What are they consuming? Exploring the Zebra Mussel Associated Bacterial Community

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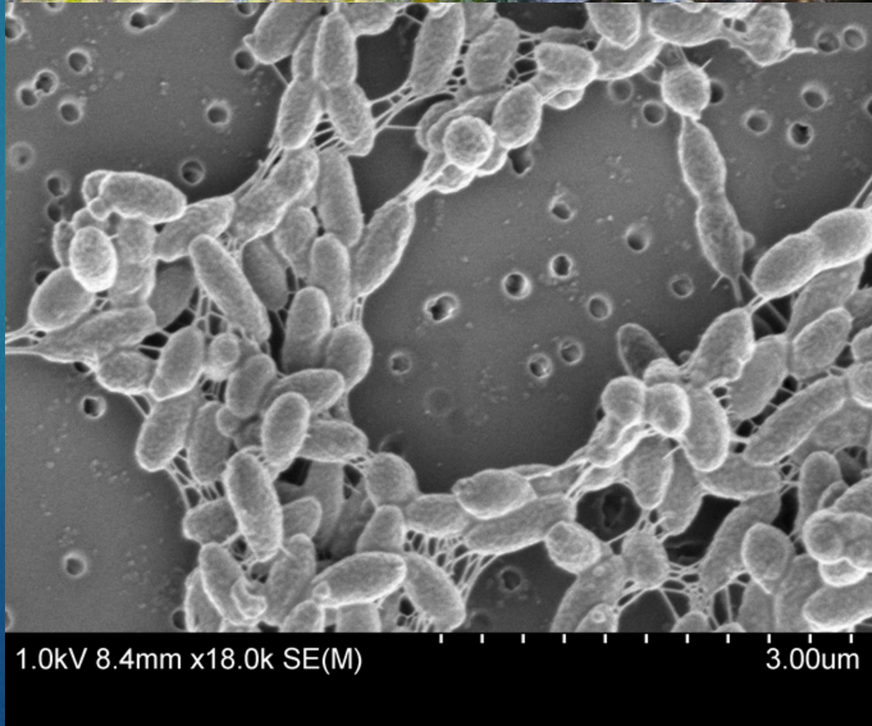
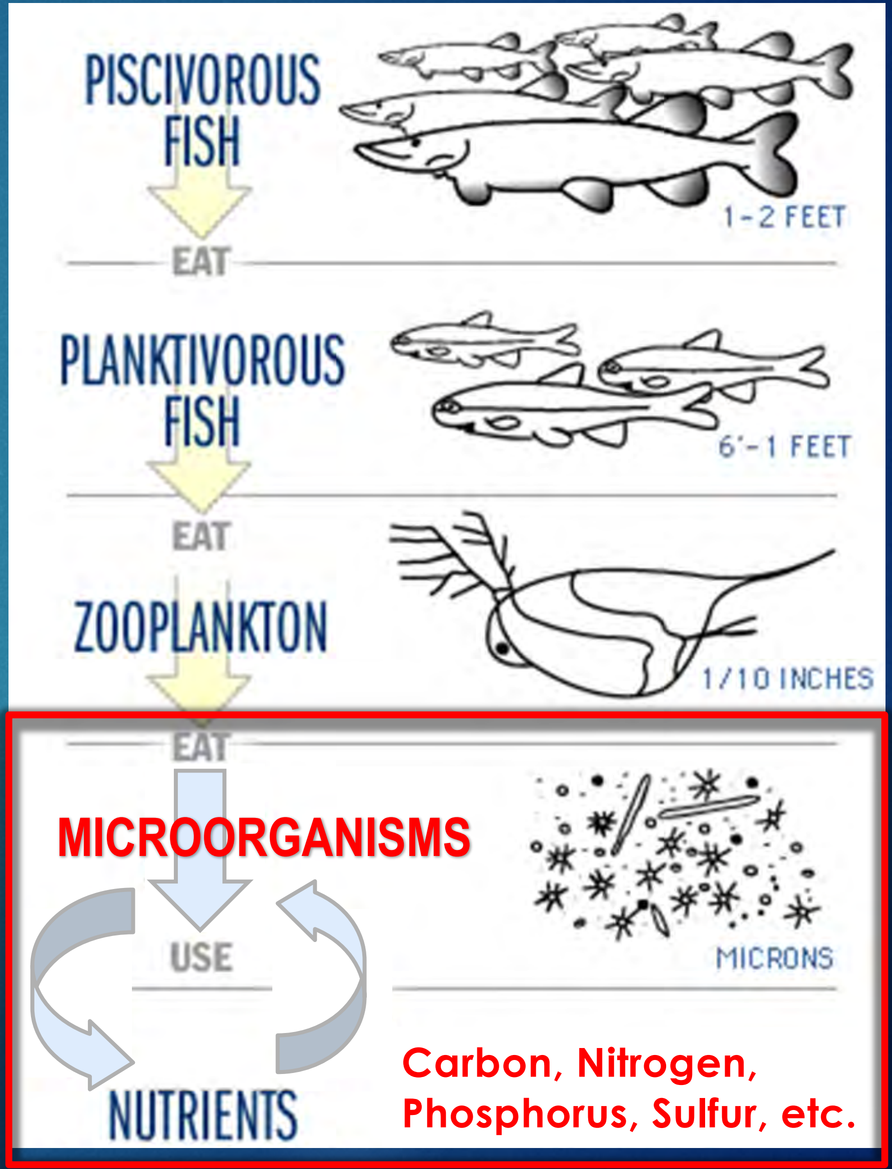
Lake Winnipeg Research Consortium Workshop

University of Manitoba



Base of the aquatic food chain: **Microbial Primary Productivity**

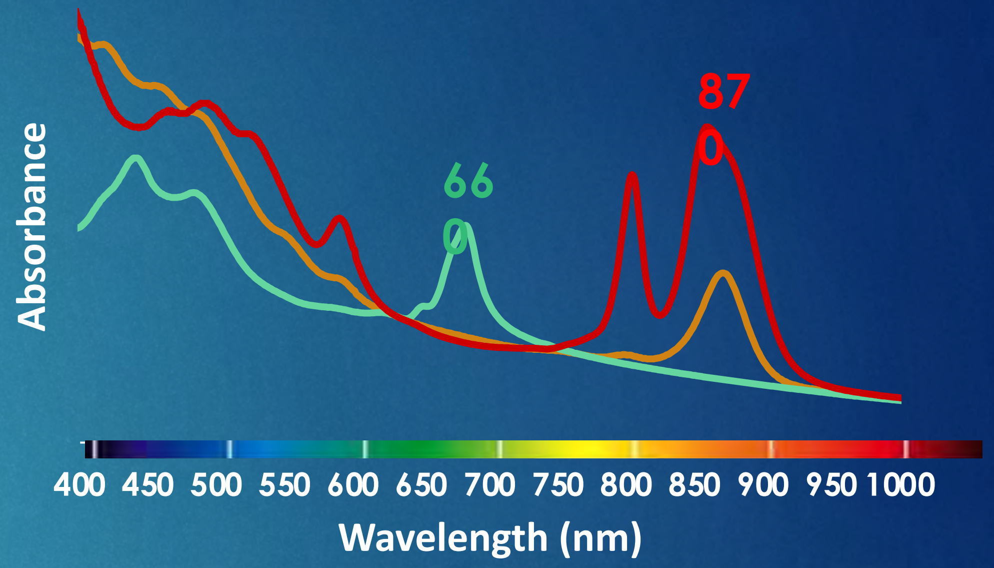
- Introduction of raw organic elements, organic carbon, nitrates, phosphates, etc.
- **Microbes** are the primary producers of usable biological nutrients as well as primary recyclers
- **Higher level** organisms have been well studied
- Knowledge gaps remain for **primary producers**
  - **AAP likely a major part of microbial community**



http://www.waterontheweb.org/under/lakeecology/11_foodweb.html

Aerobic Anoxygenic Phototrophs

- Photoheterotrophic bacteria
  - Require organic carbon
  - Supplement energy via **phototrophy**
  - Anoxygenic phototrophy with a reaction center complexed with bacteriochlorophyll *a* (Bchl *a)*
- Cannot fix CO_2 or grow anaerobically
- Likely primary recyclers of organics in association of oxygenic phototrophs
- Make up **10-37%** of the total bacteria in aquatic ecosystems



