

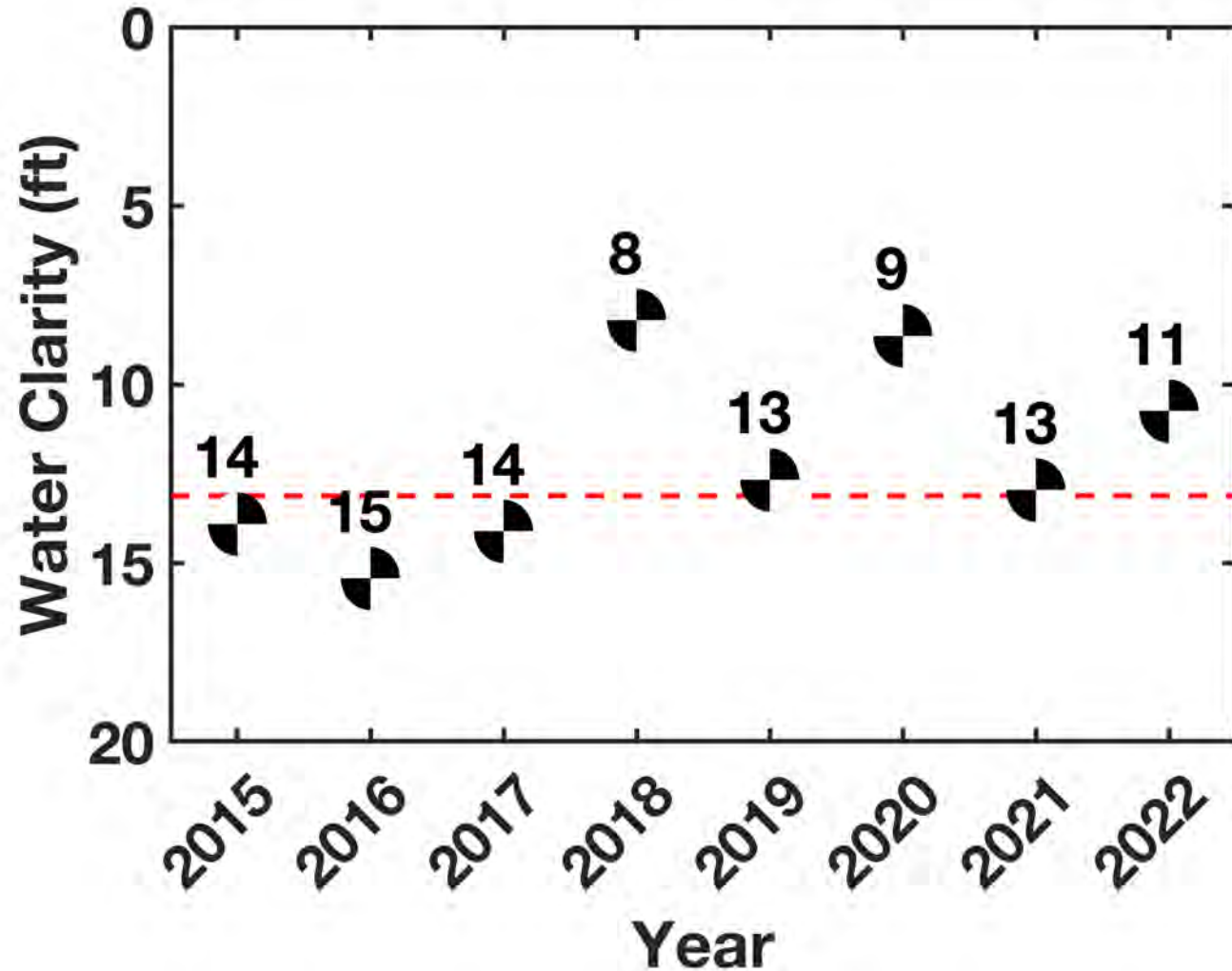
Using caffeine as a tracer for septic contamination in lakes

