

Using a high-throughput sequencing assay to assess dreissenid mussel communities

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Quagga

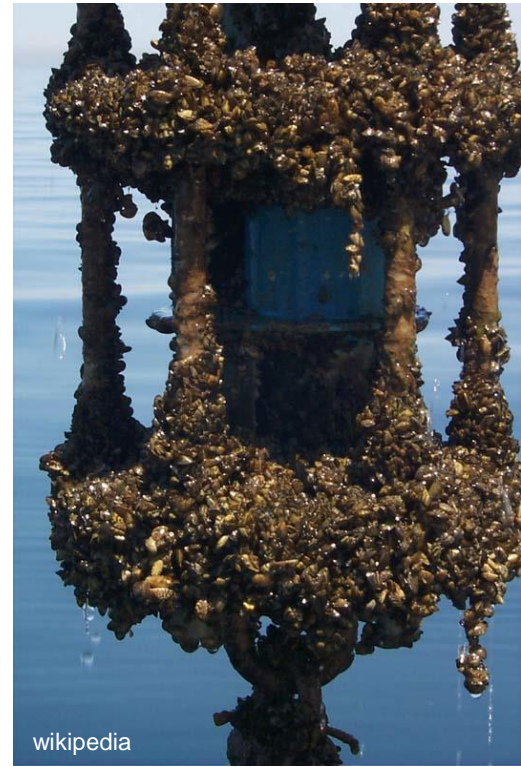


Zebra



Outline

- *Dreissena* invasion and background
- **HTS assay design**
- Evaluating assay
 1. Mock Communities
 2. Aquaria Samples
 3. Field Samples
- **Ecological Implications**



NOAA GLERL

Dreissena Invasion

- Native to Ponto-Caspian region
- **Zebra mussel invade GL ~1986**
- Quagga mussel invade GL ~1989



Zebra

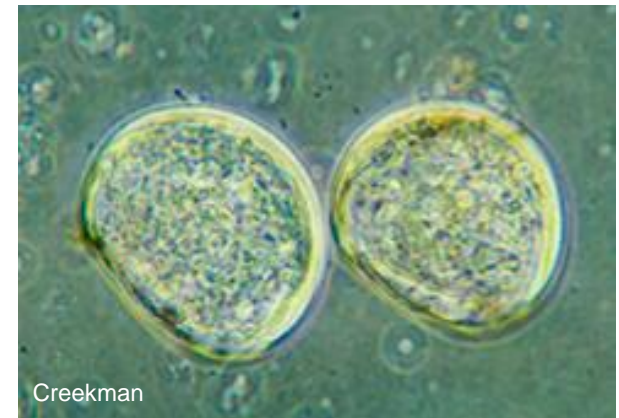


Quagga

- **Negative economic and ecological impacts**

Dreissena Invasion

- **Difficult to distinguish species morphologically**
 - High phenotypic plasticity
 - Especially difficult at veliger larvae stage



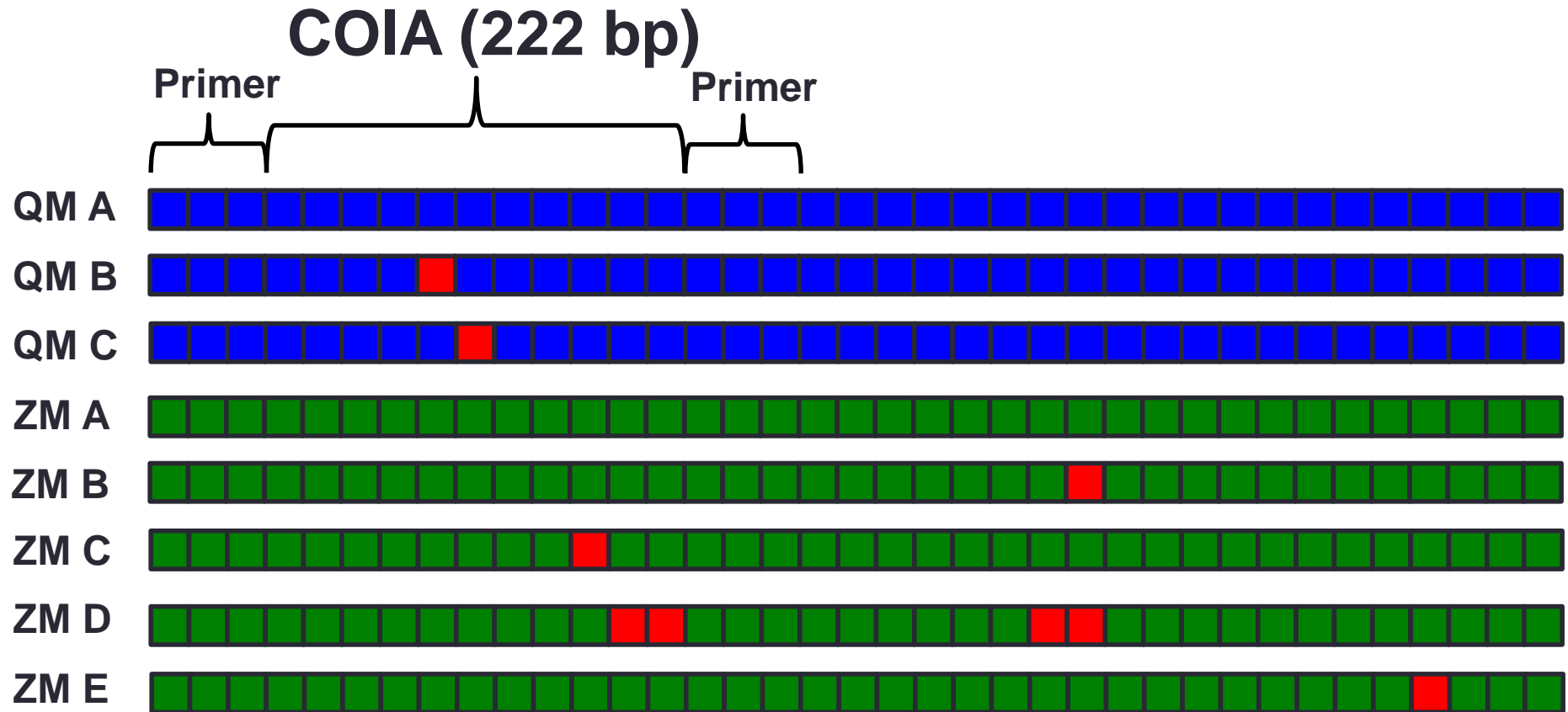


QM = Quagga
Dreissena rostriformis



ZM = Zebra
D. polymorpha

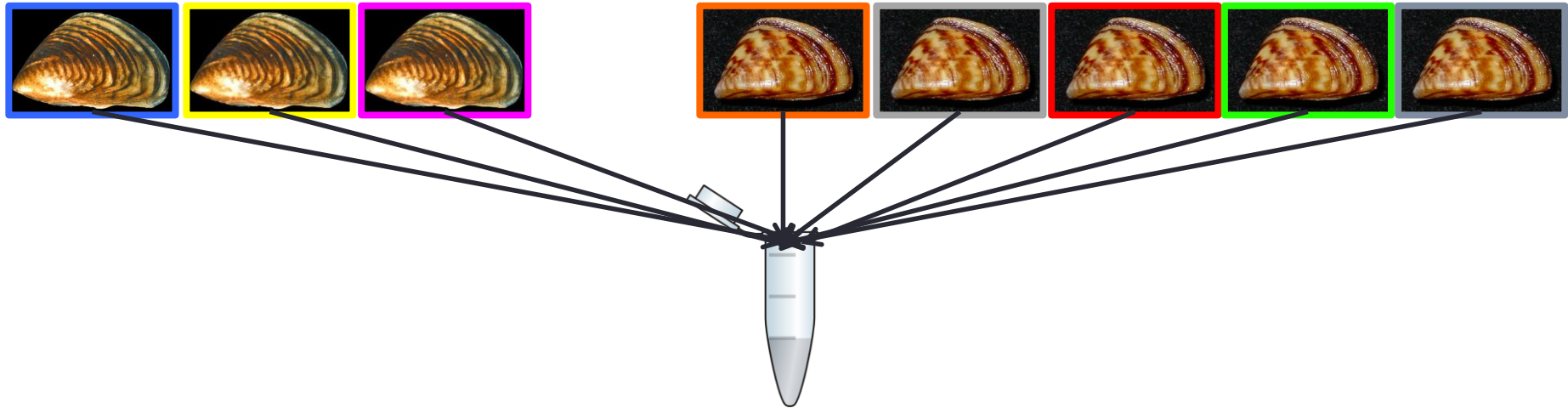
COI Assay Design



■ = Single Nucleotide Polymorphism (SNP)

Evaluating the Assay

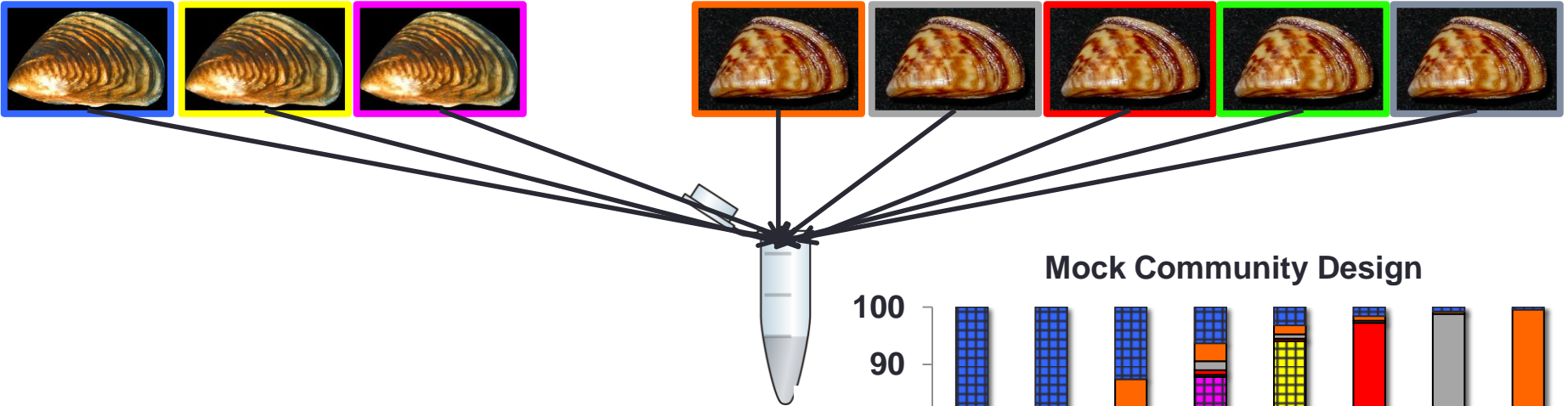
1. **Mock Communities**
2. **Aquaria Experiments**
3. **Field Samples**