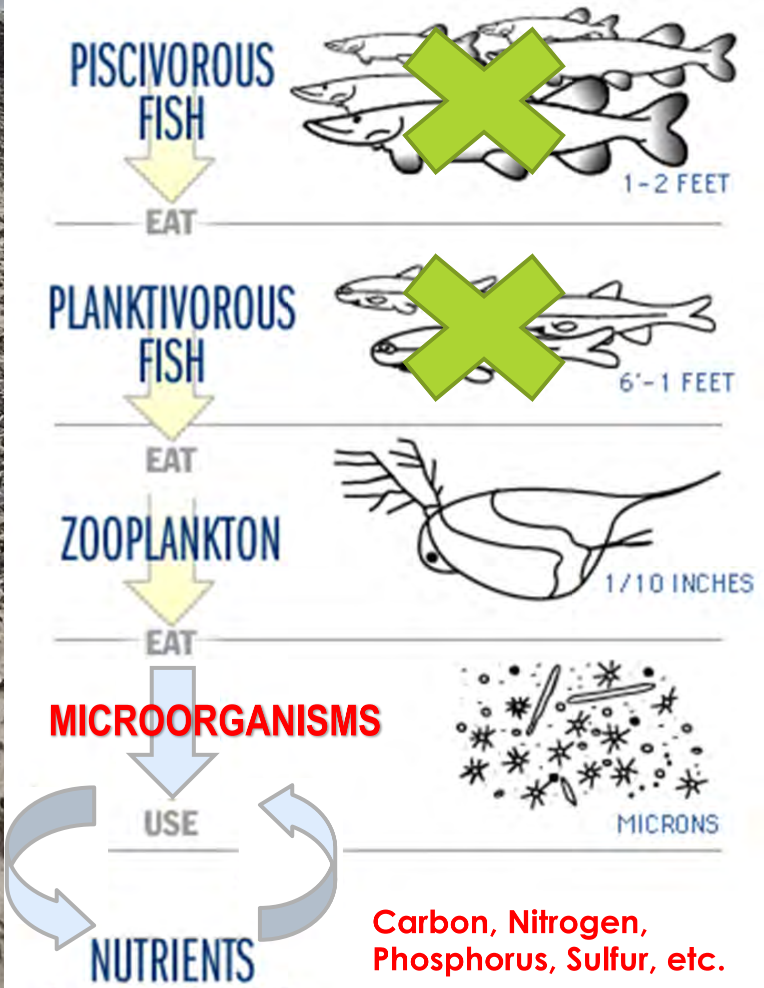
Unedited photo of AAP cultures

Grand Beach October 9th 2019

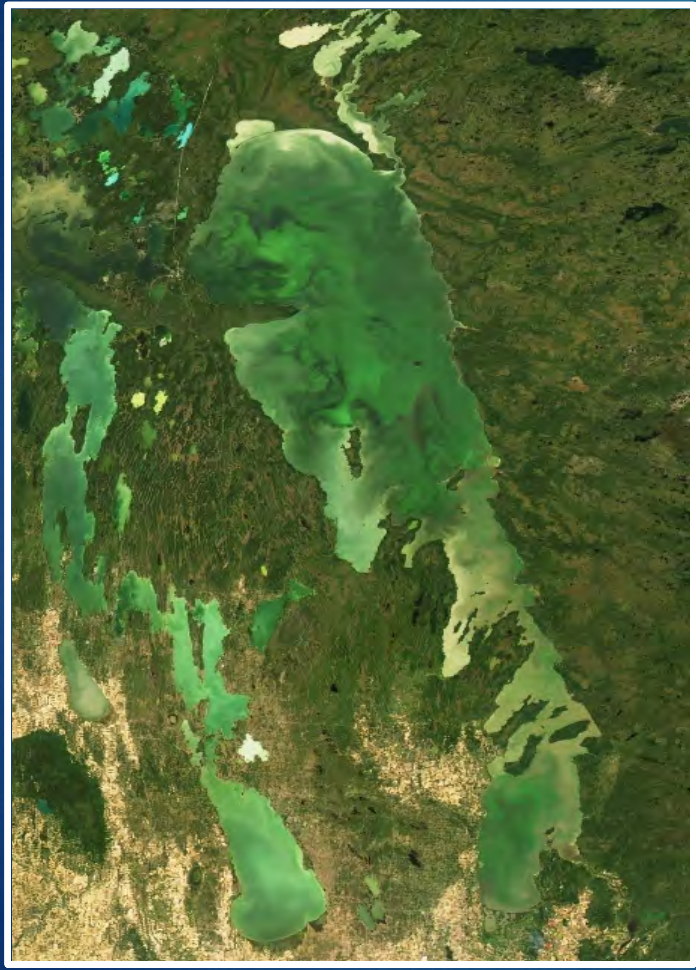
- What happens when the natural system is interrupted?