A pilot study on North Pond, Smithfield ME

Brian DiMento (Colby College)

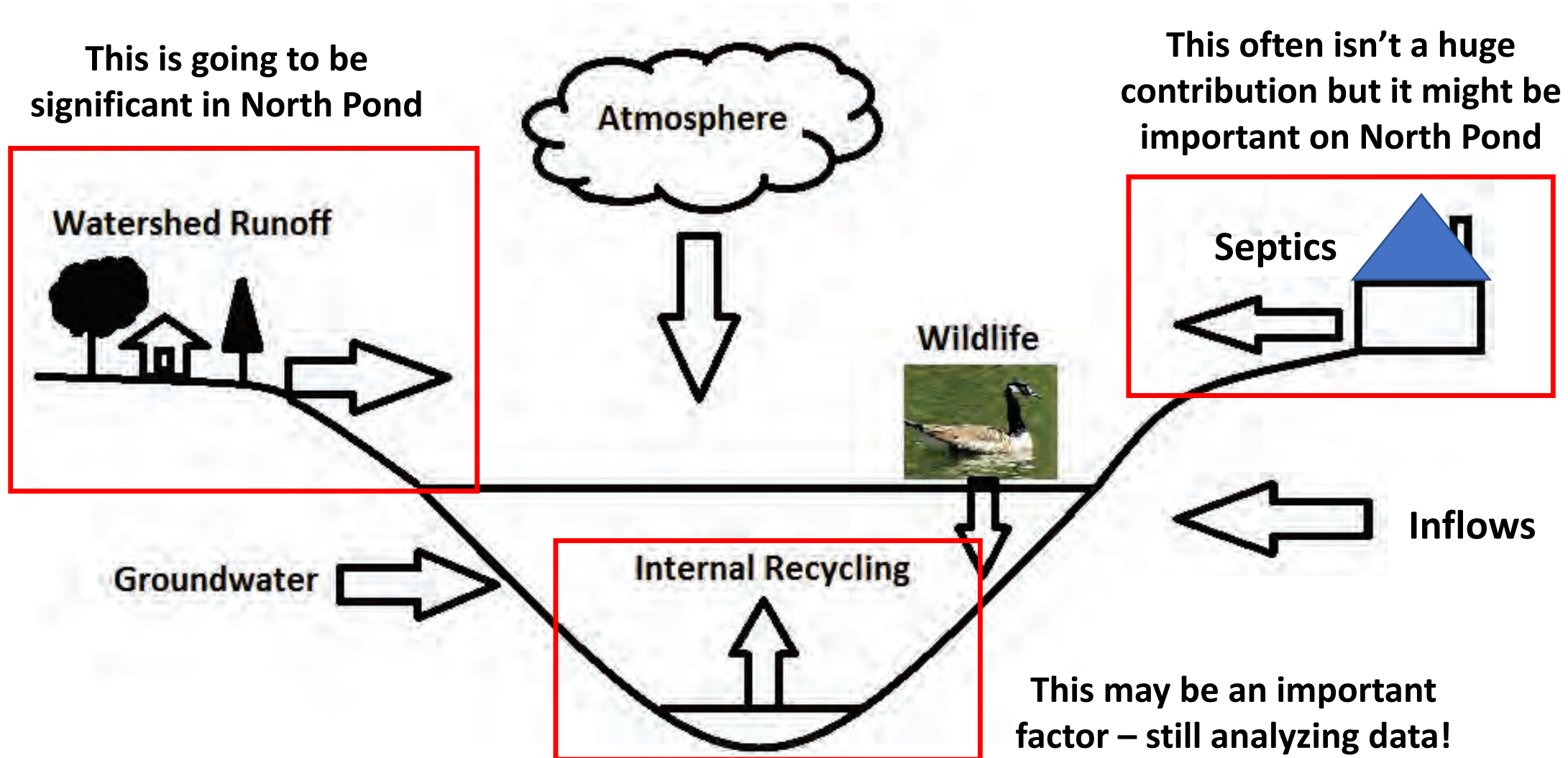
Whitney King (Colby College), Danielle Wain (7 Lakes Alliance), Claire Yu
(Colby College), Margo Kenyon (Colby College), Julie Millard (Colby College)
and Charlie Baeder (7 Lakes Alliance)

