Novel methods for early detection of the bloody red shrimp, *Hemimysis anomala*

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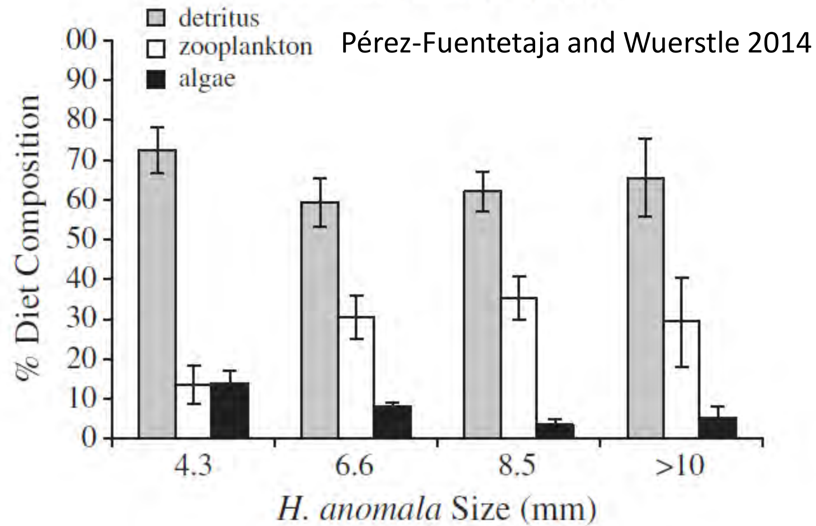
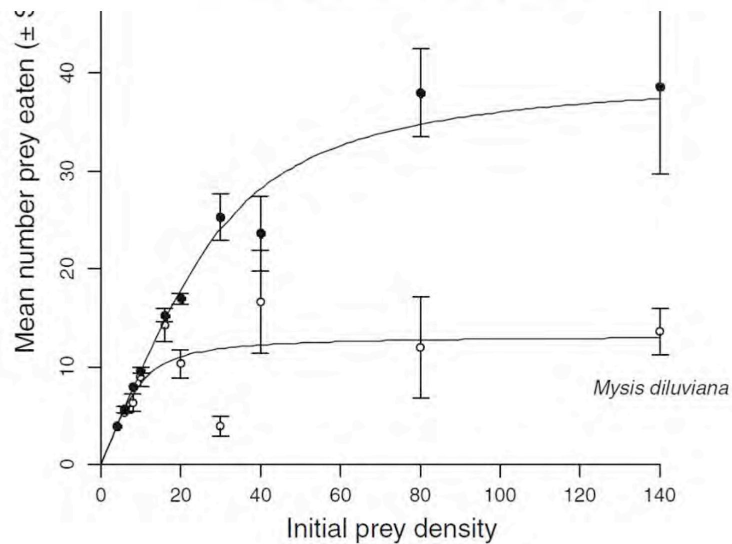
An emerging invader: The Bloody Red Shrimp, *Hemimysis anomala*