Zebra mussels introduced in 2013-15

Unknown predation.
Filter 5-45µm particles

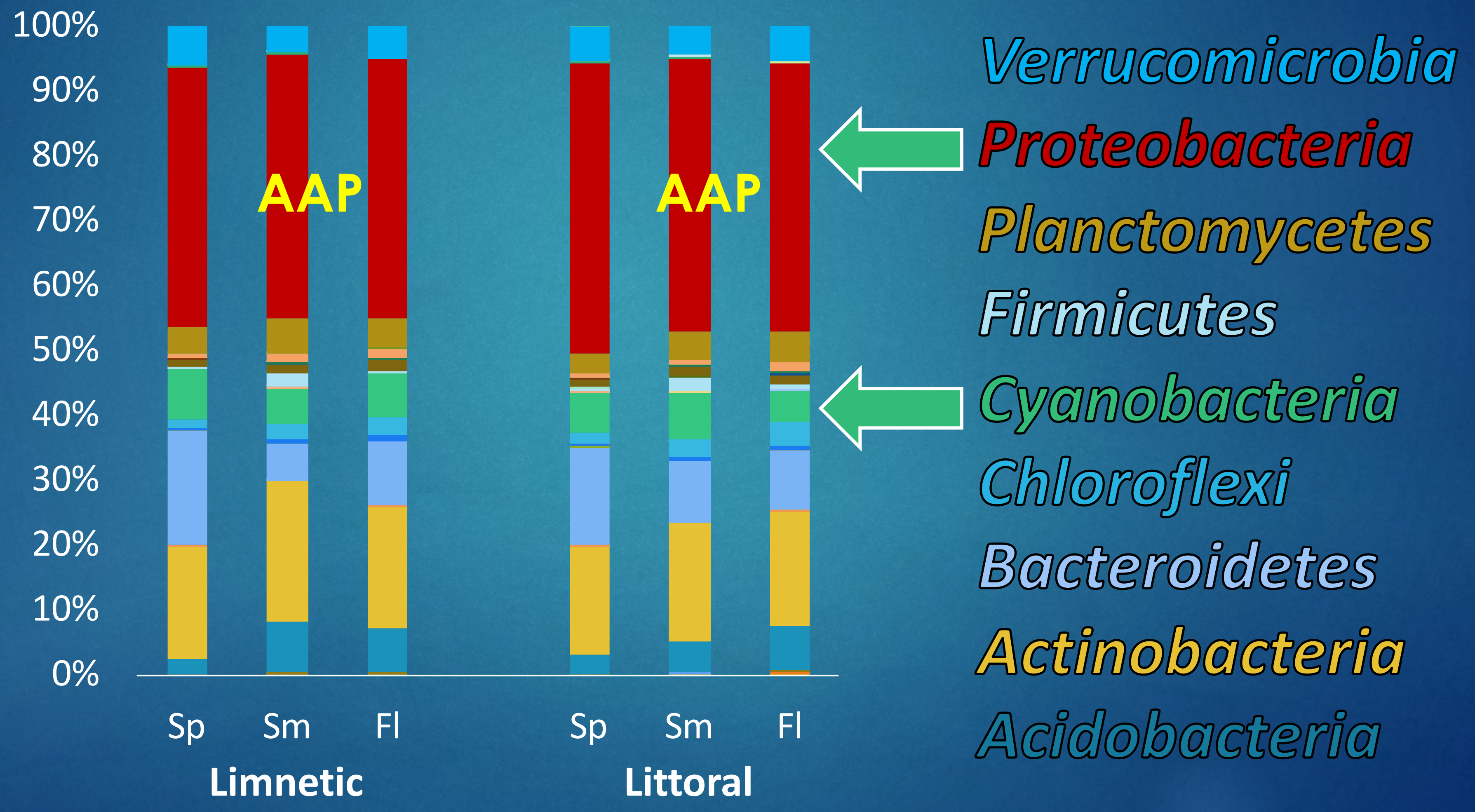


PhD Methodology Overview:

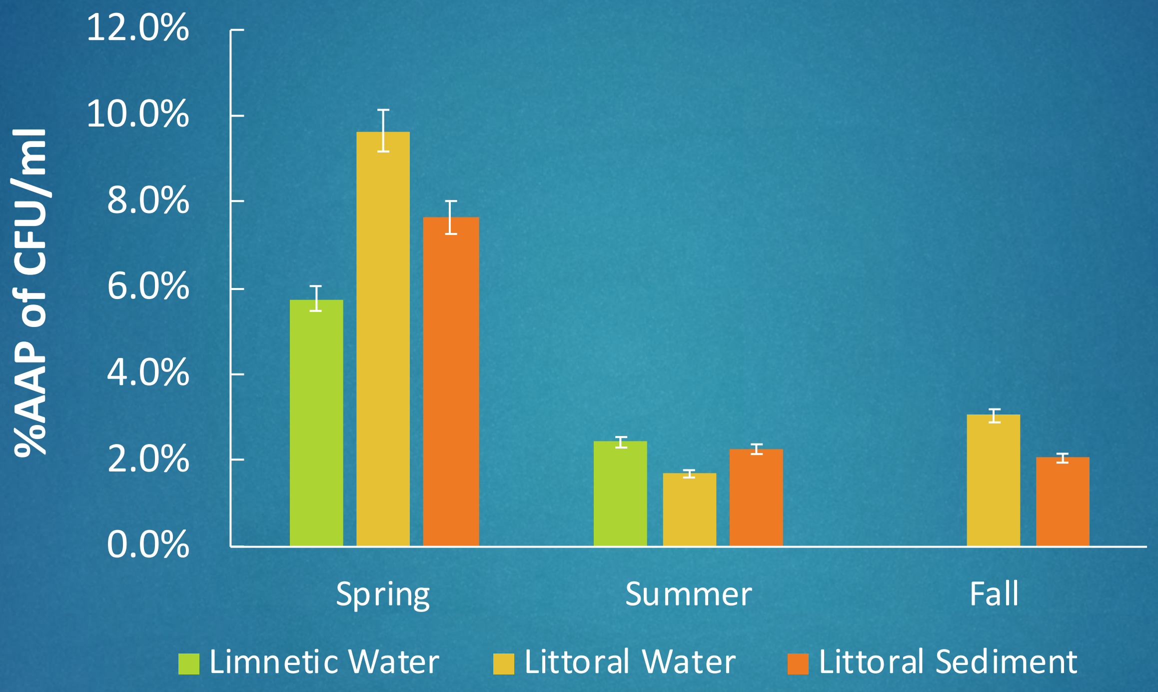


- Sampling Lake Winnipeg spatially and temporally
- Grow and enumerate bacteria
- Extract and sequence environmental DNA (eDNA)
- Determine proportion that make up AAP
- Note Functional Capacity of Isolates

Sequenced eDNA of 16S V4 rRNA: Stable community seen throughout seasons



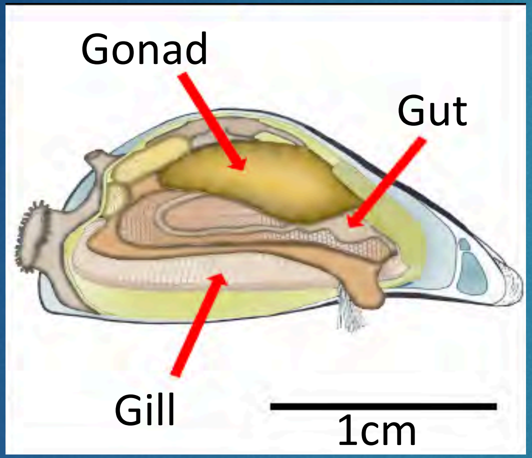
Proportion of cultured pigmented strains that are AAP reveal maximum in the spring