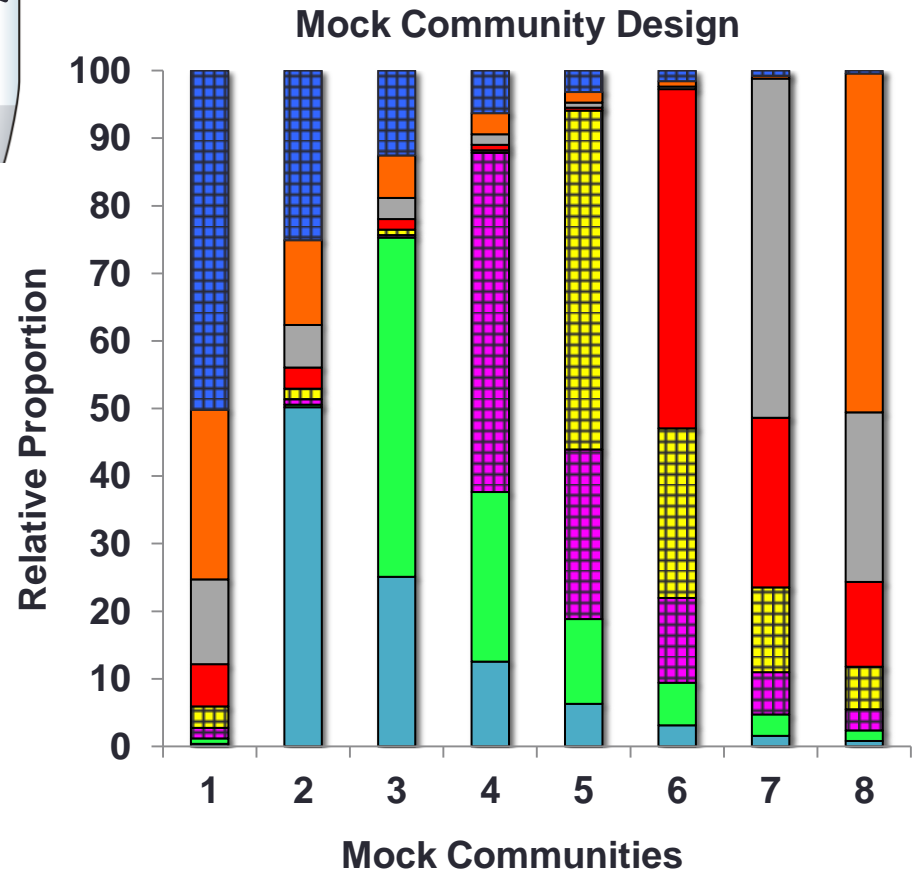
Mock Communities

QM=Quagga

ZM=Zebra



	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8
QMA	6000	3000	1500	750	375	188	94	47
ZMA	3000	1500	750	375	188	94	47	6000
ZMB	1500	750	375	188	94	47	6000	3000
ZMC	750	375	188	94	47	6000	3000	1500
QMB	375	188	94	47	6000	3000	1500	750
QMC	188	94	47	6000	3000	1500	750	375
ZMD	94	47	6000	3000	1500	750	375	188
ZME	47	6000	3000	1500	750	375	188	94

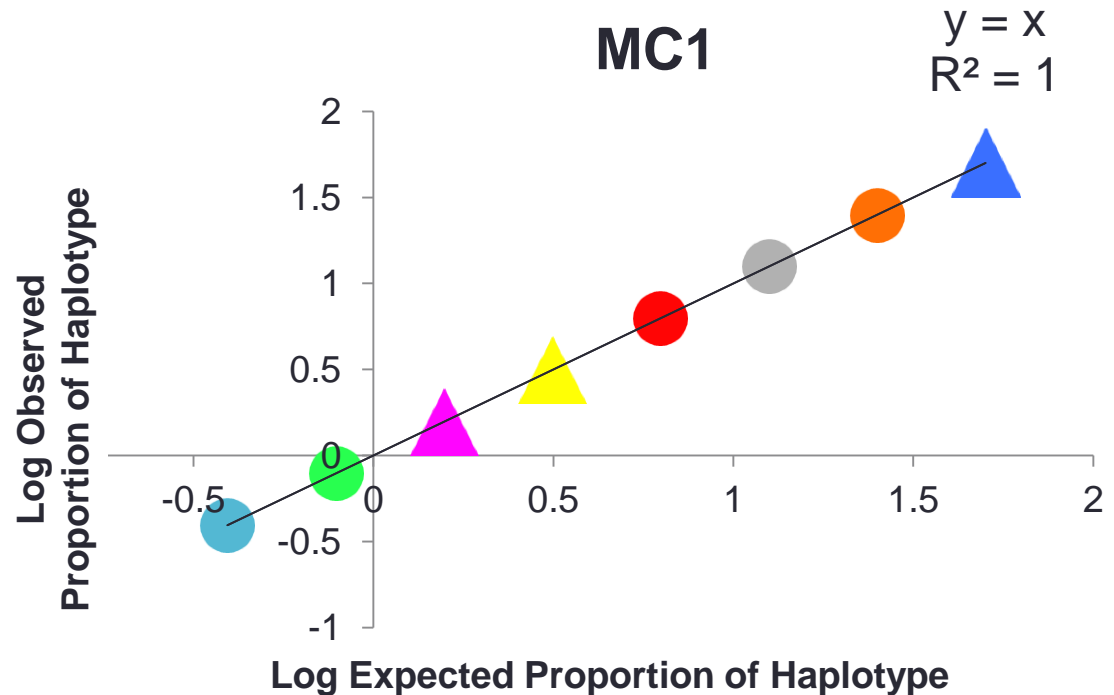
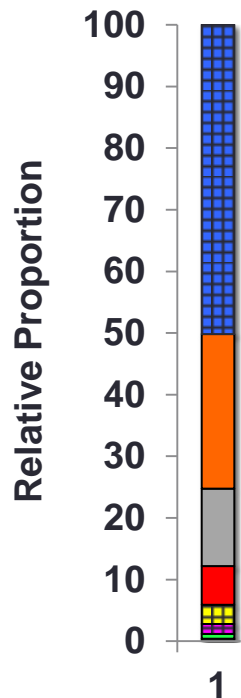


Testing the Assay

	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8
QMA	6000	3000	1500	750	375	188	94	47
ZMA	3000	1500	750	375	188	94	47	6000
ZMB	1500	750	375	188	94	47	6000	3000
ZMC	750	375	188	94	47	6000	3000	1500
QMB	375	188	94	47	6000	3000	1500	750
QMC	188	94	47	6000	3000	1500	750	375
ZMD	94	47	6000	3000	1500	750	375	188
ZME	47	6000	3000	1500	750	375	188	94