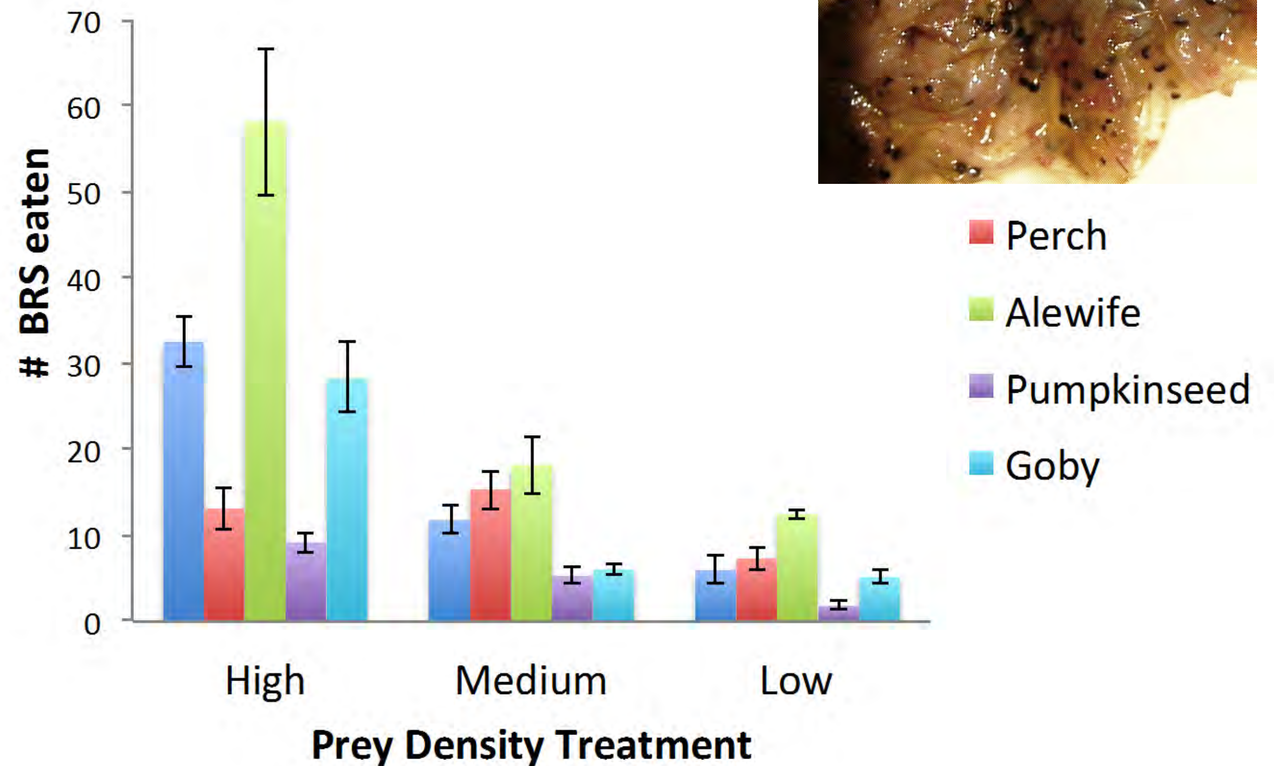
Pink detected prior to 2014
Red detected in 2015
Blue not detected

Hemimysis is an omnivore with high consumption rates.

And, a prey item for fish.







Limitations of plankton net collections.

Light-based traps are an effective detection method

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The light at the end of the funnel?: Using light-based traps for the detection and collection of a nearshore aquatic, invasive invertebrate, *Hemimysis anomala*