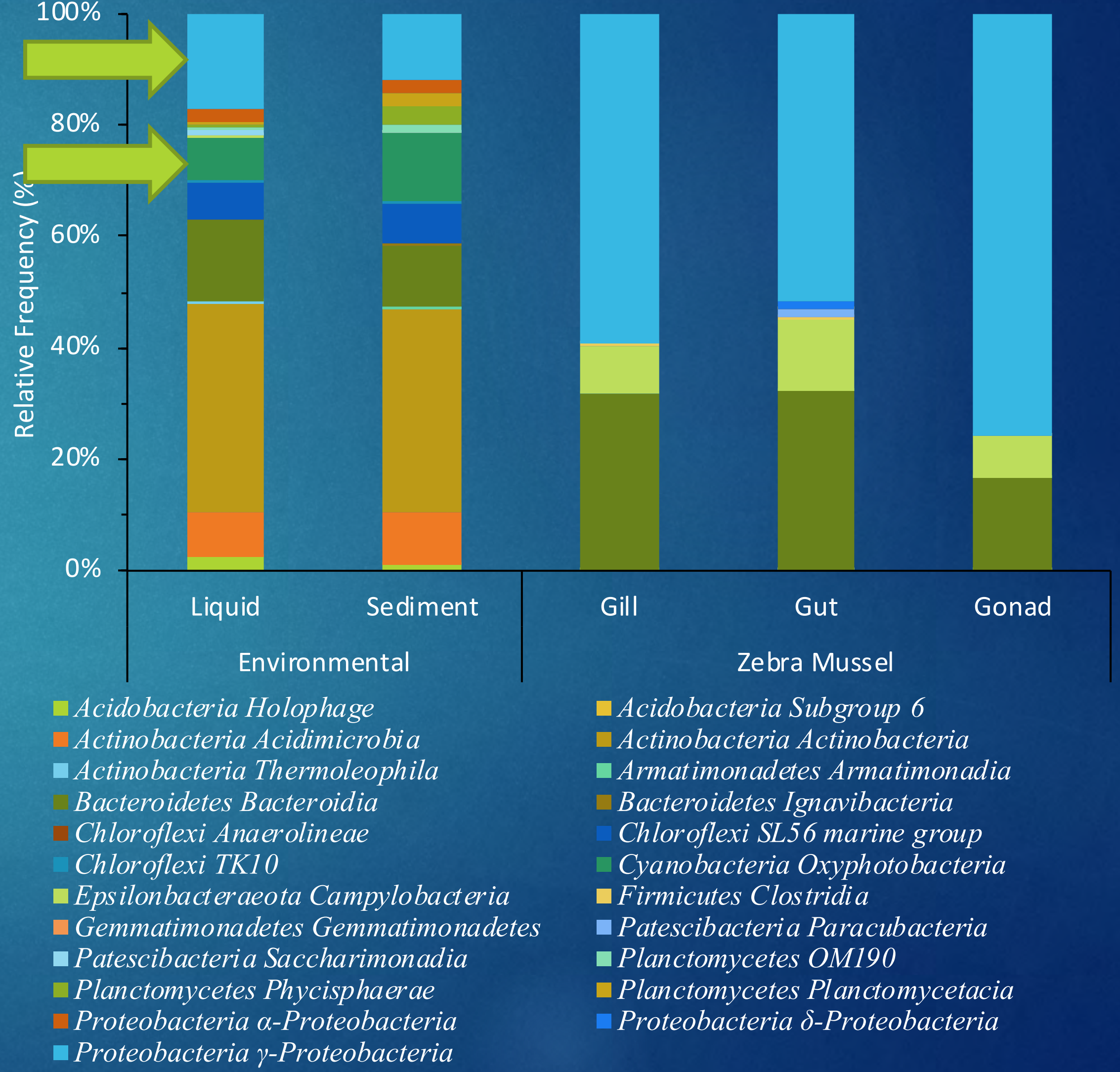


- **Highest abundance of AAP at littoral sites**
- **Both littoral and limnetic AAP are prevalant during the spring**

D. polymorpha associated bacterial community

- Sampled zebra mussel tissues for bacteria
- Culture based enumeration and community analysis via 16S eDNA sequencing





- *Proteobacteria, Epsilonbacteria, Bacteroidetes* predominant in mussels.
- Cyanobacterial present in lake, absent in zebra mussel
- Bivalve consumption of **AAP and other bacteria?**

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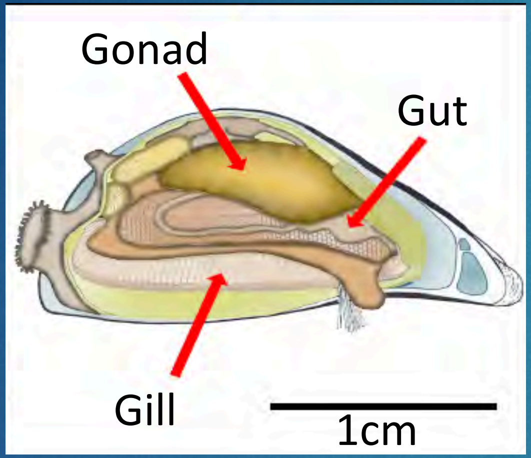

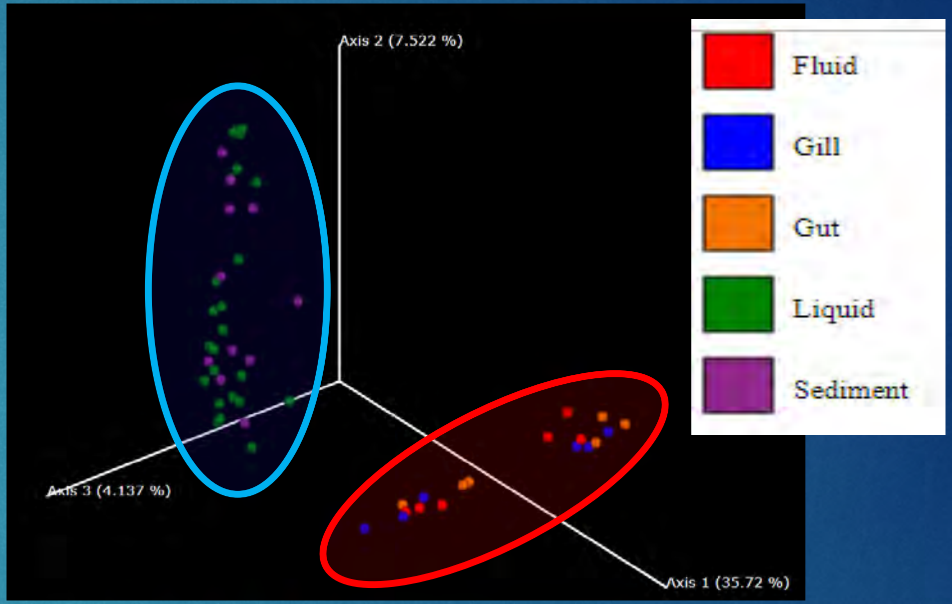

- β-diversity revealed the microbial community within zebra mussel tissues more similar to one another than to their surrounding lake water and sediments

D. Polymorpha associated bacterial community

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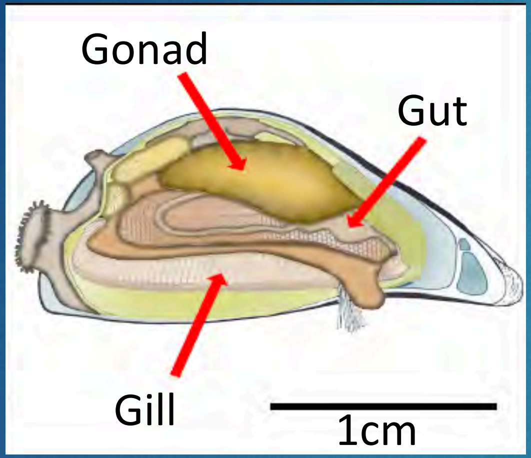
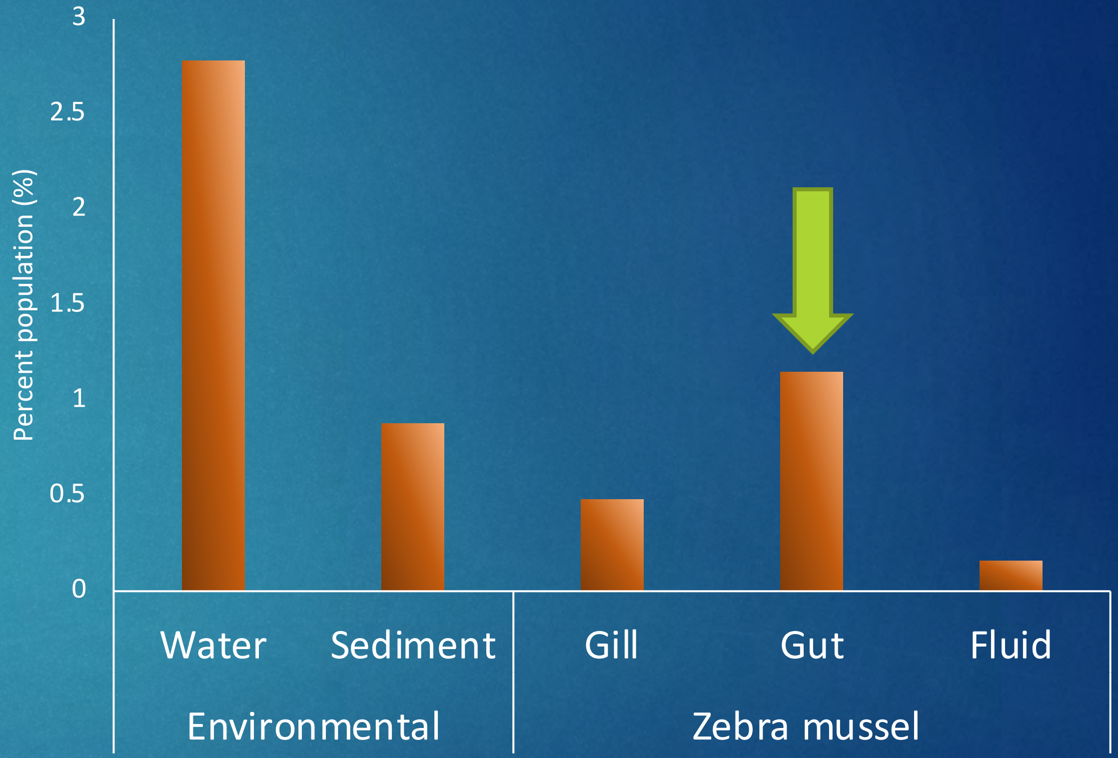
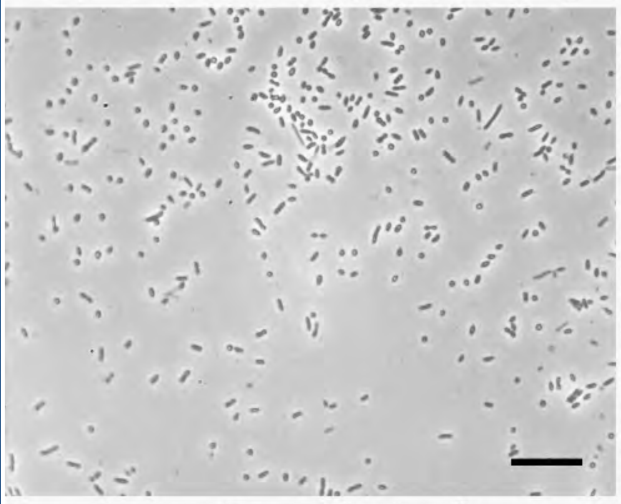