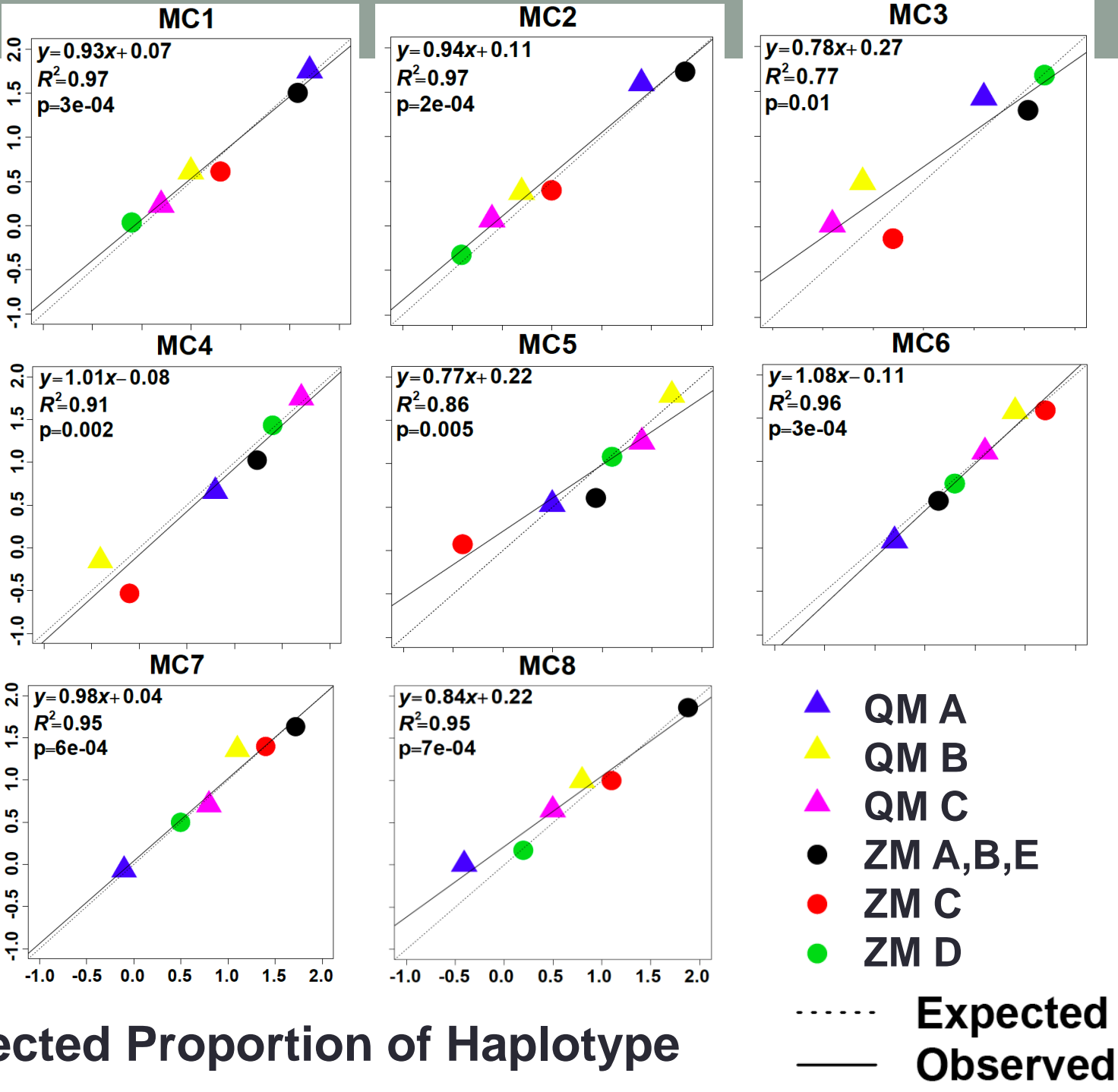
△ QM=Quagga

○ ZM=Zebra



COIA

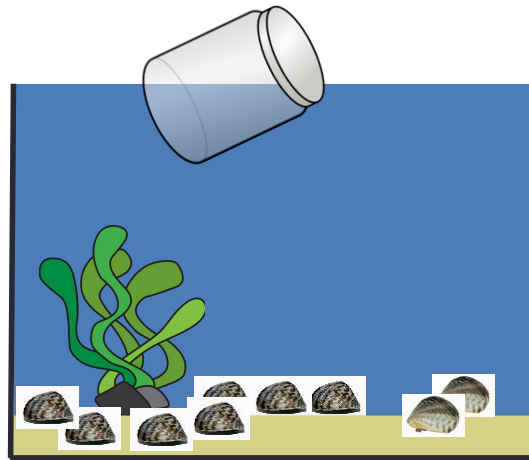
Log Observed Proportion of Haplotype



Log Expected Proportion of Haplotype

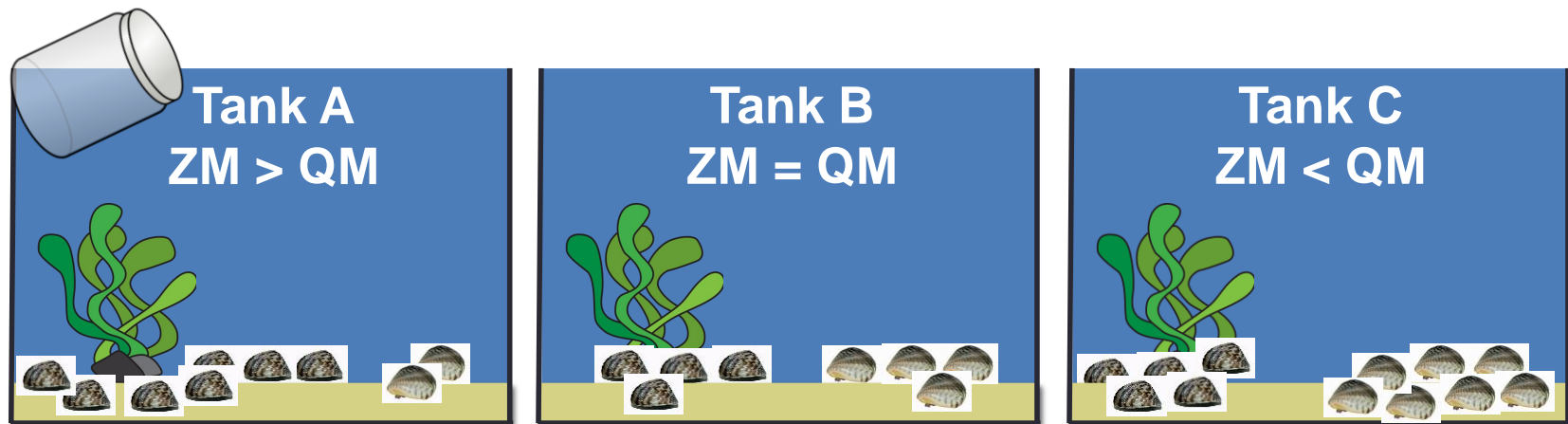
Evaluating the Assay

1. Mock Communities
2. **Aquaria Experiments**
3. Field Samples

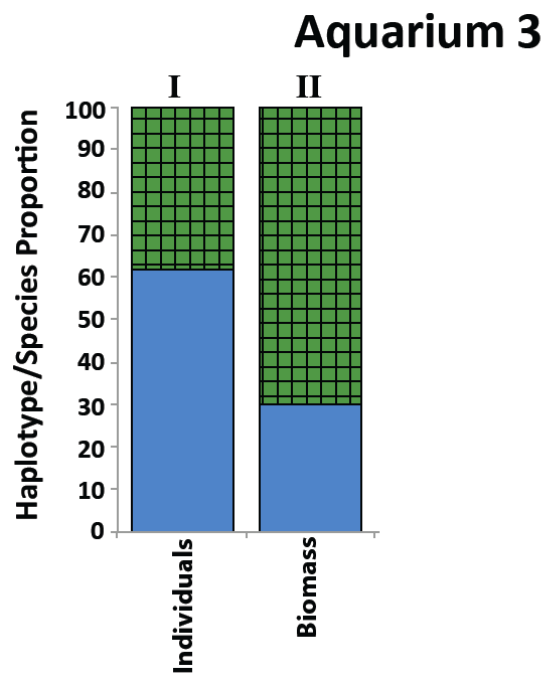
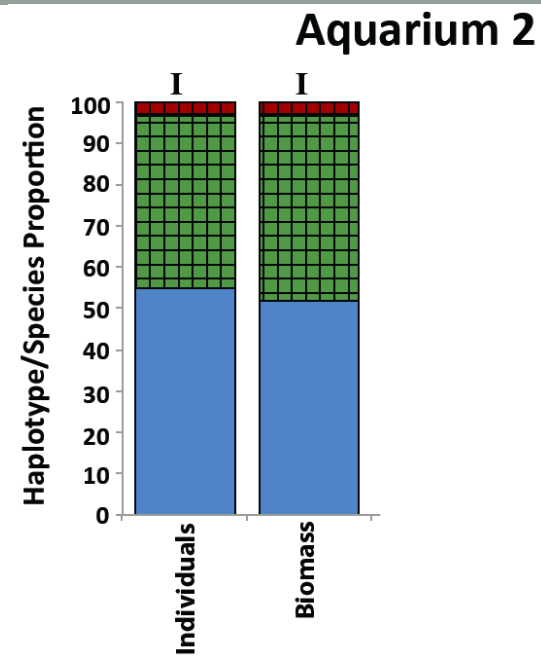
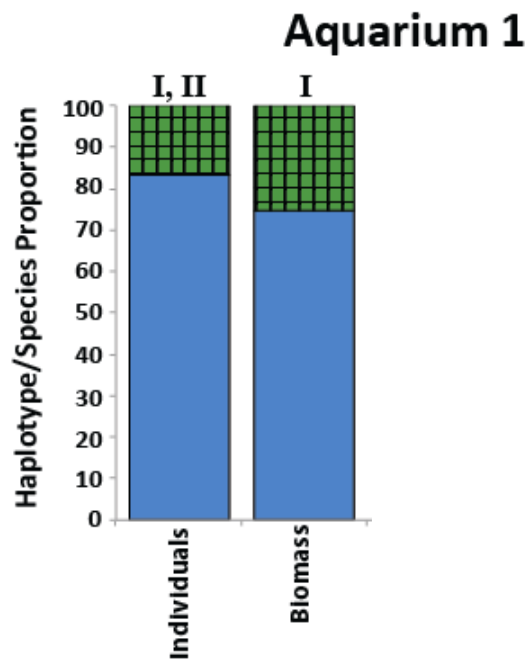


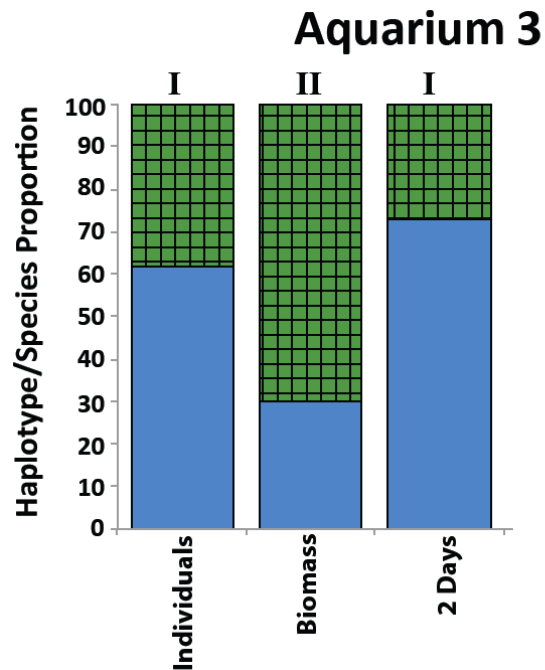
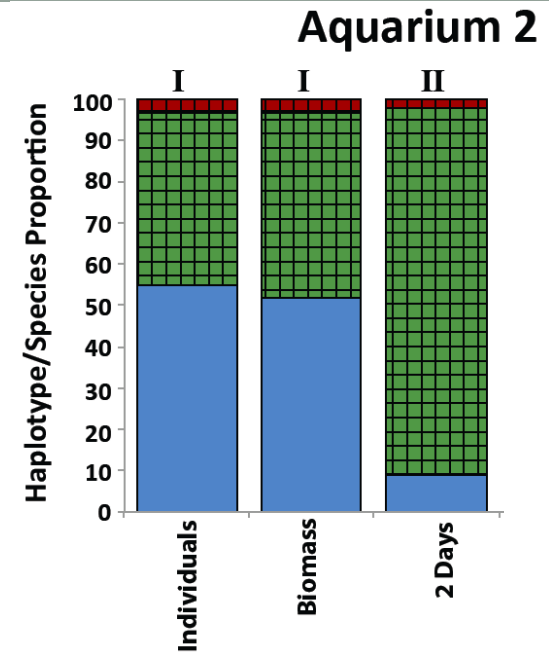
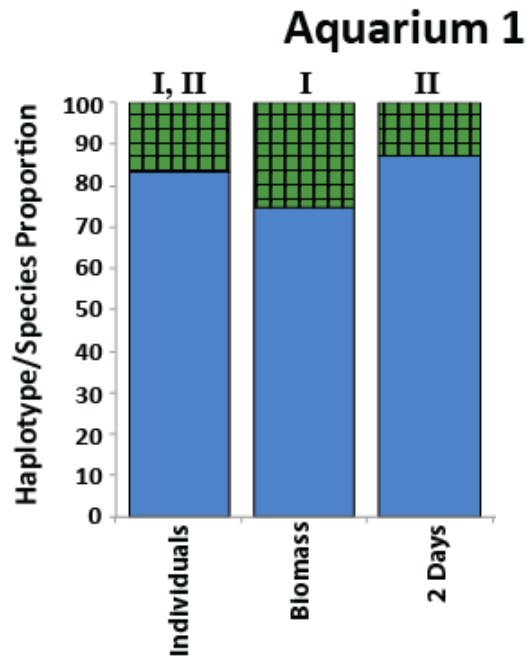
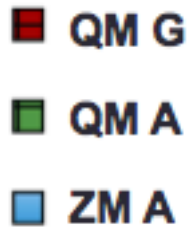
Aquaria Experiments

- Each tank filled with 15 L water
- 500 mL water sampled at 0, 2, 7, and 14 days
- Water filtered through 0.2µm PES filter
- Resultant eDNA run with marker COIA

