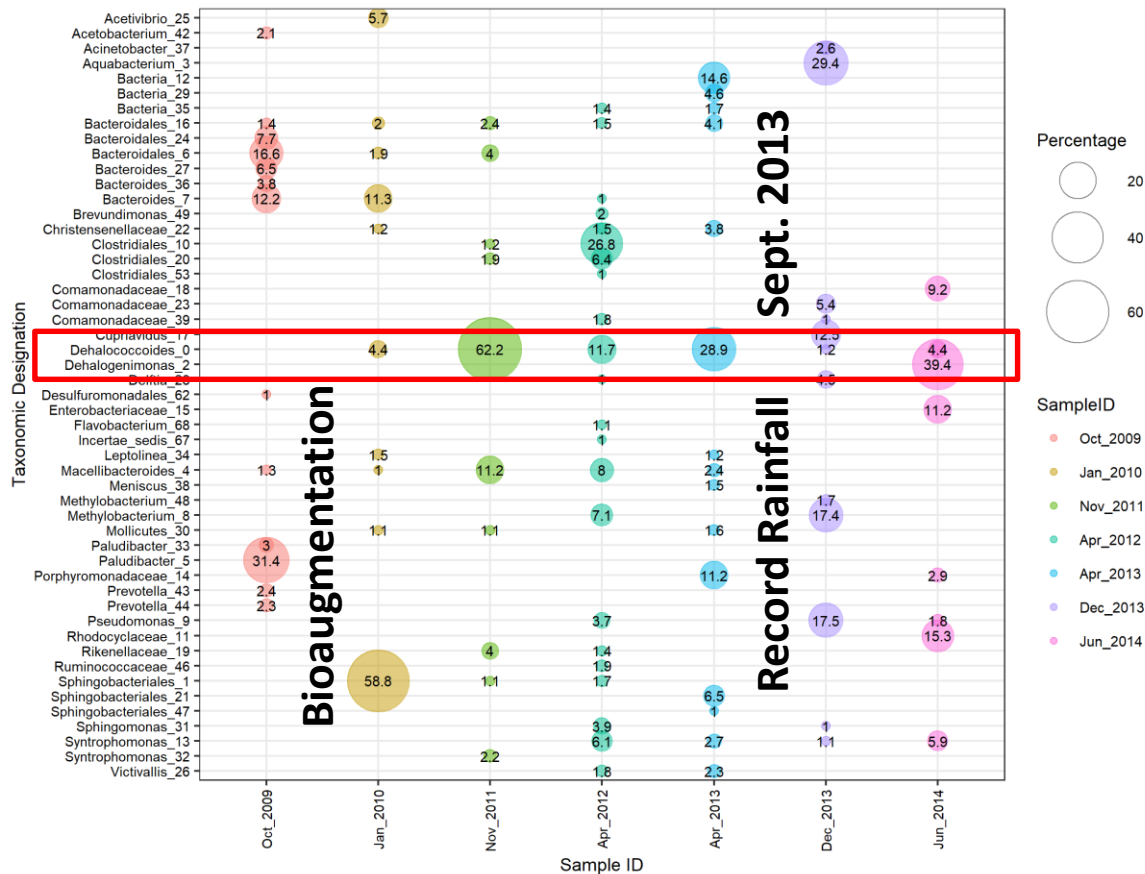
- Bioaugmentation site KB-1 (Dhc)
- >99% TCE mass removal after one year
- Residual cDCE and VC are declining
- Long term Dhc monitoring and NGS study