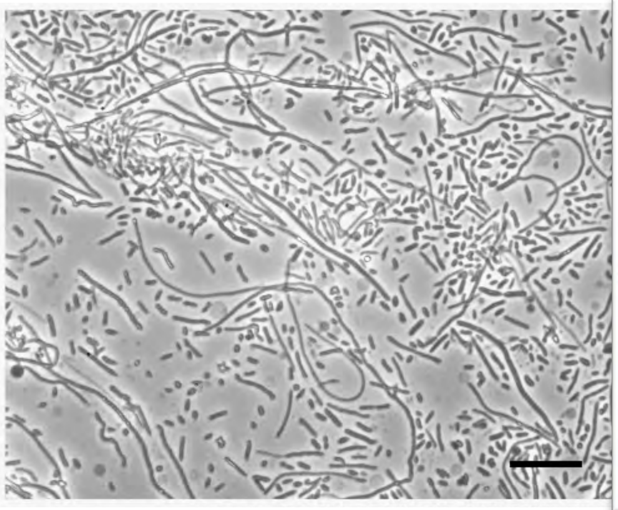
- AAP are found predominantly in gut tissues of zebra mussels

Variable growth of AAP leads to consumption by higher level organisms?

- Variable cellular size of pelagic fresh water AAP
- Elongation in stationary capable of filtration (5-45 µm) by *D. polymorpha*
- Zebra mussels consuming **AAP and other bacteria?**

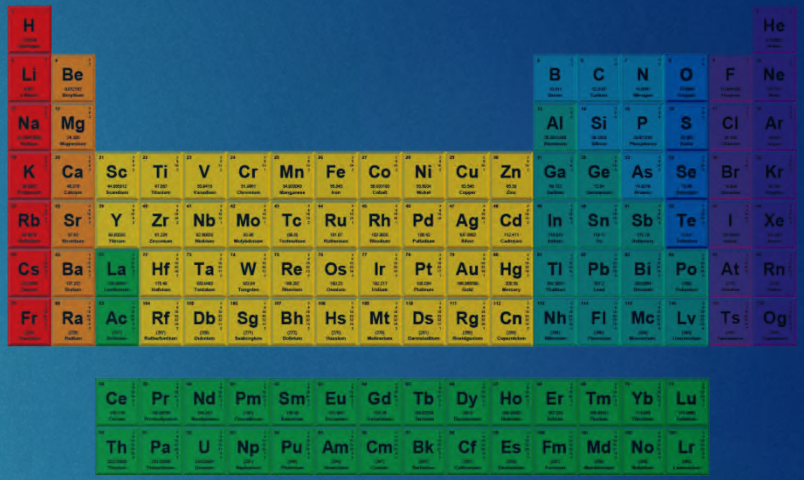


Exponential growth phase



Stationary growth phase

Bioaccumulation in Zebra Mussels

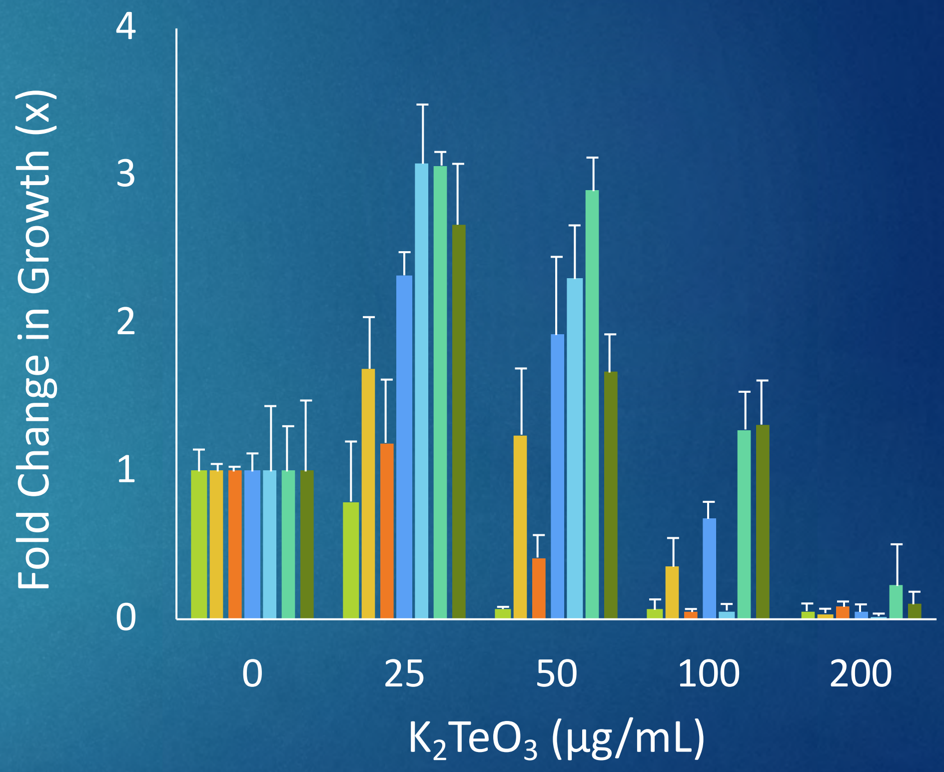


- Collision/reaction cell based inductively coupled plasma mass spectrometry (CRC ICPMS)
- Examined water, sediment, and mollusc tissues
- **39 elements down to ppb:**
  Al, Sb, As, Ba, Be, Bi, B, Cd, Ca, Cs, Cr, Co, Cu, Fe, Pb, Li,
  Mg, Mn, Mo, Ni, K, P, Rb, Se, Si, Ag, Na, Sr, S, Te, Tl, Th, Sn, Ti, W, U, V, Zn, Zr
- Within water, **ONLY** Ca, S, Na, Mg, and K were over 1 ppm
- In contrast to **lake water**, all elements were detected **10x higher in numbers within zebra mussels**, except for Mg, which was only 6x more

Bacterial AAP isolates resistant to metal oxides

- Tellurite resistance among zebra mussel AAP isolates. AAP were cultivated in RO medium with increasing levels of K_2TeO_3.