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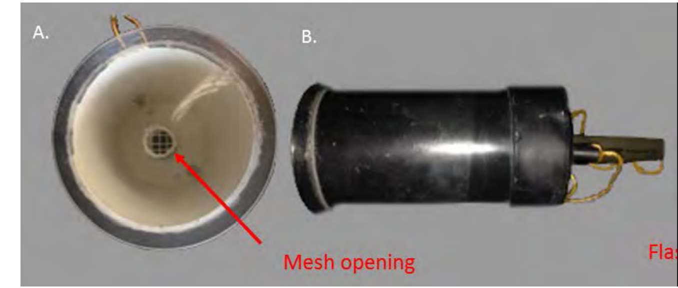
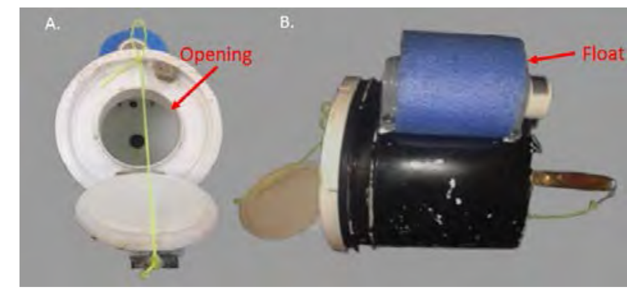
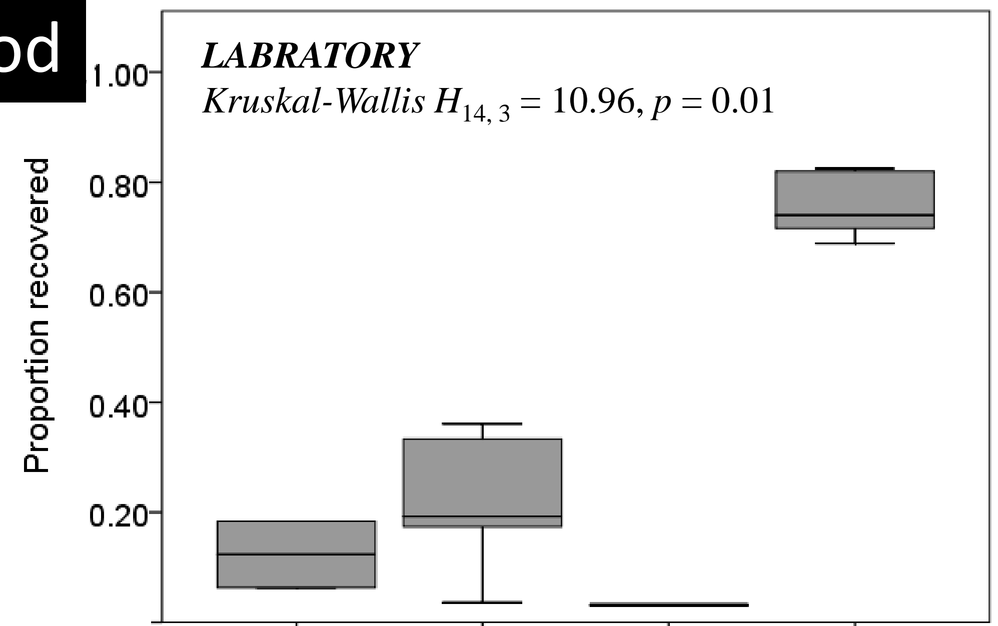
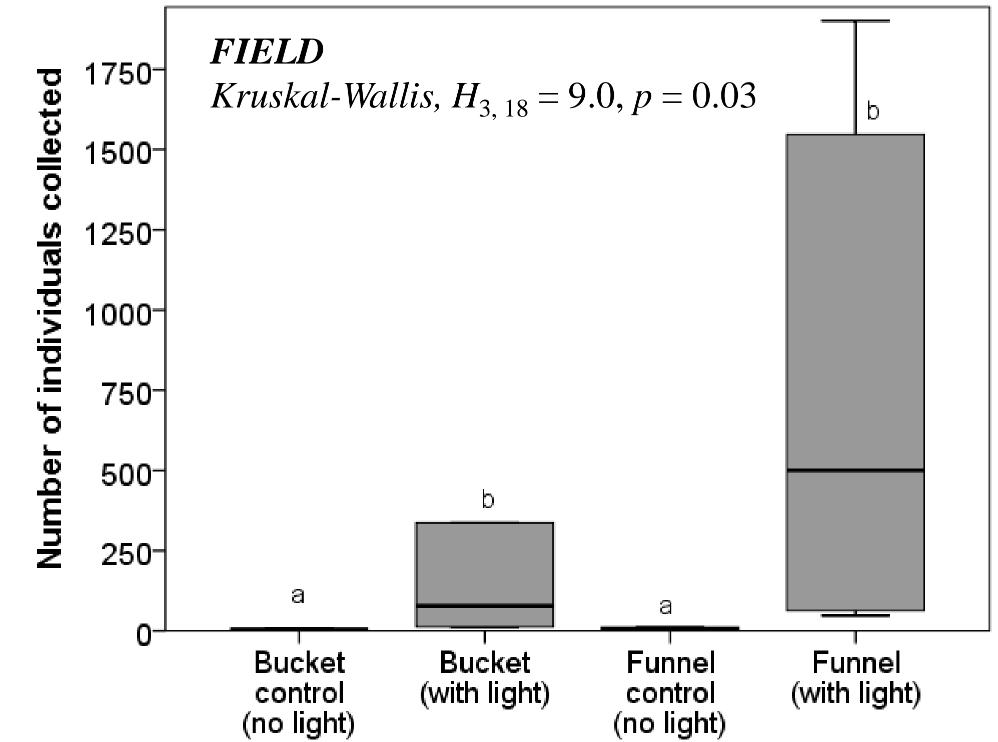