North Pond in Smithfield, ME has been experiencing algal blooms since 2018

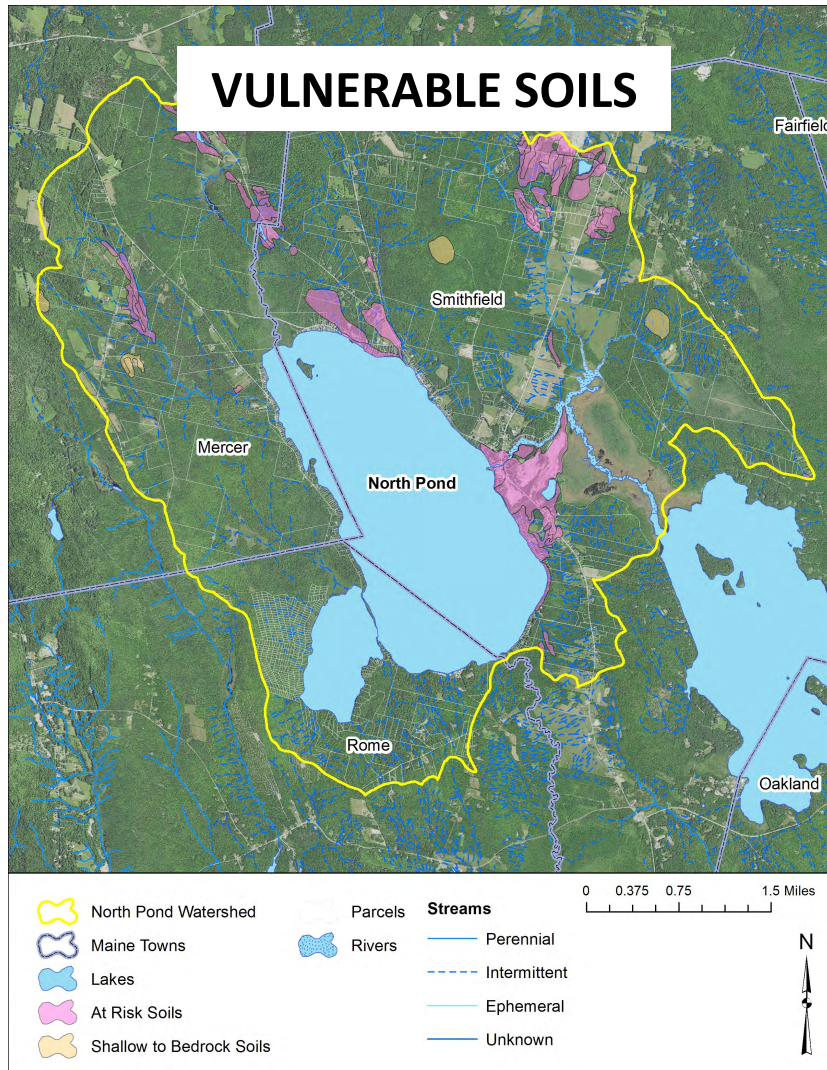


In 2021, team received an NPS 604b grant to develop a watershed based management plan

We need to keep phosphorus out of the lake, but we don't know exactly where it is coming from yet!