- Growth promotion at sub inhibitory levels?
- Protect host by performing metal transformations



Summary and Significance

- Bioaccumulation occurs in zebra mussels.
- Zebra mussel prey selectivity is largely unknown
  - AAP and other bacteria are associated with this bivalve.
  - AAP have elongated size and metal transformation capabilities may associate these bacteria with *D. polymorpha*

- Analysis of filter feeders like zebra mussels, what they eat, and the rate at which they consume, is critical to comprehend their infestation and influence
- Future work may help to prevent or restrict the proliferation of these mussels in environments to which they originally do not belong

Thank you!

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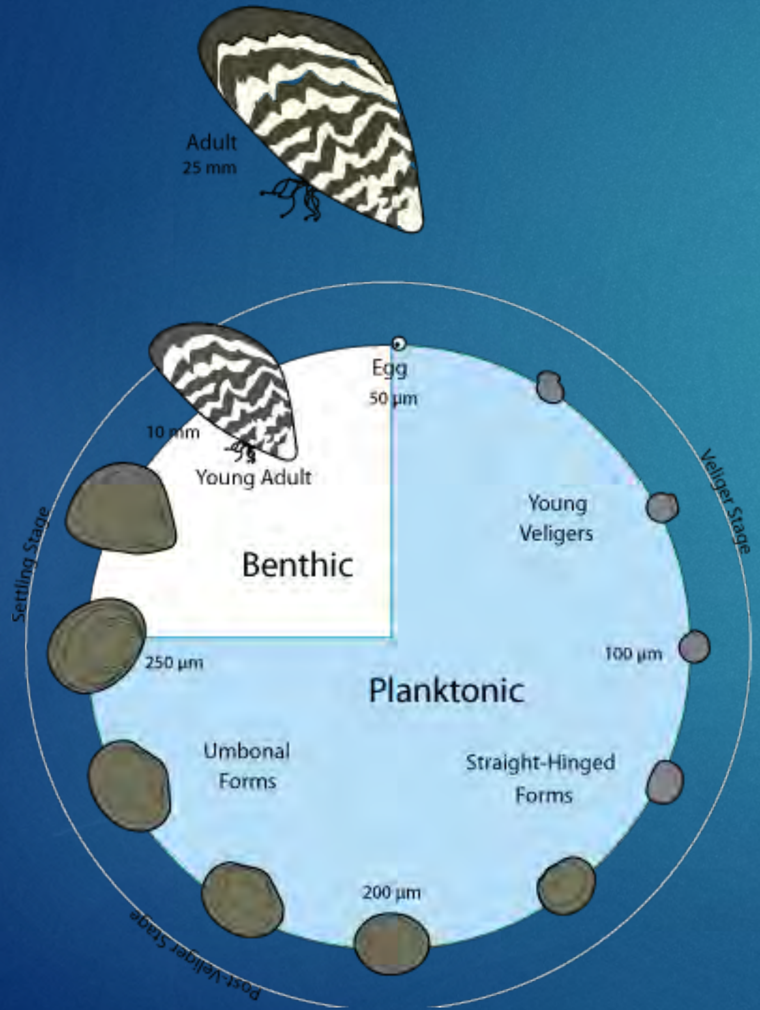
Zebra Mussel: *Dreissena polymorpha* Invasive freshwater bivalve



- Native of Eastern Europe/ Ukraine
- Anthropenically introduced west
- Introduction decimates local fish population
- Adults filter 1 litre/day
- Life span 5 years
- **No natural predators** in North America
- Filter 5 - 45µm particles
- **Potential as selective grazers**

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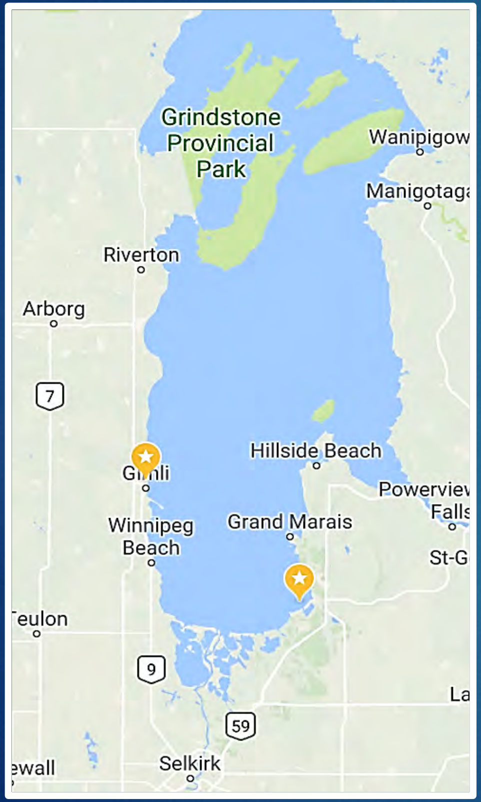
Step 1:

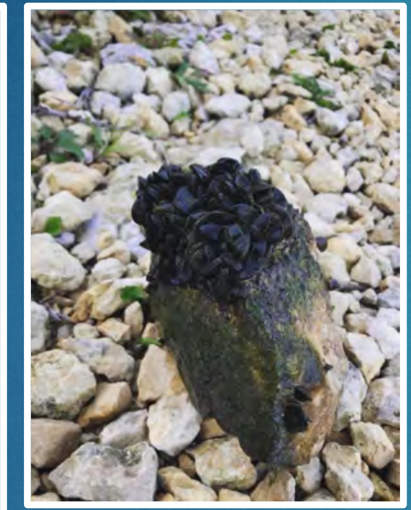
Pick strains representing major phyla in lake water to test for grazing, and isolate

Phyla	**Percent population**
Proteobacteria	41.4
Actinobacteria	18.1
Bacteroidetes	11.1
Cyanobacteria	6.2
Verromicrobia	5.0
Acidobacteria	5.2
Planctomycetes	4.4
Chloroflexi	2.4
Gemmatimonadetes	1.3
Firmicutes	0.8
	96.5

- Use sequencing data from PhD to determine abundant species
- Choose representatives and known media compositions / growth conditions
- Embark upon sampling trip specifically for culturing of phyla representatives