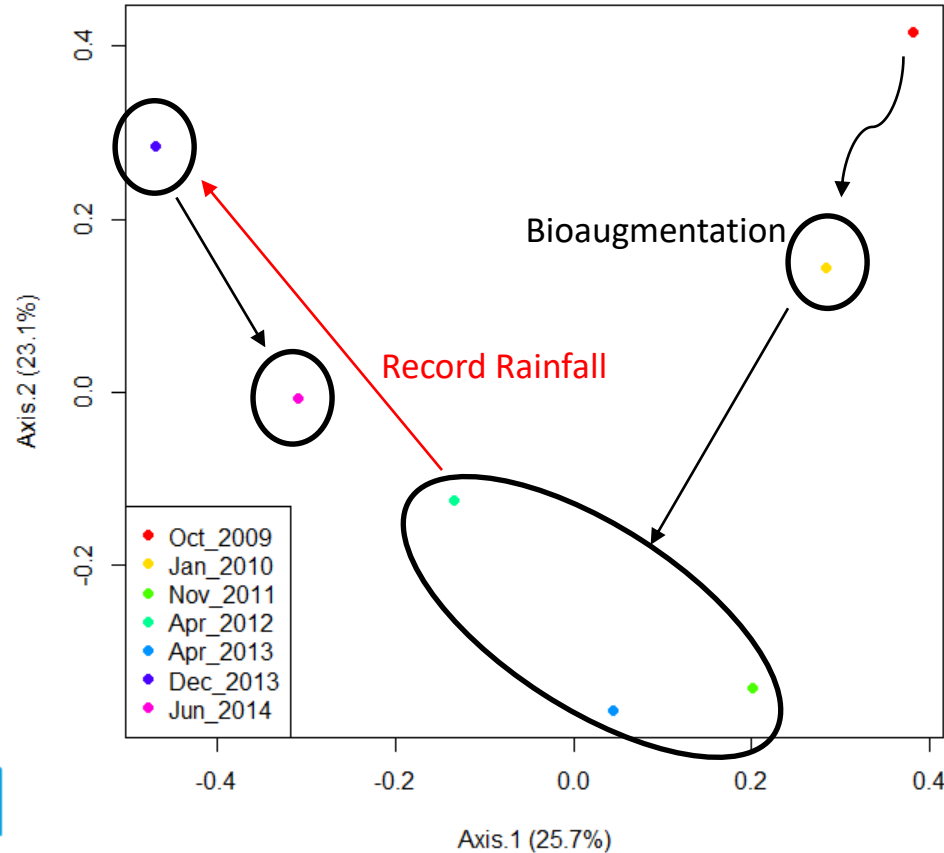
Oregon Site NGS

- *Dehalococcoides* (Dhc) increases after bioaugmentation
- *Dehalococcoides* (Dhc) decline after April 2013
Why?
- September 2013 was the wettest on record in Oregon, 17 cm rain = surface water infiltration





Oregon Site NGS





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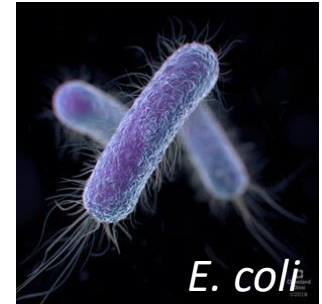
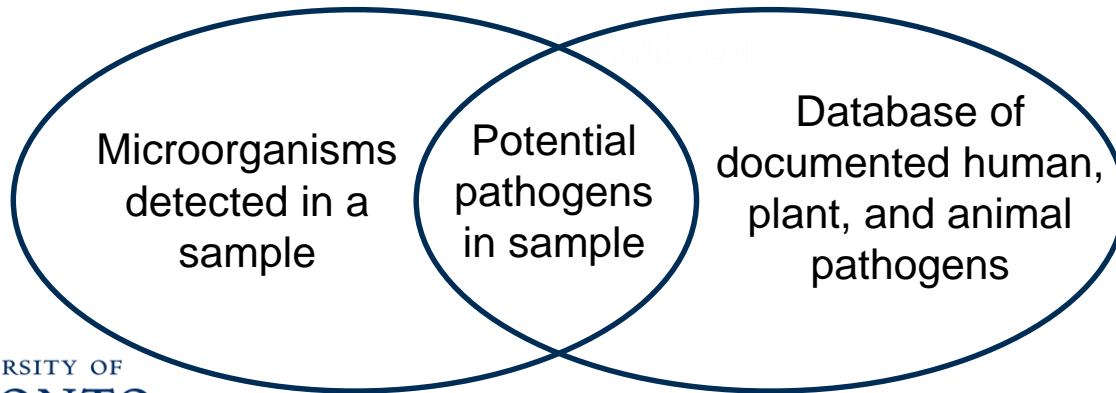


Bioaugmentation Culture Characterization



Are pathogens present?

- Pathogens are microorganisms that can cause disease
- NGS can be used to detect the DNA signature of pathogens in a sample





Using NGS to Characterize Cultures

- NGS data is used to ensure our bioaugmentation cultures meet Canada's New Substances Notification (NSN) Regulations
 - Characterize all microorganisms present in the culture
 - Ensure the absence of dangerous microorganisms

Canada 

Example: NSN Approval of DGG-Plus in 2022, an anaerobic microbial consortium for environmental treatment of benzene, toluene, and xylene

- We confirmed that there was no significant evidence of pathogens

DGGTM PLUS

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Potential Pathogens in Landfill Leachate



LEGEND

GREEN = major ASV (>1% of archaea or bacteria in 1 or more sample)

YELLOW = minor ASV (0.1 - 1% of archaea or bacteria in 1 or more sample)

RED = trace ASV (<0.1% of archaea or bacteria in 1 or more sample)