eDNA as a potential early-detection tool

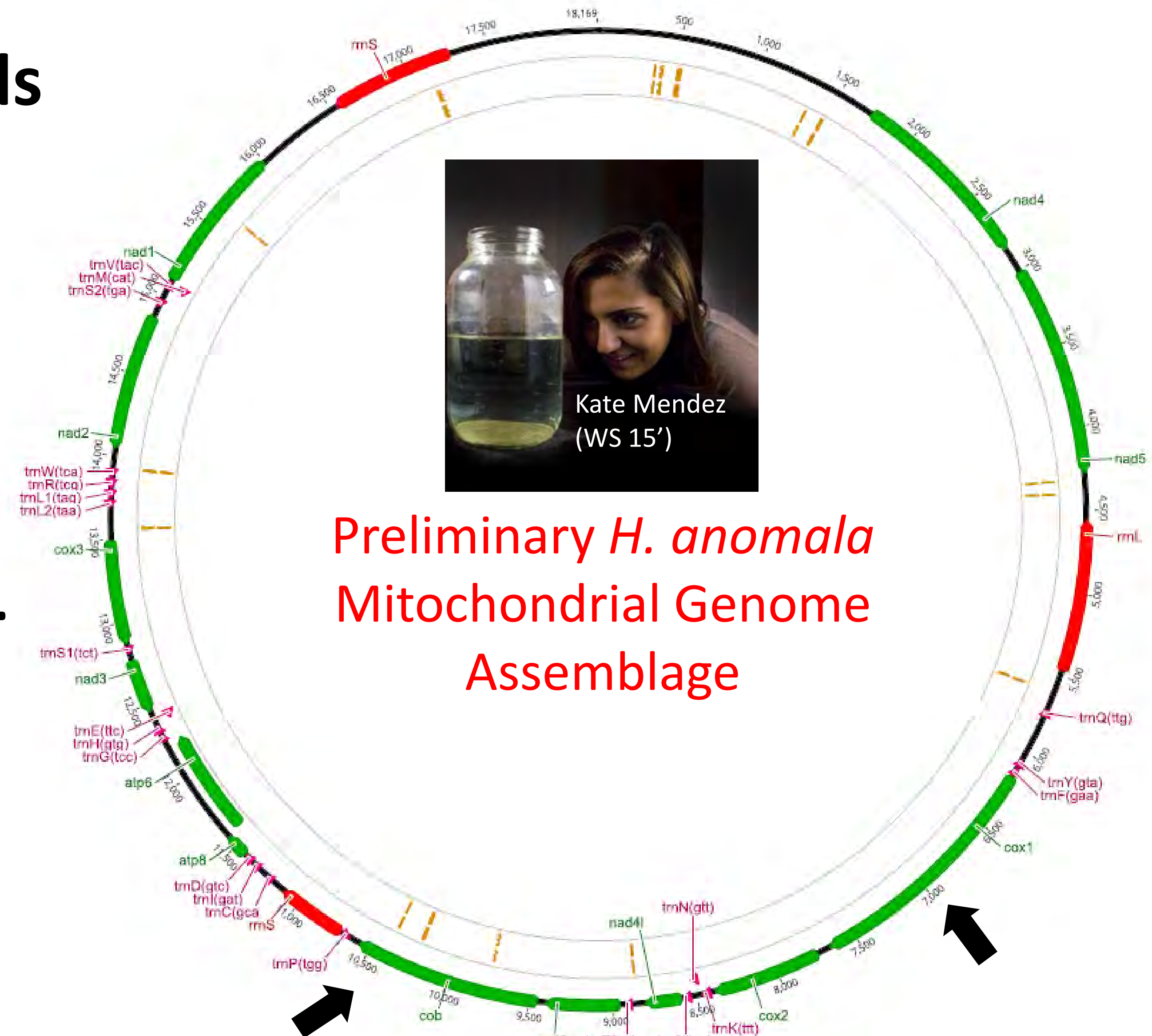
DNA extraction and qPCR methods

Field studies to compare

- habitat types,
- times of day, and
- locations in the water column.

Lab experiments to compare

- *Hemimysis* density and
- incubation time.



What are the most suitable primer for qPCR detection of *Hemimysis*?

mt-DNA, subunit I of cytochrome oxidase gene

HAco1-4AF & HAco1-4BF (two variants)

- 35 thermal cycles, most reliable time frame and avoided primer dimers
- 54 -55°C ideal for annealing

```
HA ---------------------------Gggttagtaggttcttctttgagaattttaatt
MD ctctattttgtgtttggggcttgggctggaatagttggatcttctttaagagttttaatt
                              ** ****  ** ******** *** ********

HA cgacttgaattgggtcagcccggtaggttaattggggatgatcagatttataacgtgatt
MD cggttagagttagggcaacctgggcatttgattggggacagacaactttataacgtaatt
   **  * ** ** ** ** ** **    ** ********    **  ********** ***

HA gtgacagcgcatgcgtttgtaataatttttttatagttataccaaccataattggtggt
MD gttactgcgcatgcttttgttataatttctttatagttatacctattacagttggtggg
   ** ** ******** ***** ******** ************** *  * * *******

HA ttcggcaactggttagttcctttaataattggggccccagatatagcgtttcctcgaata
MD tttggaaattggttagtaccgttgatattaggagctcctgatatagcttttcctcgtata