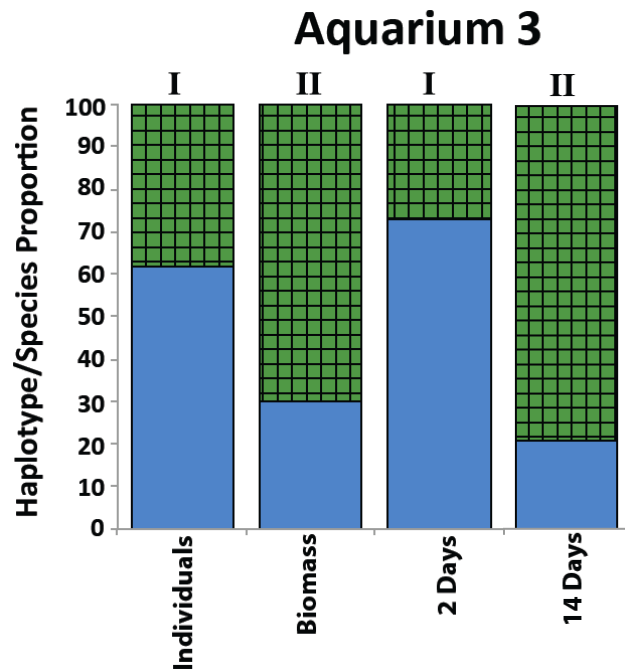
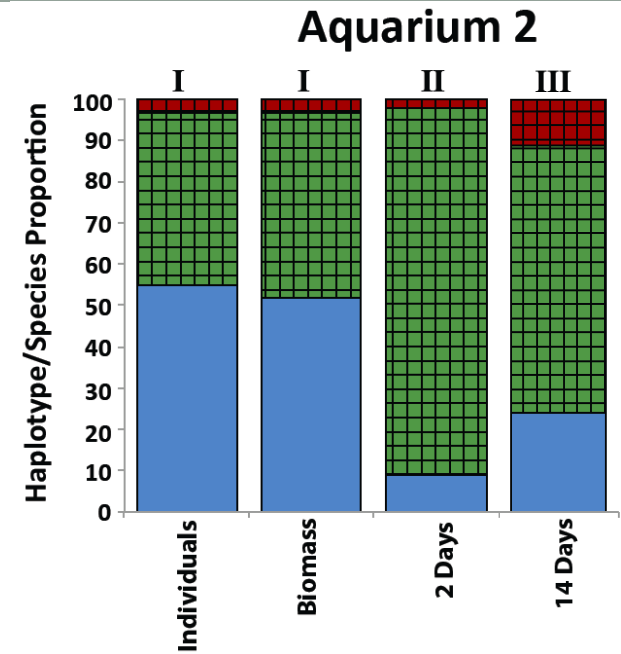
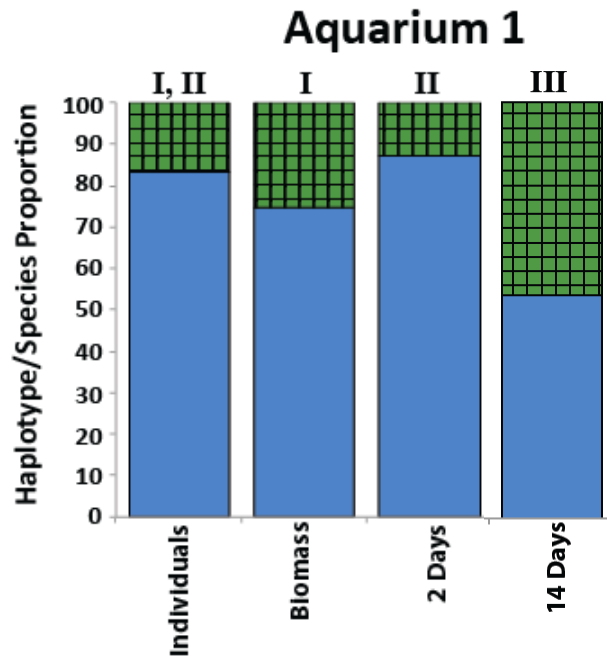


	Tank A ZM > QM		Tank B ZM = QM		Tank C ZM < QM	
	Zebra	Quagga	Zebra	Quagga	Zebra	Quagga
Individuals (N)	30.0 (83%)	6.0 (17%)	17.0 (55%)	14.0 (45%)	16.0 (62%)	10.0 (38%)
Biomass (g)	5.8 (75%)	2.0 (25%)	2.8 (52%)	2.6 (48%)	4.2 (30%)	9.8 (70%)





- Tanks A & C represented individuals early,



- Tanks A & C represented individuals early
- But shifted to biomass late
- Tank B shifted to closer representation over time, but still off. Possibly due to mussel death.

Evaluating the Assay

1. Mock Communities
2. Aquaria Experiments
3. **Field Samples**



Dreissenid eDNA (water & plankton)

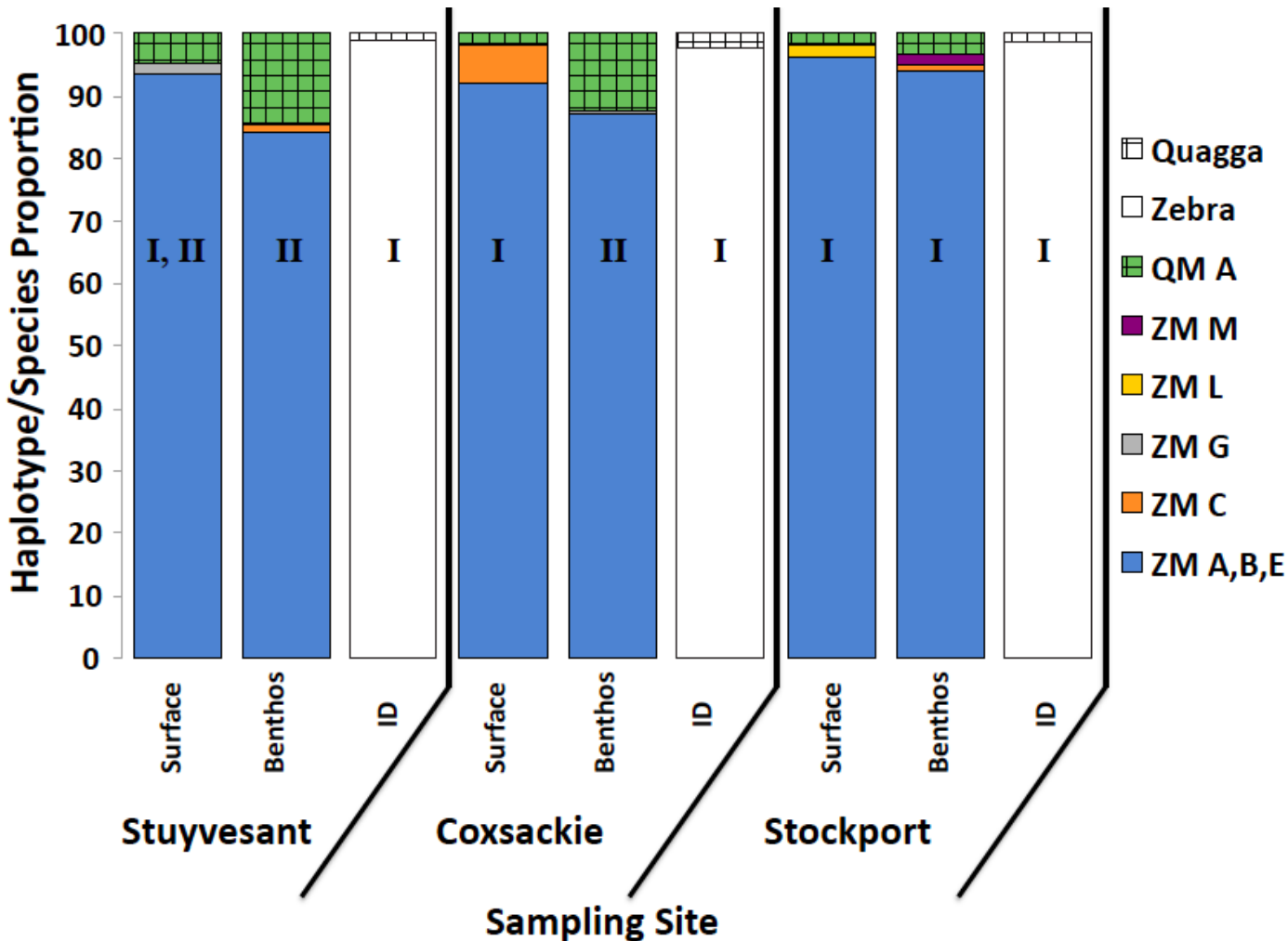


- Water from benthos & surface at 3 mid-river sites
- Sequencing compared with extensive morphological survey by the Cary Institute
 - >3,000 mussels/site

Plankton collected

- Bimonthly in Lake Erie May-September 2016
- Once in late May 2016 from 4 Hudson River sites