NCBI Accession Number	Sequence Description	Percent Identity	ASV ID	Number of Reads	Percent Total Reads
NR_044743.1	<i>Bartonella bacilliformis</i> strain KC583 16S ribosomal RNA gene	97.863	118	603	3.11%
NR_114518.1	<i>Acidovorax konjaci</i> strain ICMP 7733 16S ribosomal RNA gene, partial sequence	97.419	49	181	0.93%
AY922323.1	<i>Brucella melitensis</i> isolate Kars1 16S ribosomal RNA gene, partial sequence	97.441	342	116	0.6%
NR_103934.2	<i>Pseudomonas stutzeri</i> strain ATCC 17588 16S ribosomal RNA, complete sequence	99.785	131	62	0.32%
KF378762.1	<i>Mycobacterium phlei</i> strain CM32 16S ribosomal RNA gene, partial sequence	99.142	1160	42	0.22%
NR_113238.1	<i>Brevundimonas diminuta</i> strain JCM 2788 16S ribosomal RNA gene, partial sequence	100	512	27	0.14%
AF017749.1	<i>Stenotrophomonas maltophilia</i> 16S ribosomal RNA gene, complete sequence	98.287	581	25	0.13%
AJ278450.1	<i>Bordetella parapertussis</i> partial 16S rRNA gene, strain DSM 4922	100	981	26	0.13%
NR_103934.2	<i>Pseudomonas stutzeri</i> strain ATCC 17588 16S ribosomal RNA, complete sequence	99.571	408	21	0.11%
NR_041756.1	<i>Acidovorax cattleyae</i> strain ICMP 2826 16S ribosomal RNA gene, partial sequence	97.204	706	18	0.09%
NR_103934.2	<i>Pseudomonas stutzeri</i> strain ATCC 17588 16S ribosomal RNA, complete sequence	97.854	902	17	0.09%
AB682670.1	<i>Bordetella pertussis</i> gene for 16S rRNA, partial sequence, strain: NBRC 107857	98.048	684	14	0.07%
AB682670.1	<i>Bordetella pertussis</i> gene for 16S rRNA, partial sequence, strain: NBRC 107857	99.349	754	14	0.07%
NR_103934.2	<i>Pseudomonas stutzeri</i> strain ATCC 17588 16S ribosomal RNA, complete sequence	99.142	279	14	0.07%
JX129161.2	<i>Bordetella bronchiseptica</i> strain 78B1 16S ribosomal RNA gene, partial sequence	97.419	2504	9	0.05%
NR_113238.1	<i>Brevundimonas diminuta</i> strain JCM 2788 16S ribosomal RNA gene, partial sequence	99.548	599	10	0.05%
NR_118052.1	<i>Burkholderia andropogonis</i> strain LMG 2129 16S ribosomal RNA gene, partial sequence	97.222	2500	9	0.05%



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Looking for Plant Associated Microbes



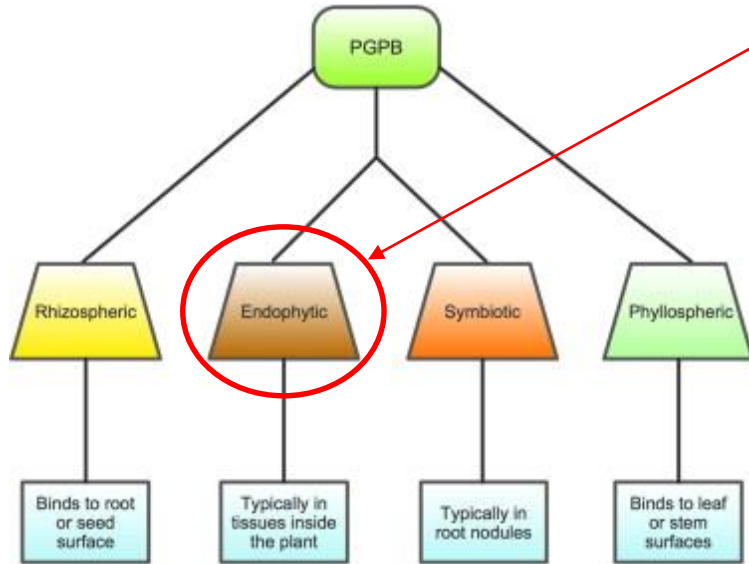
Using NGS with Phytoremediation

- In general, plant-associated microbes aid in plant growth, help acquire nutrients and moisture from soil, confer resistance to stresses, and some fix N_2
- **Phytoremediation Sites**
 - Petroleum Hydrocarbon/BTEX Site in Oklahoma
 - TCE/TCA Site in Pennsylvania
- **Study Goal** - Characterize plant-associated microbes that may play a major role in contaminant degradation in phyto systems e.g., PHCs and TCE



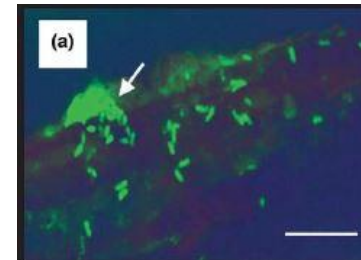


Plant Growth Promoting Bacteria



Endophytes – subset of microbes that reside within plant tissues

- Include specific bacterial species may aid in degrading certain contaminants *within* plants