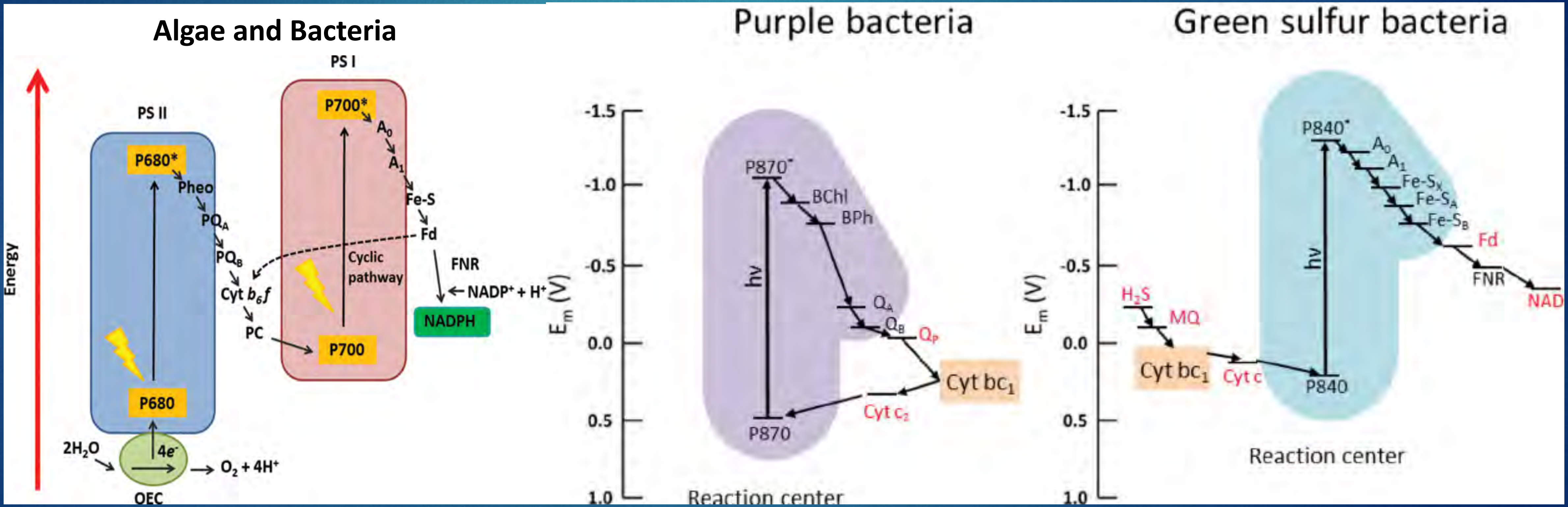
Zebra Mussel Collection



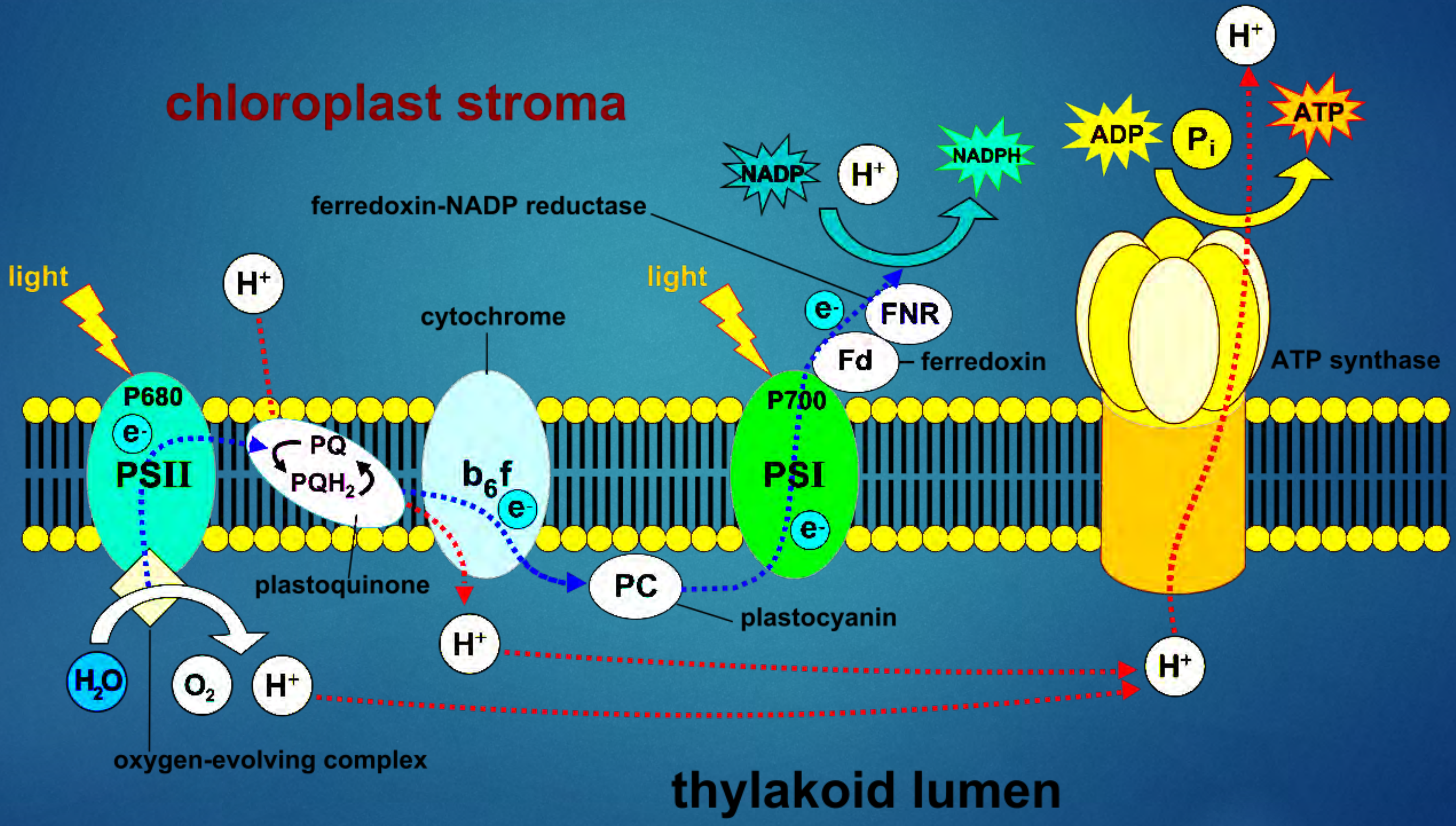


- Adult, living, bivalves for analysis of predation
- Gimli or Patricia beach
- In lake water during transport to laboratory

Photosystems



Extra Information:

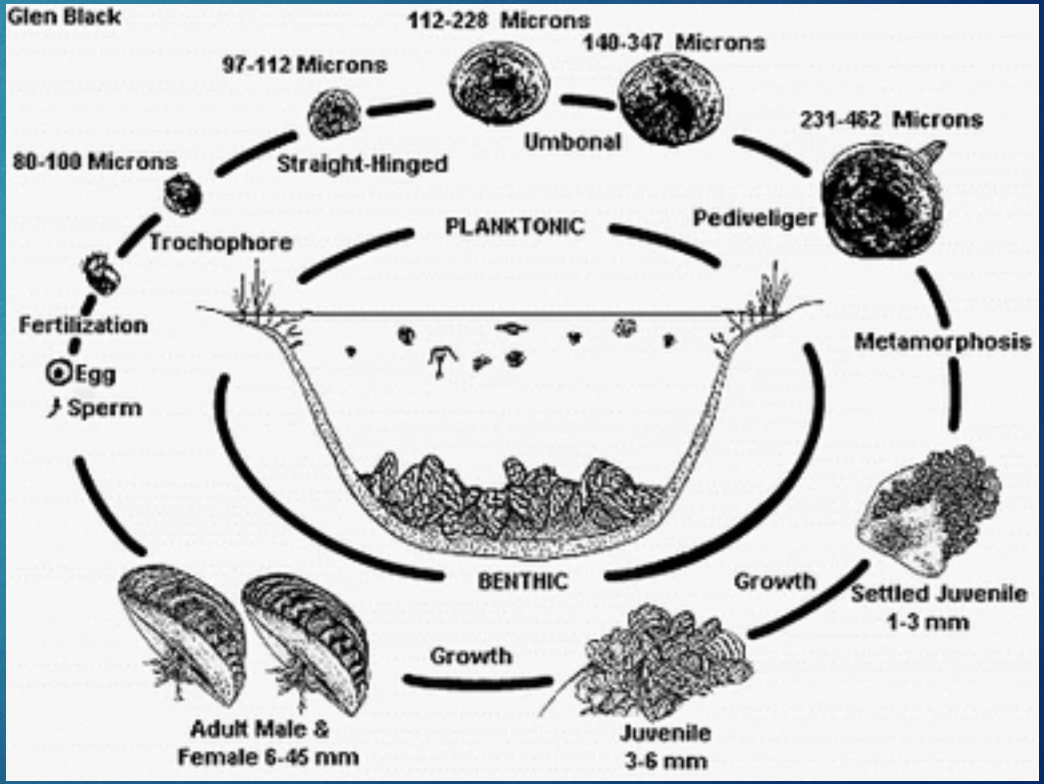


Methods of Zebra Mussel Inhibition

- Research on **natural enemies,** both in Europe and North America, has focused on predators, particularly birds (36 species) and fish (15 and 38 species eating veligers and attached mussels).
- The vast majority of the organisms that are natural enemies in Europe are **not present in North America**. Ecologically similar species do exist, but these species are unlikely to be able to eliminate those mussels already established and will have a limited role in their control.
- Crayfish could have a significant impact on the densities of 1- to 5-mm-long zebra mussels. An adult crayfish consumes around 105 zebra mussels every day, or about 6000 mussels in a season. Predation rates are significantly reduced at lower water temperatures. Fish do not seem to limit the densities of zebra mussels in European lakes. Smallmouth bass are a predator in the zebra mussels' adopted North American Great Lakes habitat.
- **Other control**
  On June 4, 2014, Canadian conservation authorities announced that a test using liquid fertilizer to kill invasive zebra mussels was successful. This test was conducted in a lakefront harbor in the western province of Manitoba. However, there continue to be outbreaks in Lake Winnipeg.