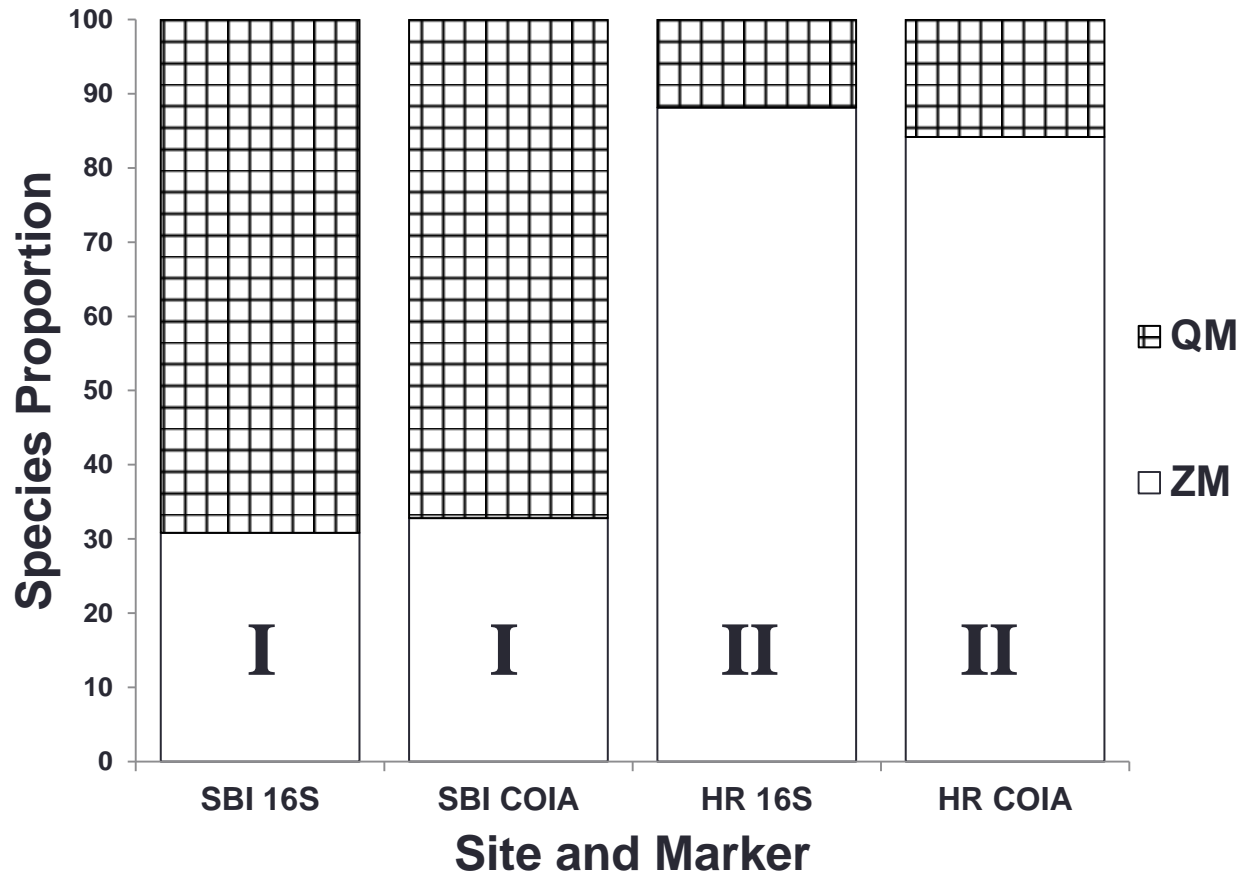
Hudson River Water Samples



- Water Samples taken from surface better represented morphological ID
- Surface and benthos samples showed different intraspecific variation

Dreissenid Veligers in Plankton

Comparison of dreissenid composition between 16S and COIA assays

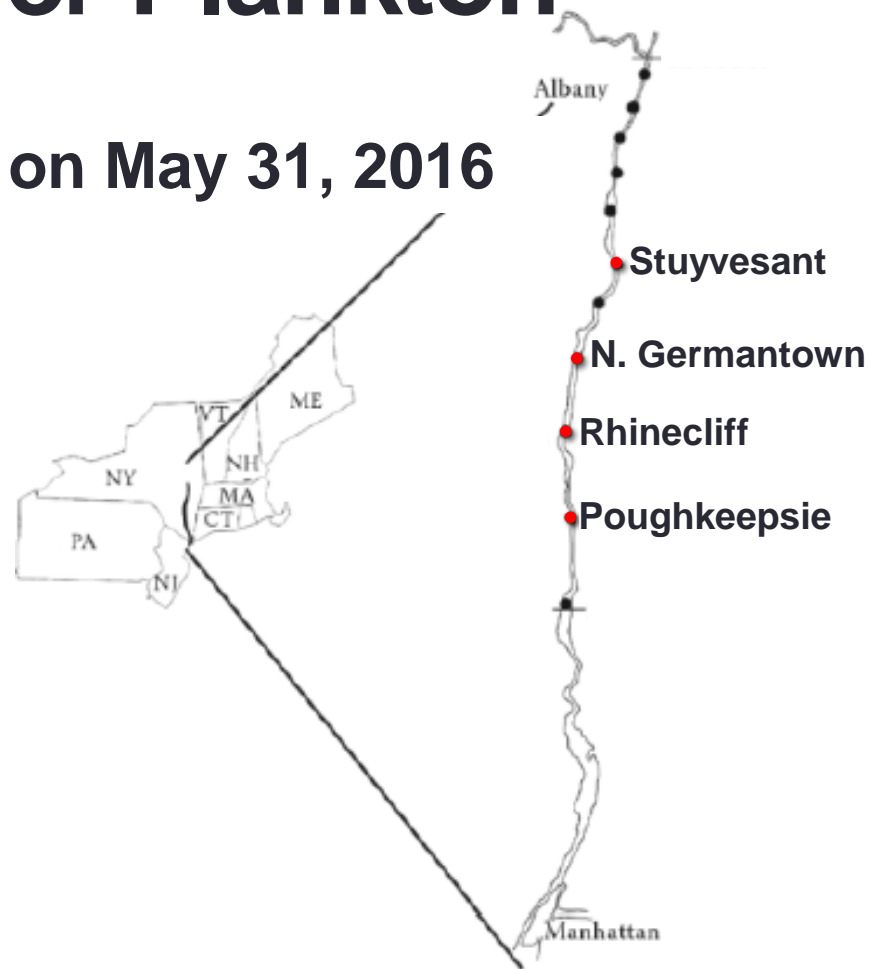
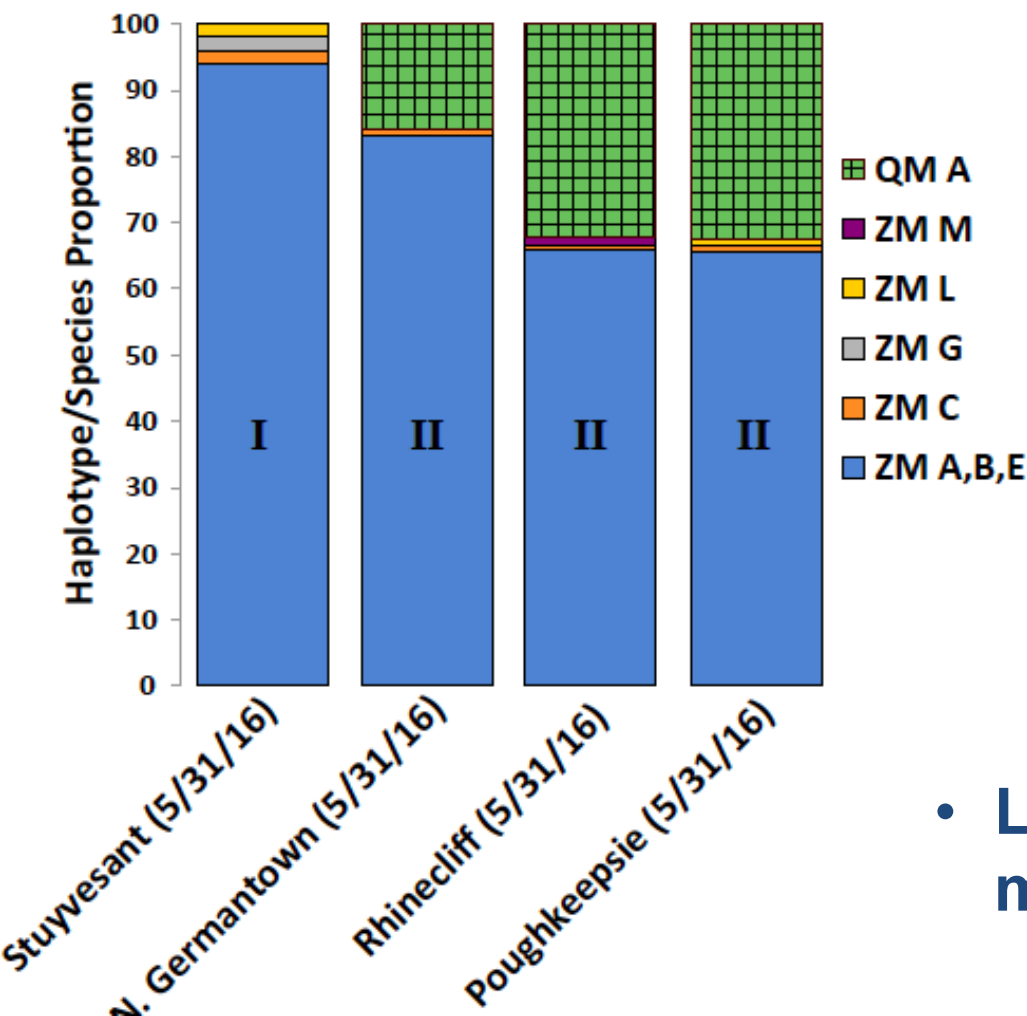


- Compared proportion of dreissenid DNA from plankton with our DreCOIA and Mol16S assays
- Samples from the Hudson River and South Bass Is., Lake Erie
- Species proportions are significantly different between the two sites
- Species proportions did not differ between the markers at either site



Hudson River Plankton

- Plankton collected at four sites on May 31, 2016



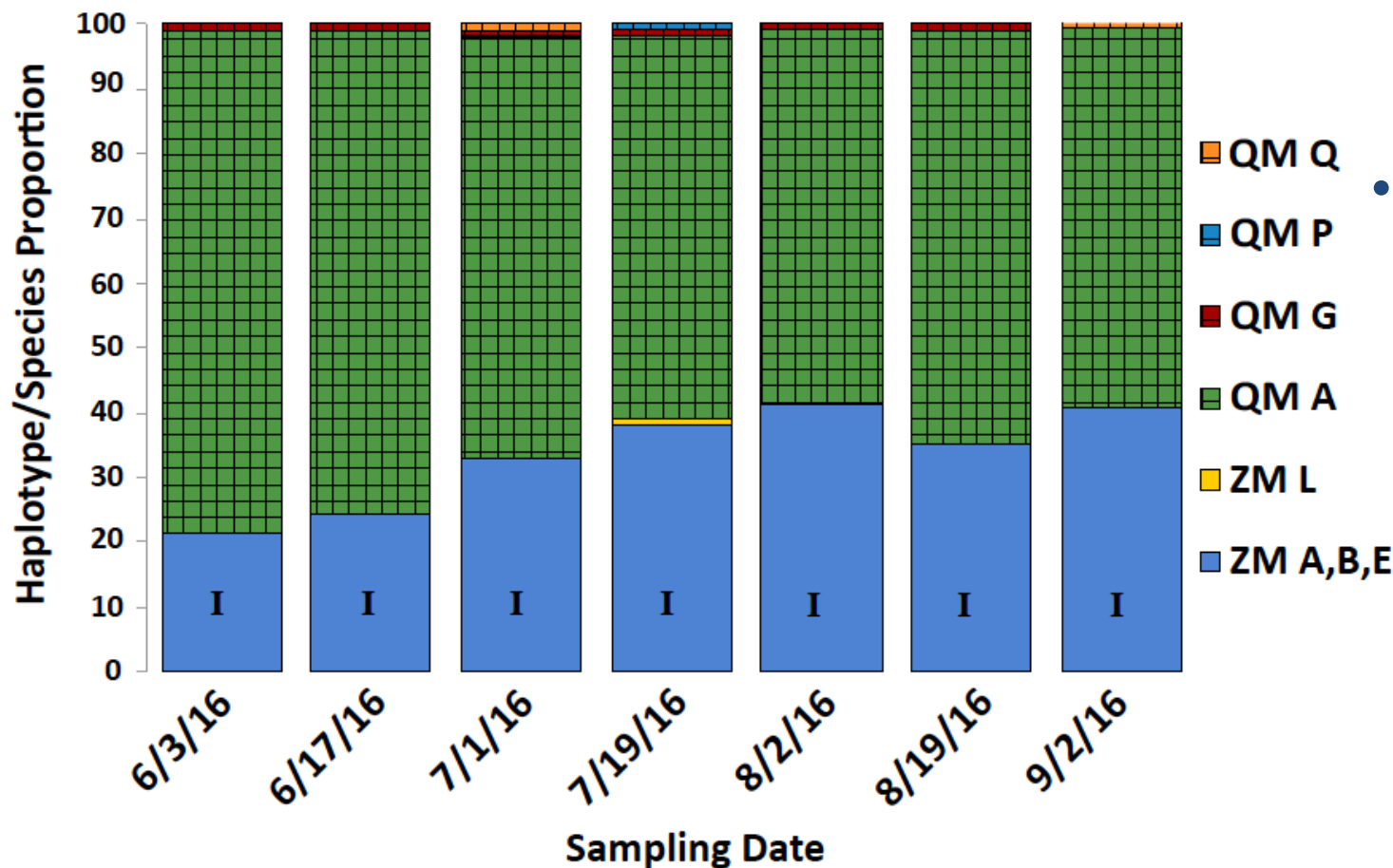
- Lower number of quagga mussel veligers up river