   ** ** ** ******** ** ** *** * ** ** ** ******** ******** ***

HA aataatataagattttgattattacctccatcgttaagattgcttttagctagagggtta
MD aataatataaggttttgacttttgccaccttctttagctcttatactaataagagggata
   *********** ****** * ** ** ** ** ***    *  *  **   ****** **

HA gtggaggggggggttggtacggggtggacagtctaccctcccttagcggtaacgtgtca
MD gtagagagaggggtagggactggttgaaccgtttatccaccattggcttcaaacattgct
   ** *** * ****** ** ** ** ** ** ** ** ** ** ** **    *** *  *

HA cacatgggtgcagctgtggatataggaatttttctttgcatttagctggcgcctcttct
MD catgcaggggcagcagtagatataggaatttttctttacatttagctggggcttcttca
   **    ** ***** ** ******************** *********** ** *****

HA attttaggtgctgttaattttatttctacagttattaatatacgtgctgtaggaataggg
MD attttaggtgctgtaaattttatttcaactgttattaatatacgggcacctggggtaggt
   ************** *********** ** ************** **    **  ****

HA tttgacagtataccactatttgtttggtctgtgtttattactgctattttactacttctg
MD atagatagactacctctttttgtgtggtcaattttattacagcaattcttttattactt
    * ** **  **** ** ***** *****  * ******** ** *** *  ** * **

HA tctttacctgttttagcaggggctattactatgctt------------------------
MD tctttaccagtgttagcaggggctattacaatacttttaacagaccgtaatttaaatact
   ******** ** ***************** ** ***

HA ----------------------------------
MD tctttctttgaccctgtaggtgggggtgaccta
```

What densities and consequent concentration of eDNA are detectable?