There are a few reasons to suspect that septics might contribute more to North Pond than other lakes



UNKNOWN SEPTIC INVENTORY

**YES, THAT'S AN OLD OCEAN BUOY
(FROM NPA NEWSLETTER)**

Usage of caffeine as an indicator of septic/wastewater contamination

[caffeine] (ppb)	Location	Paper	Analytical Method
< 0.021	Belgrade Lakes, ME	Kullberg et al 2021	SPE LC-MS
< 23	urban watersheds	Mizukawa et al 2019	SPE LC-PDA
< 0.5	River water	Viviano et al 2017	UHPLC-MS
< 100	septic tank effluent	Richards et al 2017	SPE LC-MS/MS
< 50	urban	Goncalves et al 2017	HPLC-DAD
< 0.15	coastal waters, estuaries	Rodriguez del Rey et al 2012	SPE GC-MS
0.02	Lake Simcoe, Ontario	Kurissery et al 2012	GC IT-MS/MS
< 0.25	Swiss lakes + rivers	Buerge et al 2003	SPE GC-MS
< 0.23	groundwater	Seiler et al 1999	Liquid-liquid extraction, GC-MS

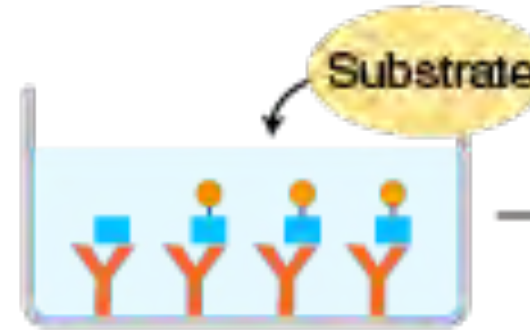
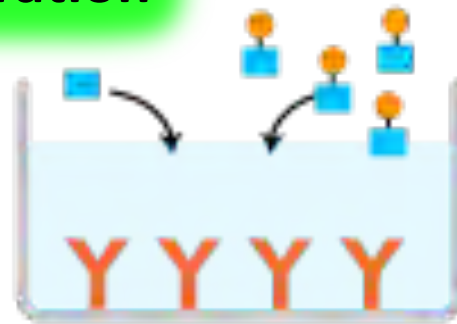
Caffeine detection methods

- Solid phase extraction followed by GCMS or HPLC/LCMS
- Enzyme-linked immunosorbent assay (ELISA)
 - Intended for blood serum, saliva, urine...

How does a competitive ELISA work?

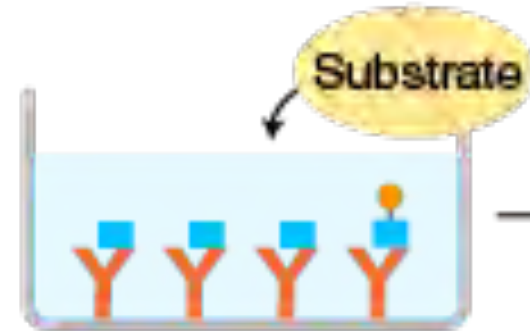
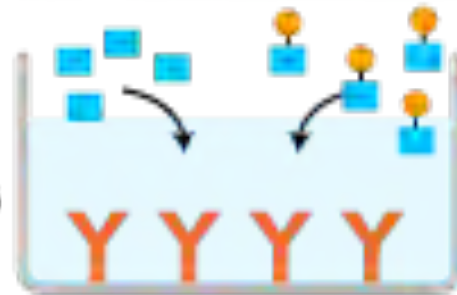
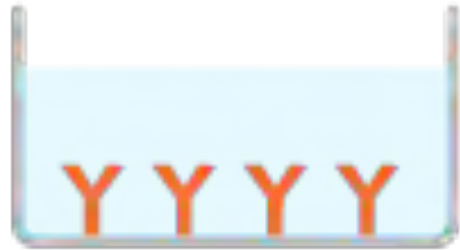
LOW concentration