Lake Erie Plankton



- Plankton collected from June-September 2016 at SBI



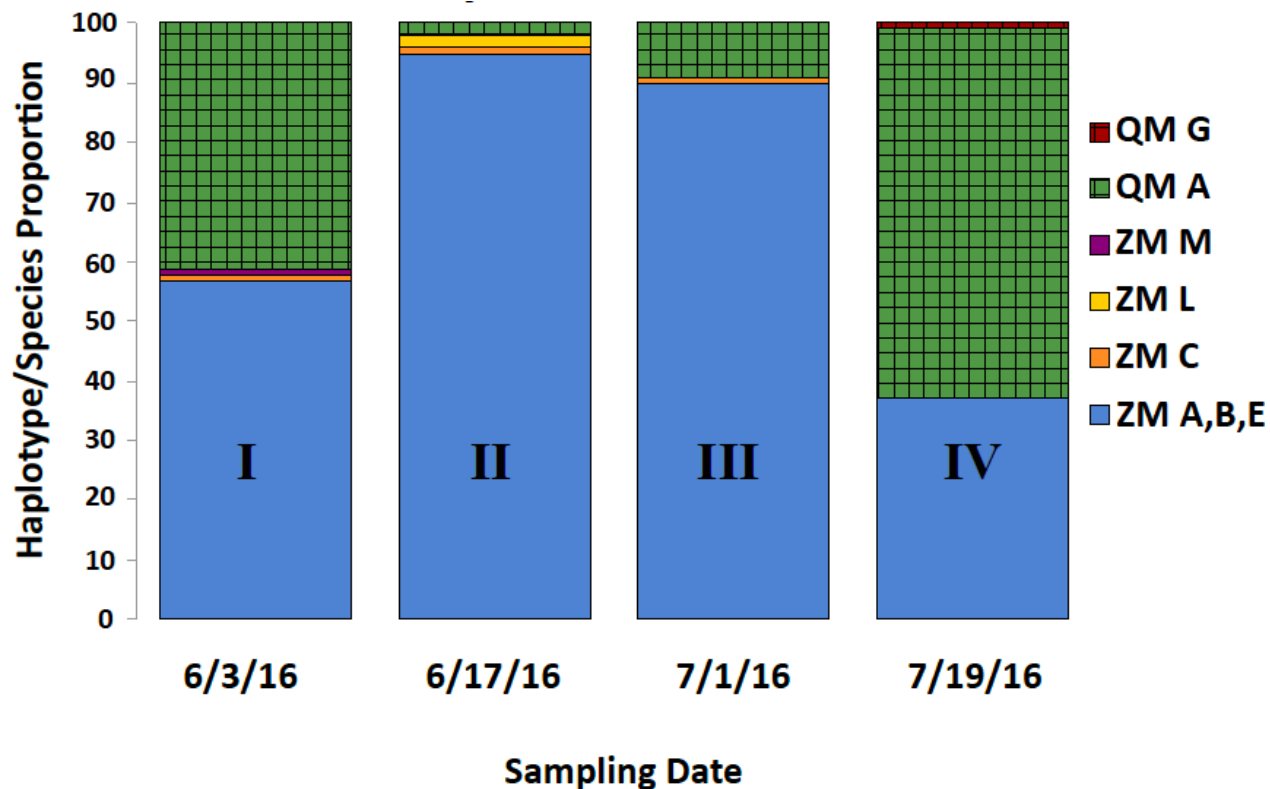
- Slight species/ community changes throughout summer



Lake Erie Plankton

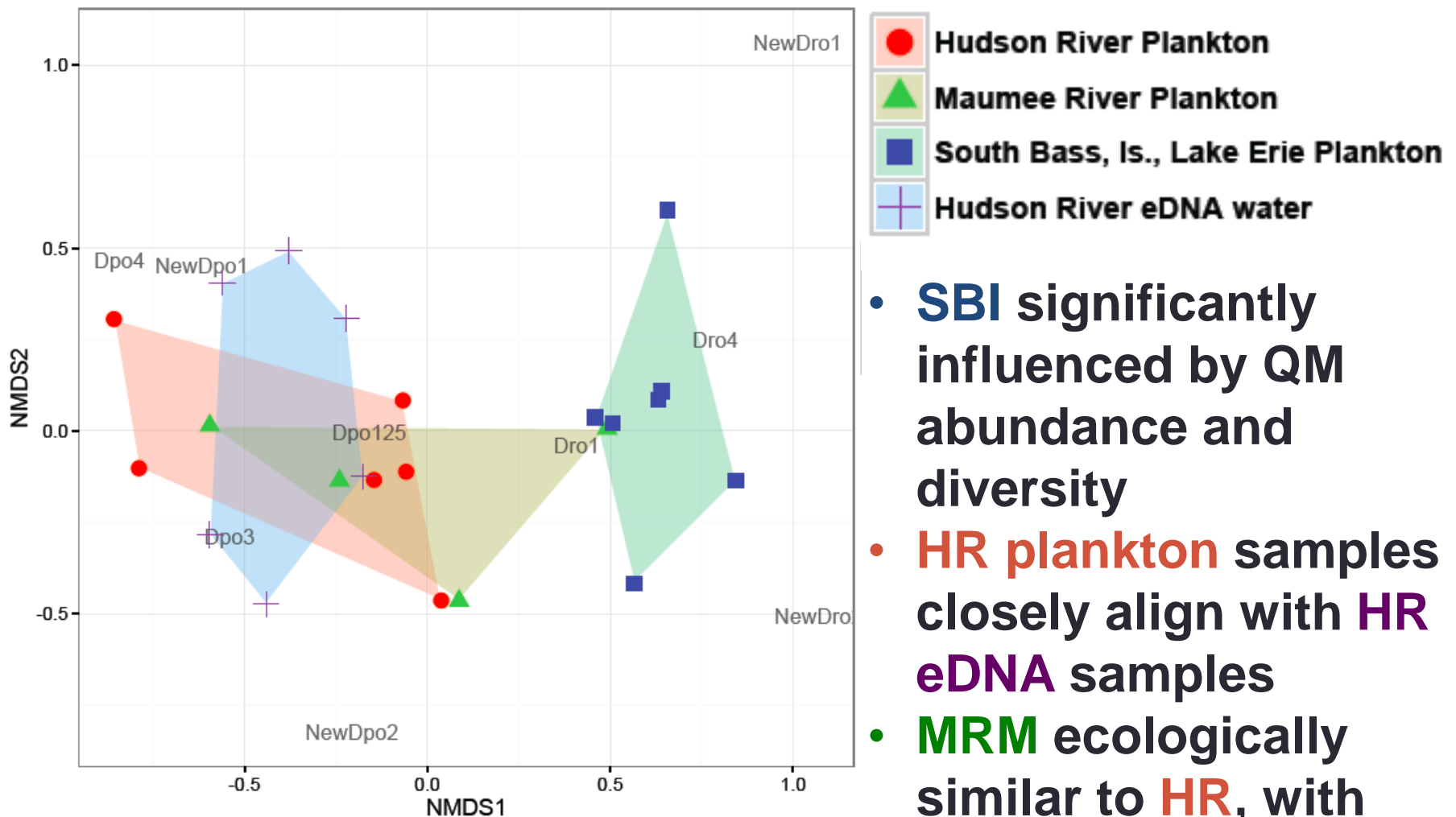


- Plankton collected from June-September 2016 at MRM



- Large species/ community changes throughout summer
- Ecologically similar to HR, geographically close to SBI

NMDS plot of environmental samples



- **SBI** significantly influenced by QM abundance and diversity
- **HR plankton** samples closely align with **HR eDNA** samples
- **MRM** ecologically similar to **HR**, with some possible influence from the east