Dilutions of pure *Hemimysis* DNA

- 1/10
- 1/100
- 1/1000

DNA Dilution Series with Various Primers

Relative Fluorescence Units (RFU)

1600
1400
1200
1000
800
600
400
200
0

0 10 15 20 22 24 26 28 30 32 34 36

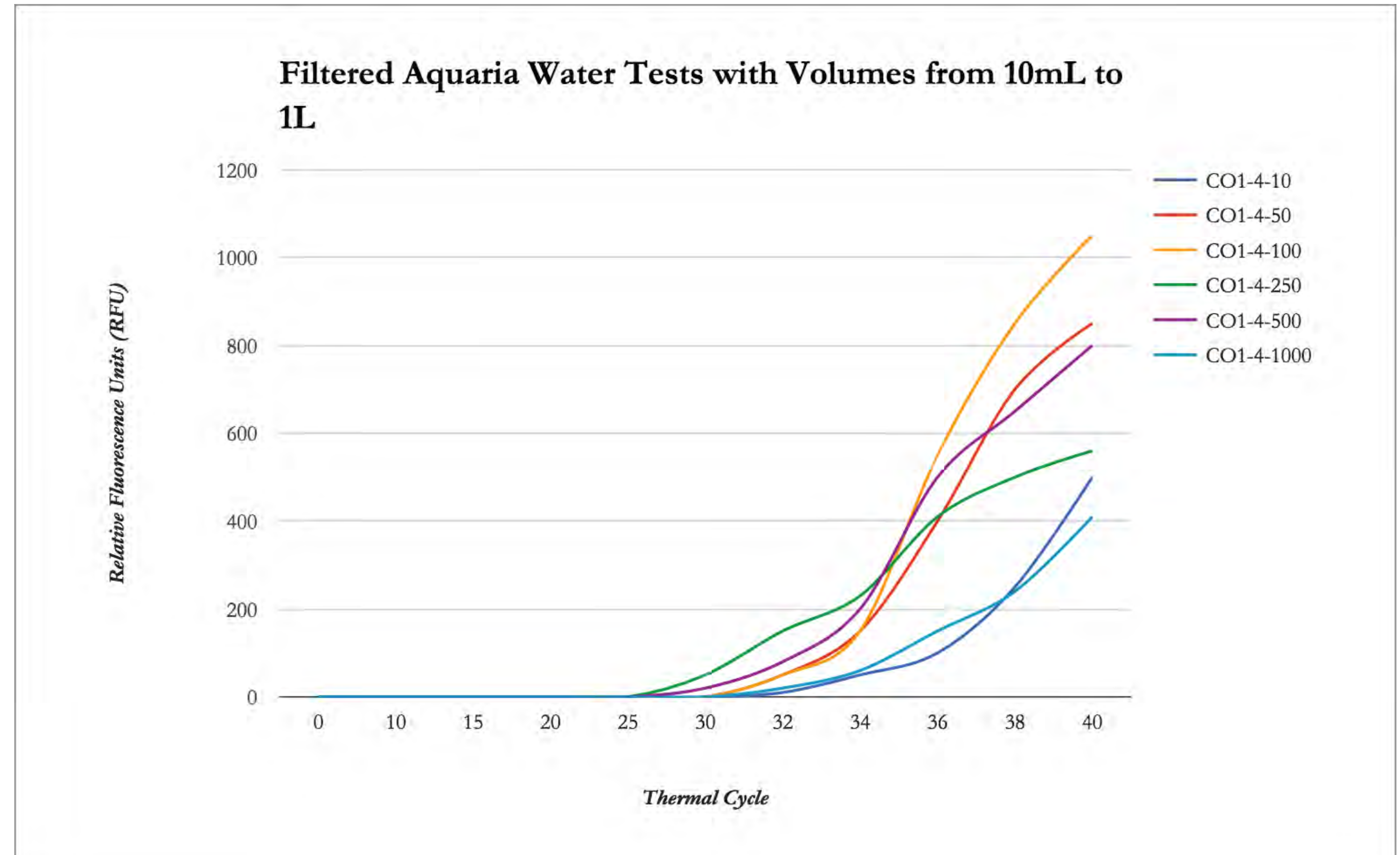
Thermal Cycle

HAEPR-conc.
HAEPR-.1
HAEPR-.01
HAEPR-.001
HAEPR-0
rRNA-conc.
rRNA-.1
rRNA-.01
rRNA-.001
rRNA-0
CO1-conc.
CO1-.1
CO1-.01
CO1-.001
CO1-0

What volume of water is needed for reliable detection?