Source: Glick, B. Bacteria with ACC deaminase can promote plant growth and help feed the world. Microbiological Research, Vol. 169, Issue 1, Jan. 2014, pp: 30-39





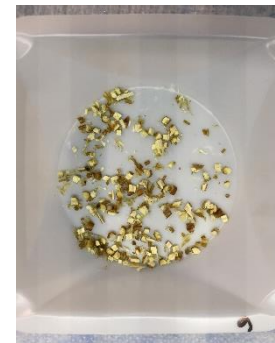
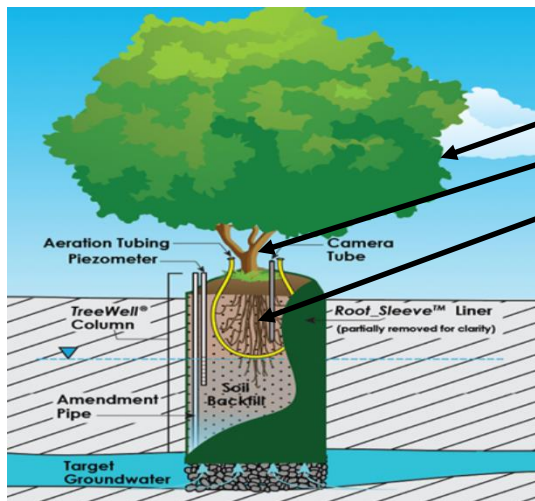
Tree Samples

Collect samples at three key locations:

Stems

Tree Cores

Roots





Are contaminant degraders present in tree tissue?

Common Endophyte Genera Detected:

- *Burkholderia*, *Azoarcus*, *Rahnella*, *Pseudomonas*, *Pantoea*, *Enterobacter*, *Arthrobacter*, *Streptomyces*, *Bradyrhizobium*

Other Specific Genera or Species Detected with Biodegradation potential:

- *Dehalococcoides* spp., - known CVOC degraders
- *Pseudonocardia* spp., - include 1,4-Dioxane degraders
- *Polaromonas* spp., – aerobic degraders of Naphthalene & DCE
- *Phenylobacterium* – include obligate herbicide degraders
- *Methylibium* spp., - known MTBE degraders
- *Variovorax paradoxus* – known benzene degrader
- *Enterobacteracea* spp. – wide range of biodegradation activities
- *Pseudomonas* – wide range of biodegradation activities





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Detecting Larger Organisms with Environmental DNA



What is eDNA?

- Environmental DNA is expelled and accumulates when an organism interacts with an environment
- Analysis is cheaper & easier to perform than physical bioassessment surveys
- eDNA can be used to:
 - Determine ecological health of water bodies
 - Assess changes to ecosystems over time
 - Detect presence of endangered species
 - Track invasive species





Sequencing Gene Targets

- Gene-Trac NGS (Bacteria and Archaea 16S rRNA)
 - Fish
 - Macroinvertebrates
 - Amphibians
- } eDNA Targets
12S rRNA (fish only)
Cytochrome C Oxidase I

DNA was extracted from water and sediment



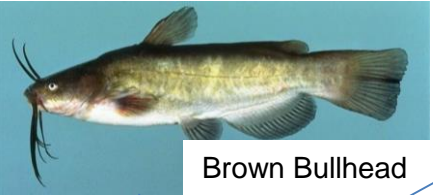
One sample, multiple organisms



Spottail Shiner



Alewife



Brown Bullhead



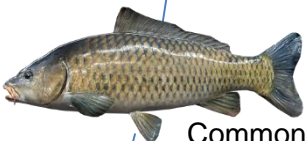
Green Sunfish



Brook Stickleback



Flathead Minnow



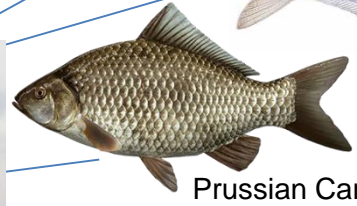
Common Carp



Gizzard Shad



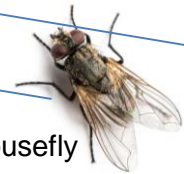
Pumpkinseed



Prussian Carp



Frog



Housefly



Canada Goose



Salamander



House Mouse



Human



Red Ear Slider



Cattle



Conclusions

Some of many applications of NGS:

- Track entire microbial communities during bioaugmentation
- Predict functions of the microbial community
- Determine whether pathogenic microorganisms are present
- Look for contaminant degrading bacteria in plants, soil, and groundwater
- Assess larger organisms including fish, macroinvertebrates, and amphibians



Questions

Thank you for your attention!



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