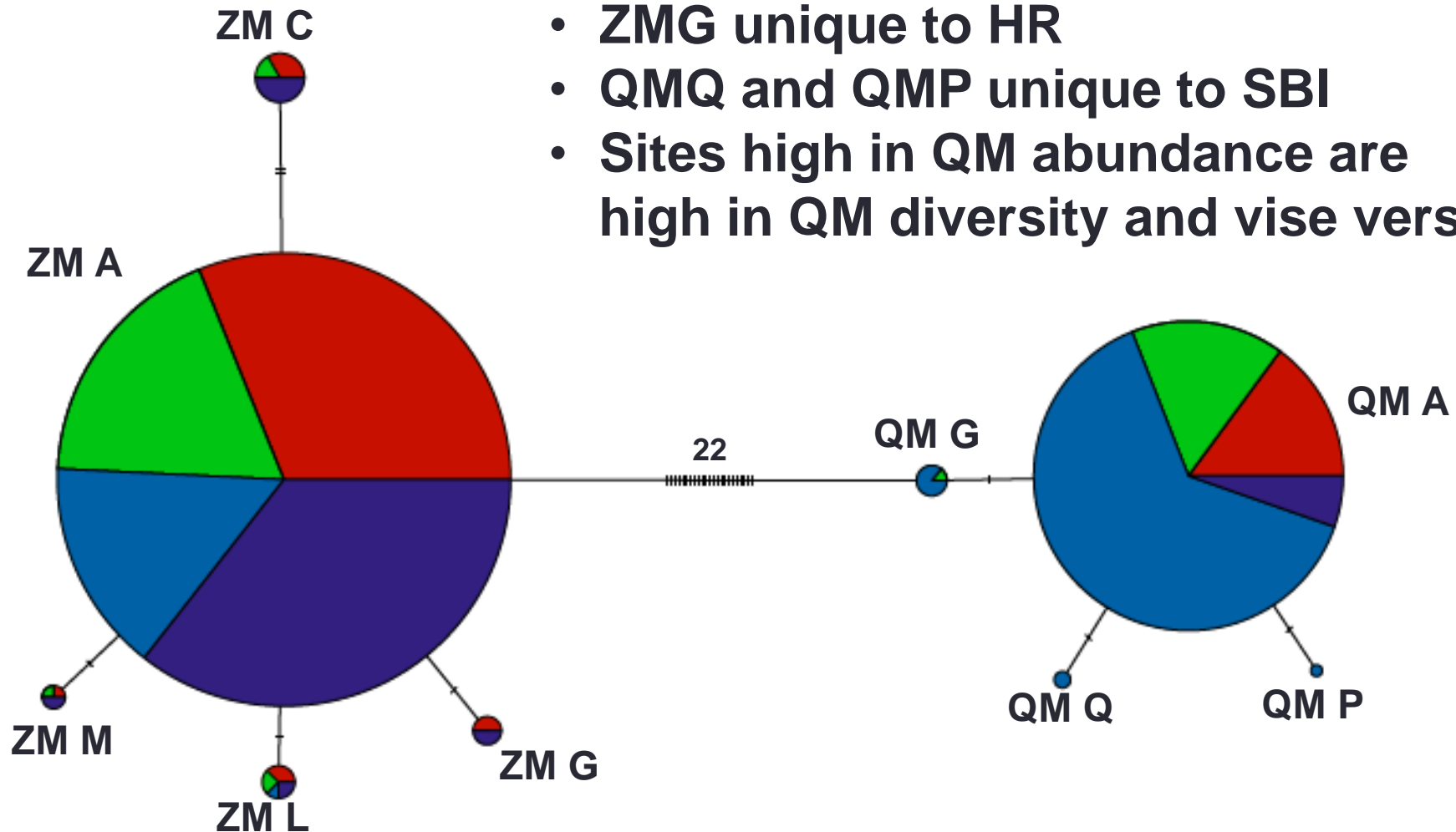
Hudson River Plankton

Hudson River eDNA

SBI Plankton

BSR Plankton

- Samples largely dominated by common haplotypes
- ZMG unique to HR
- QMQ and QMP unique to SBI
- Sites high in QM abundance are high in QM diversity and vice versa



Conclusions

1. Careful assay design required for accurate results
2. HTS can address population genetic information
3. Aquaria tests show positive relationship between eDNA and biomass/individuals
4. Surprisingly, surface water samples showed closer relationship to the mussel community
5. Assay is useful for demonstrating spatial/temporal differences in dreissenid communities



Support: Past and Present members of the Stepien Lab

Sampling: Cary Institute (Dr. Dave Strayer, Heather Malcom, David Fischer)

Funding: EPA Great Lakes Restoration Initiative

Sigma Xi Grant-In-Aid of Research

Hudson River Foundation Mark B. Bain Graduate Fellowship

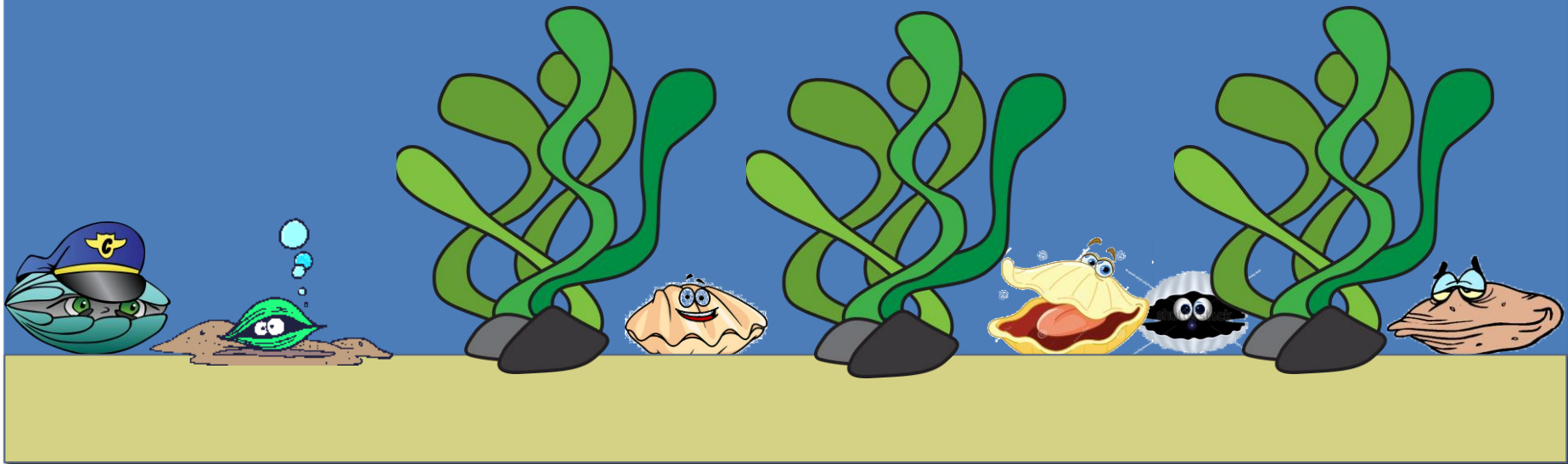
University of Toledo Robert N. Whiteford Scholarship

Malacological Society of London Travel Award

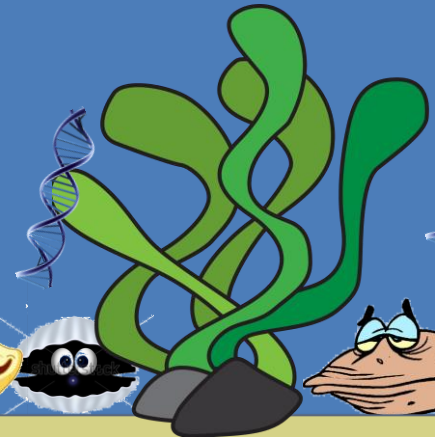
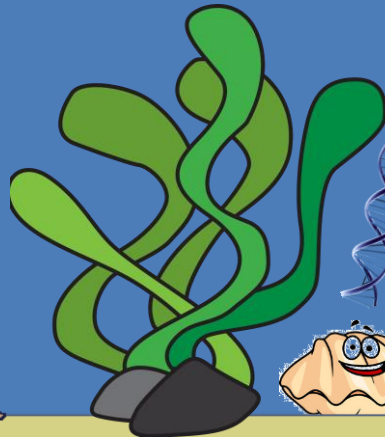
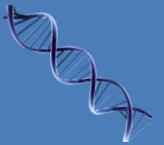


Project Goal

To design a high-throughput assay for detection and population analysis of invasive *Dreissena* using water and/or plankton samples



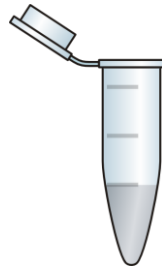
Environmental DNA (eDNA)