Lifecycle

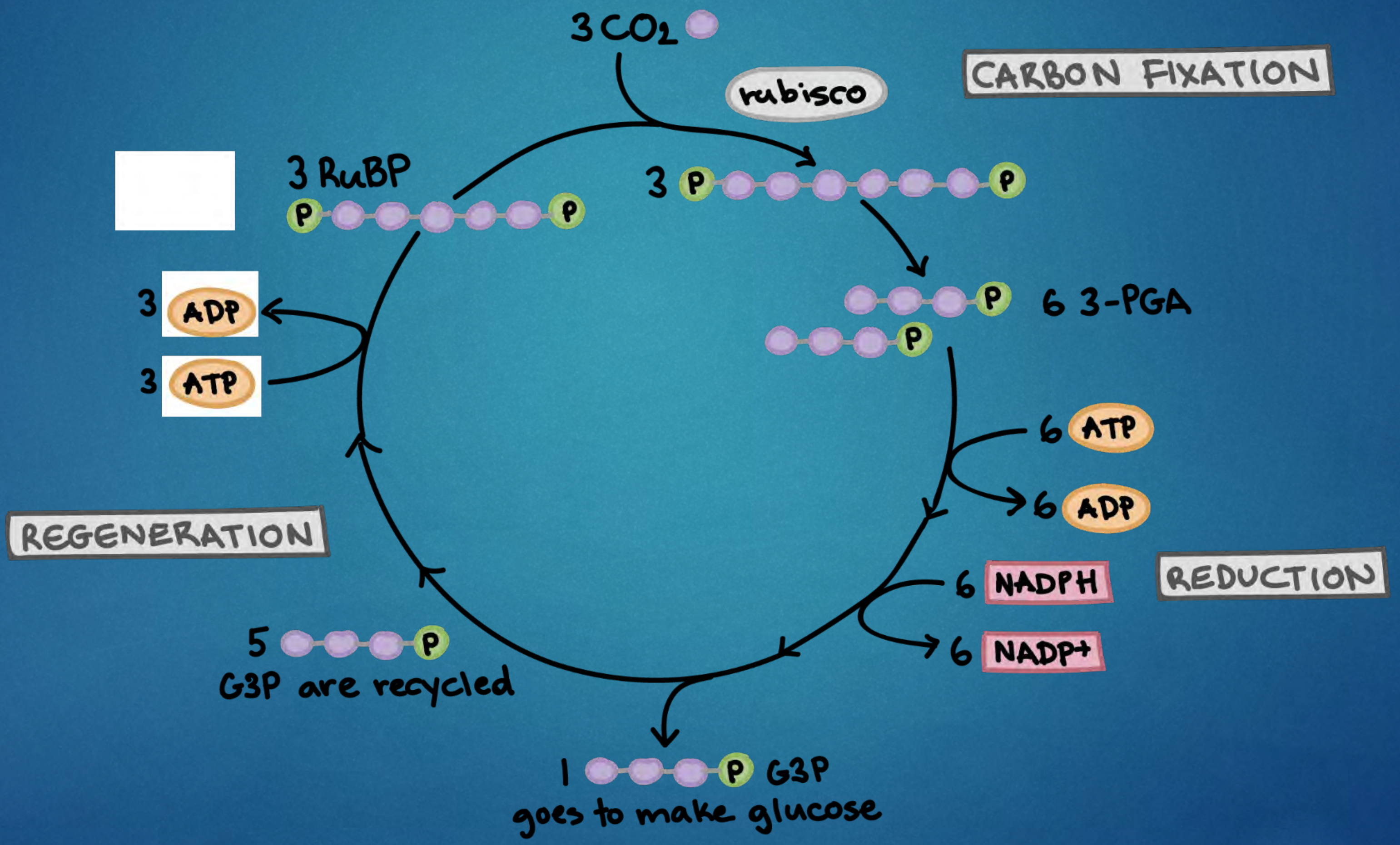
- zebra mussel lifespan is four to five years.
- A female zebra mussel begins to reproduce within 6–7 weeks of settling.
- An adult female zebra mussel can produce 30,000 to 40,000 eggs in each reproductive cycle **late spring or early summer**, and over 1 million each year.
- Free-swimming microscopic larvae, called veligers, drift in the water for several weeks and then settle onto any hard surface they can find. Zebra mussels also can tolerate a wide range of environmental conditions and adults can even survive out of water for about 7 days.



Bacteriochlorophyll varieties

Pigment	Bacterial group	in vivo infrared absorption maximum (nm)
Bacteriochlorophyll **a**	**AAP**, Purple Bacteria, Heliobacteria, Green Sulfur Bacteria, Chloroflexi, Chloracidobacterium thermophilum[1]	805, 830–890
Bacteriochlorophyll **b**	Purple bacteria	835–850, 1020–1040
Bacteriochlorophyll **c**	Green sulfur bacteria, Chloroflexi, C. thermophilum	745–755
Bacteriochlorophyll $\mathbf{c_s}$	Chloroflexi	740
Bacteriochlorophyll **d**	Green sulfur bacteria	705–740
Bacteriochlorophyll **e**	Green sulfur bacteria	719–726
Bacteriochlorophyll **f**	Green sulfur bacteria (currently found only through mutation; natural may exist)	700–710
Bacteriochlorophyll **g**	Heliobacteria	670, 788

The Calvin Cycle



Diversity

- Alpha diversity was defined by Whittaker (1972) as the species richness of a place. However, the practical development of this concept led to redefine the alpha diversity on the basis of the structure of the community. The most common expression is linked, then, both on the number of species and the proportion in which each species is represented in the community. In short, a community will have an high alpha diversity, when there is an high number of species and their abundances are much similar. You can consult a list of indexes in: Magurran & McGill 2011. (Editors) Biological diversity. Oxford University Press.

- Beta diversity was defined by Whittaker (1972) as "the extent of species replacement or biotic change along environmental gradients." The beta diversity measures the turnover of species between two sites in terms of gain or loss of species. However, although the concept of change in species composition is quite clear and intuitive, beta diversity reflects two antithetical phenomena: nestedness and turnover. The phenomenon of nestedness occurs when the biota of a site with a lower number of species is a subset of a biota with a greater number of species. In this case, the dissimilarity between two sites is related to the difference in specific richness and occurs even in the absence of a real turnover of species. In contrast, the spatial turnover implies the replacement of some species by others. Numerous indices of beta diversity are sensitive, albeit with varying degrees, to the difference in species richness, returning results related both to nestedness and turnover. Baselga (2007; 2010) has proposed a method to measure separately the two phenomena.

- **B strains** may have an additional type II secretion system not found in**K12**.