Water volumes from aquaria holding moderate density of *Hemimysis*

10-1000 mL



Field experiments

Sites: Seneca Lake (established by 2010)
Erie Canal Lock #5 (established by 2014)

Location : Water and sediment surface

Time mid-day and midnight

Controls: Skaneateles Lake, field and lab controls

Preliminary Results!

# reps with cq < 35			Seneca Lake	NYS Canal	Controls	
		Filter size (µm)			Skaneateles Lake	Field controls
Night	Deep	0.45	3/3		0/3	0/3
		0.65	3/3			
		1.2	3/3			
	Shallow	0.45	1/3		0/3	
		0.65	2/3			
		1.2	1/3			
Day	Deep	0.45	2/3			0/3
	Shallow	0.45	2/3			

Methods:
Triplicate 250 mL water samples filtered (0.45 µm pore-diameter acetate filter paper)
Filters preserved in 70% ethanol
Equipment cleaned between replicates (50% bleach and DI rinse)

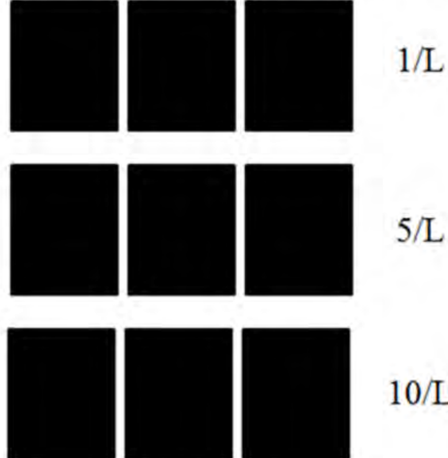
Field controls were deionized water transferred to sample bottles in the field and processed the same as field samples

Lab experiments

Mysis Control
6L buckets with 30 organisms/L

Well Water Control
6L buckets

Hemimysis anomala control
6 L buckets with varied densities

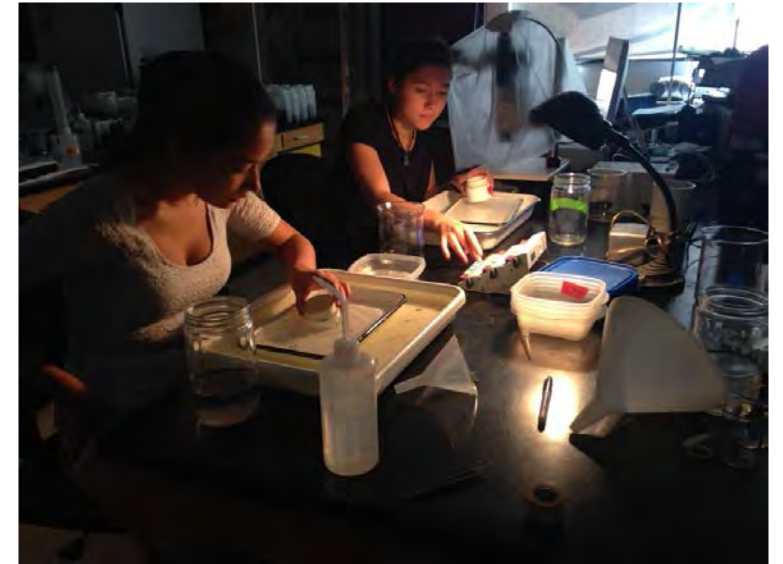


Water samples at

- 3, 5, 7, 9, 11, 13 days
- 12 hr and 2 days after *Hemimysis* removed

500 mL water samples

Filter Samples



Questions?