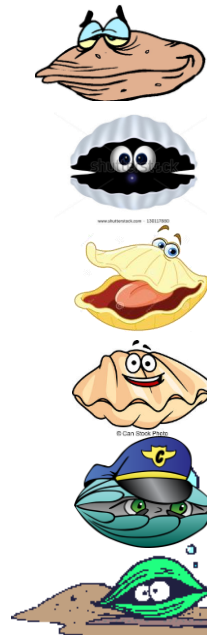
From eDNA to Sequence Data



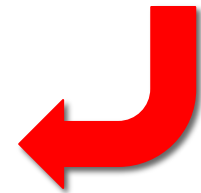
DNA
extraction



PCR and
HTS



```
>173248  
ATCGACGATGCATACGT  
>173249  
ACTGACTGACTACGATC  
>173250  
TCAGCTACGTTTACGCT  
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TGACGATCGACTATTTAC  
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>173253  
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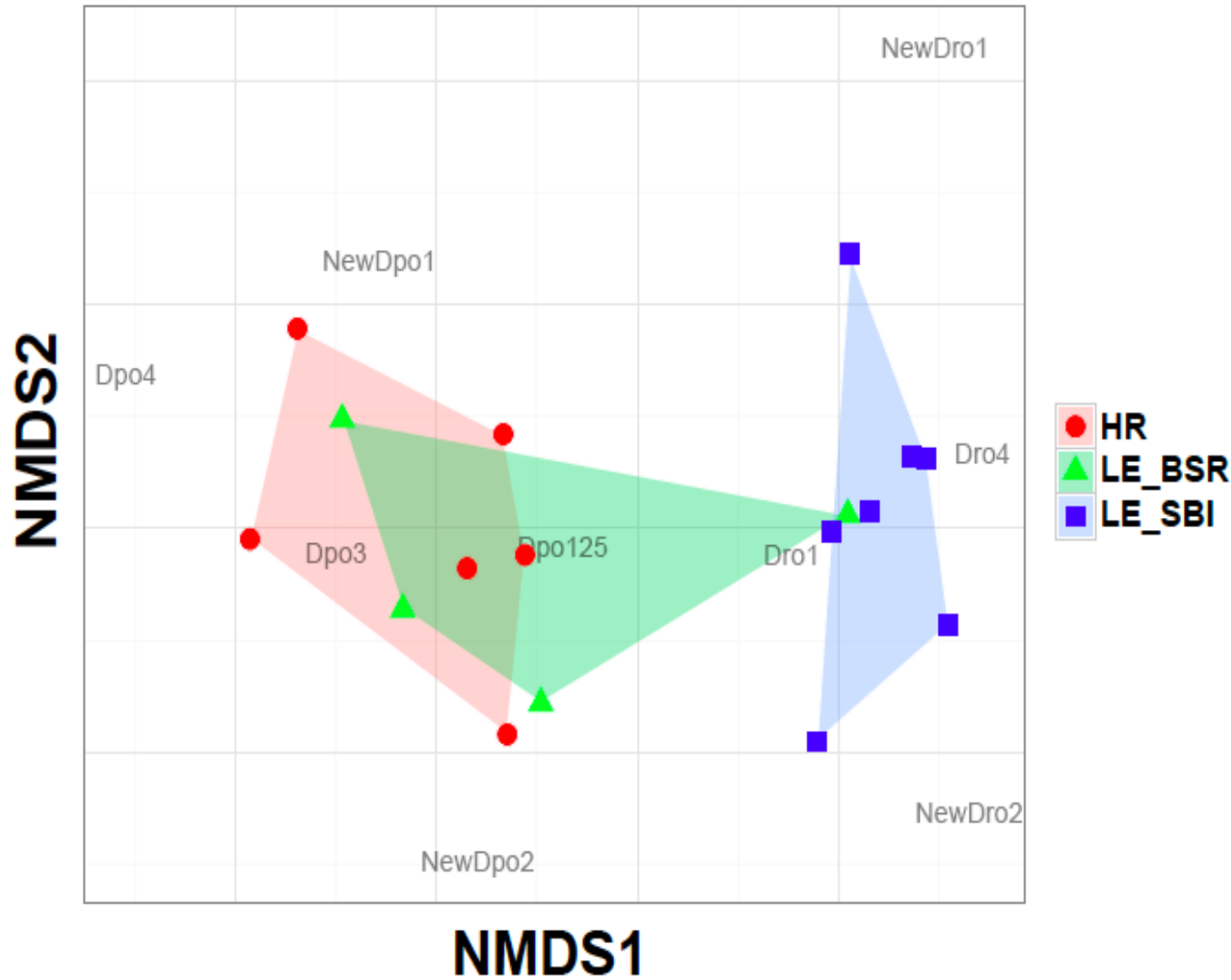


Bioinformatics
to analyze
millions of
sequences

Questions?



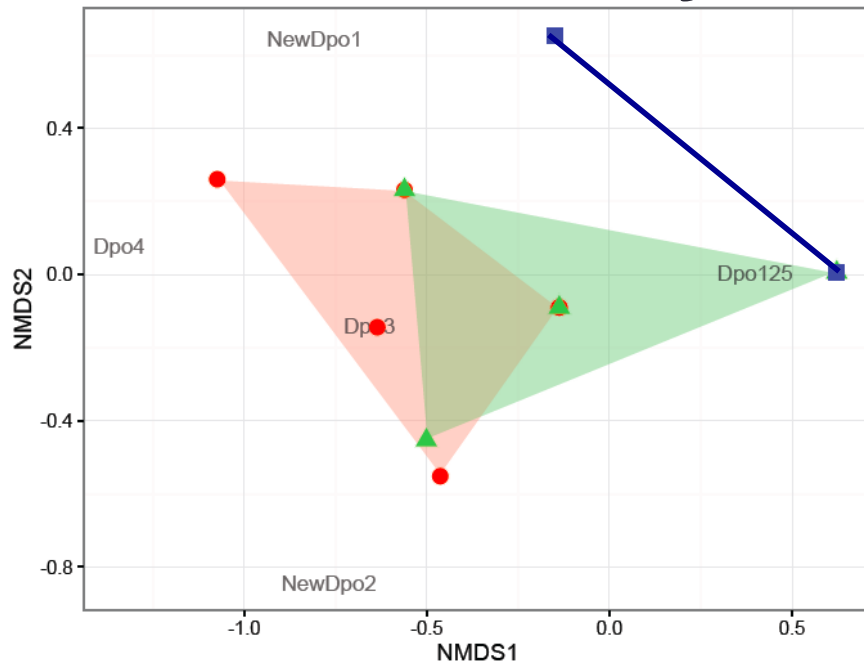
NMDS of Plankton Samples



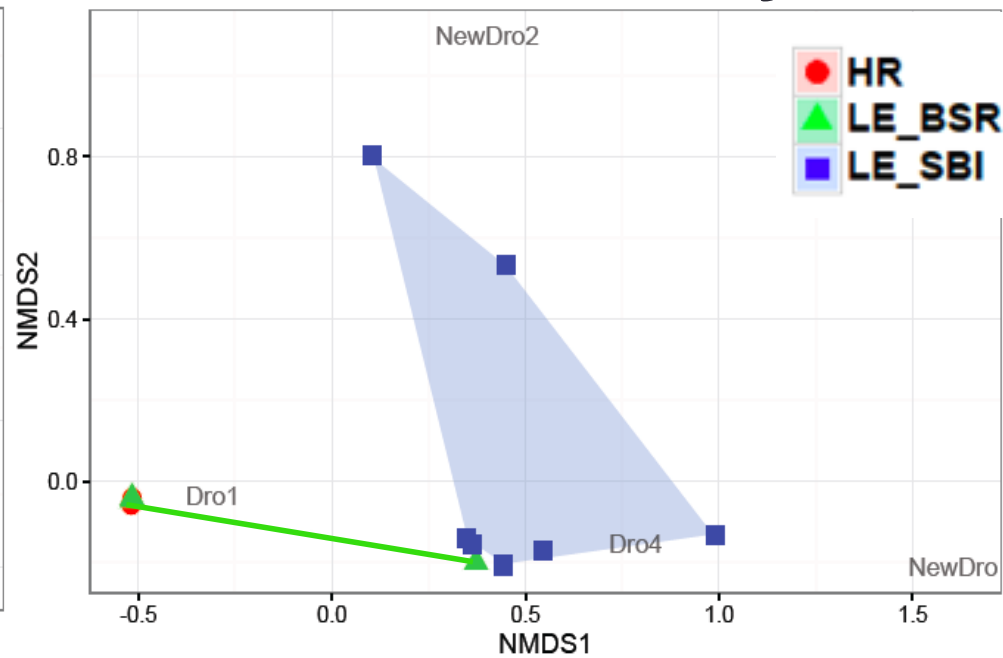
- Sites group by ecological similarity
- Differentiation driven by species abundances

nMDS of single species diversity

ZM Diversity

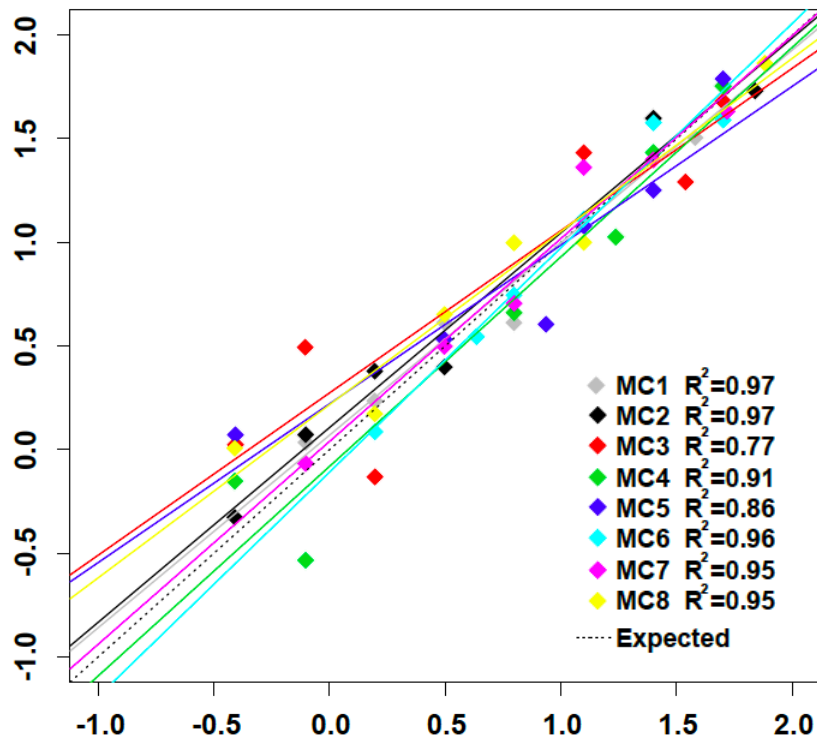


QM Diversity

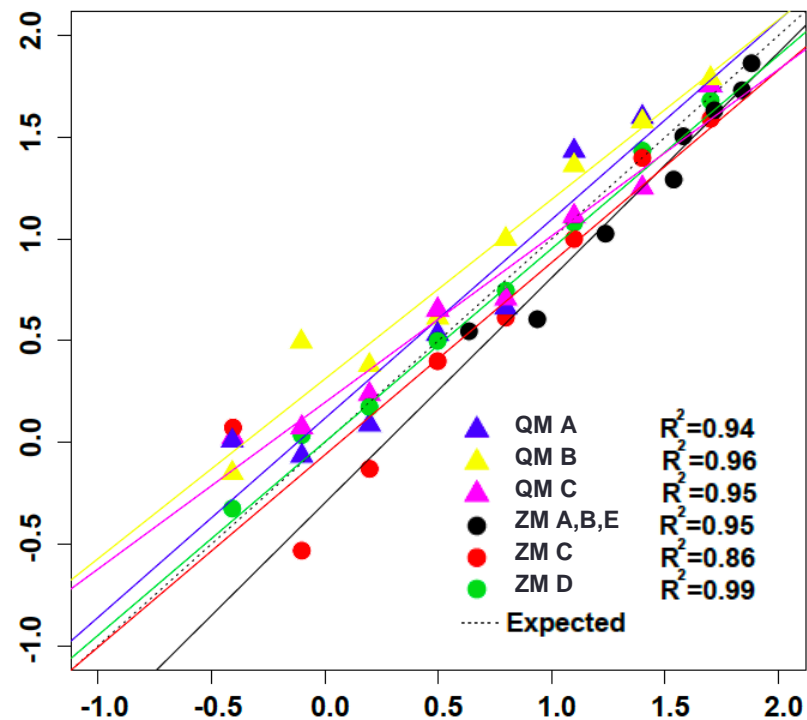


- Similar trends, **HR** grouped with **BSR**

Per MC

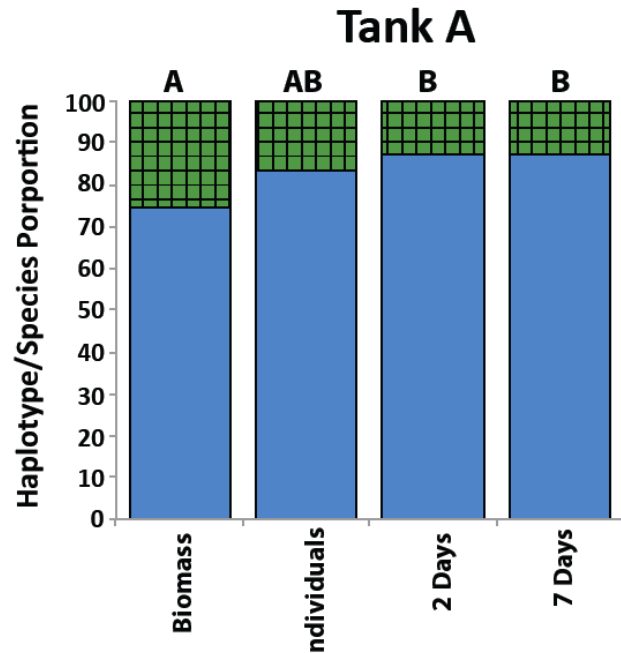
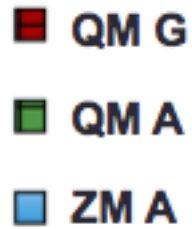


Per OTU



Log Expected Proportion of Haplotype

Log Observed Proportion of Haplotype



- Tanks A & C represented individuals early,
- But shifted to biomass late