Samples including low levels of the target protein



HIGH concentration

Samples including high levels of the target protein



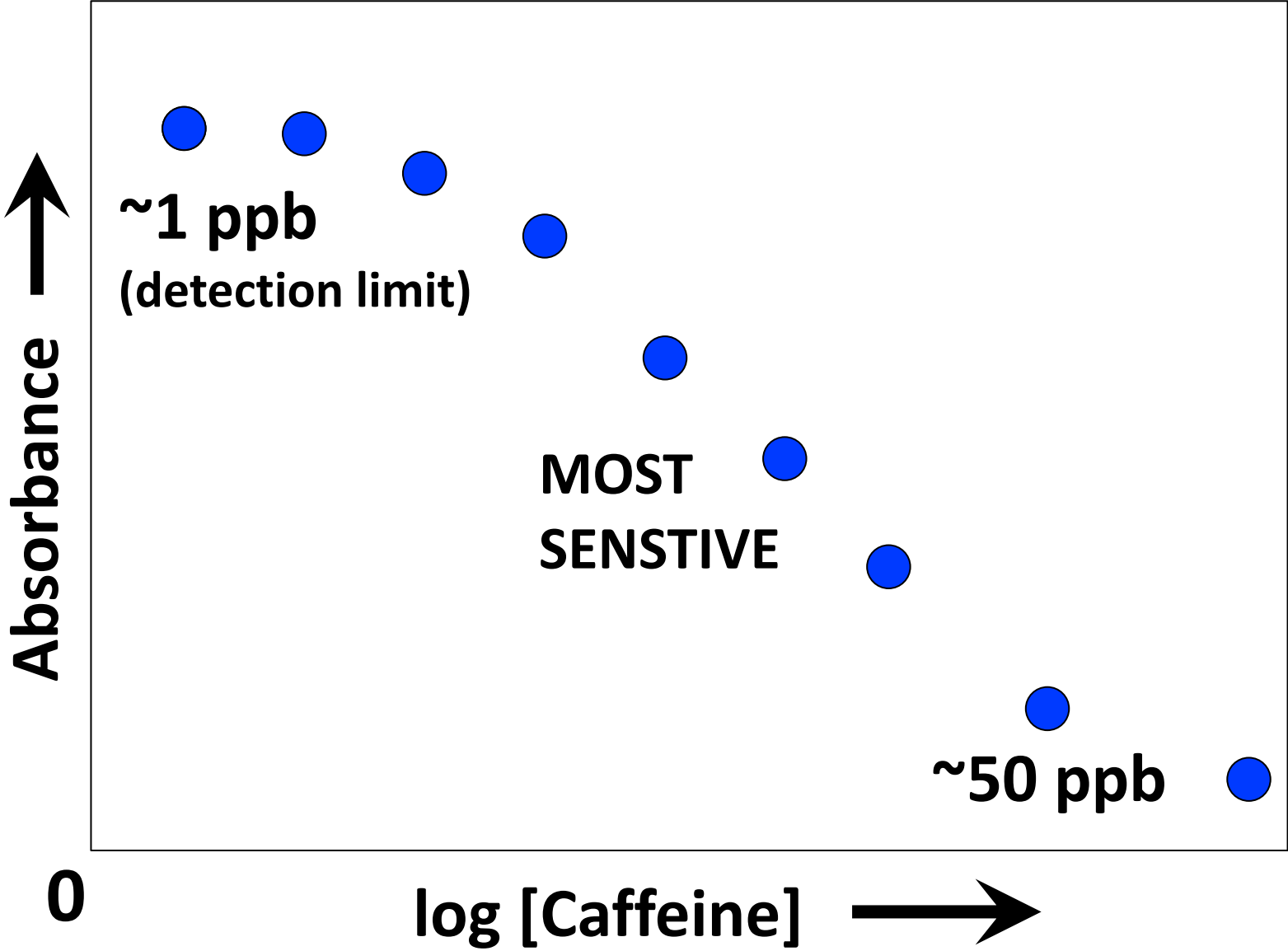
Y Antibody

■ Target analyte

● Enzyme-labeled antigen

● Enzyme reaction

How does a (competitive) ELISA work?

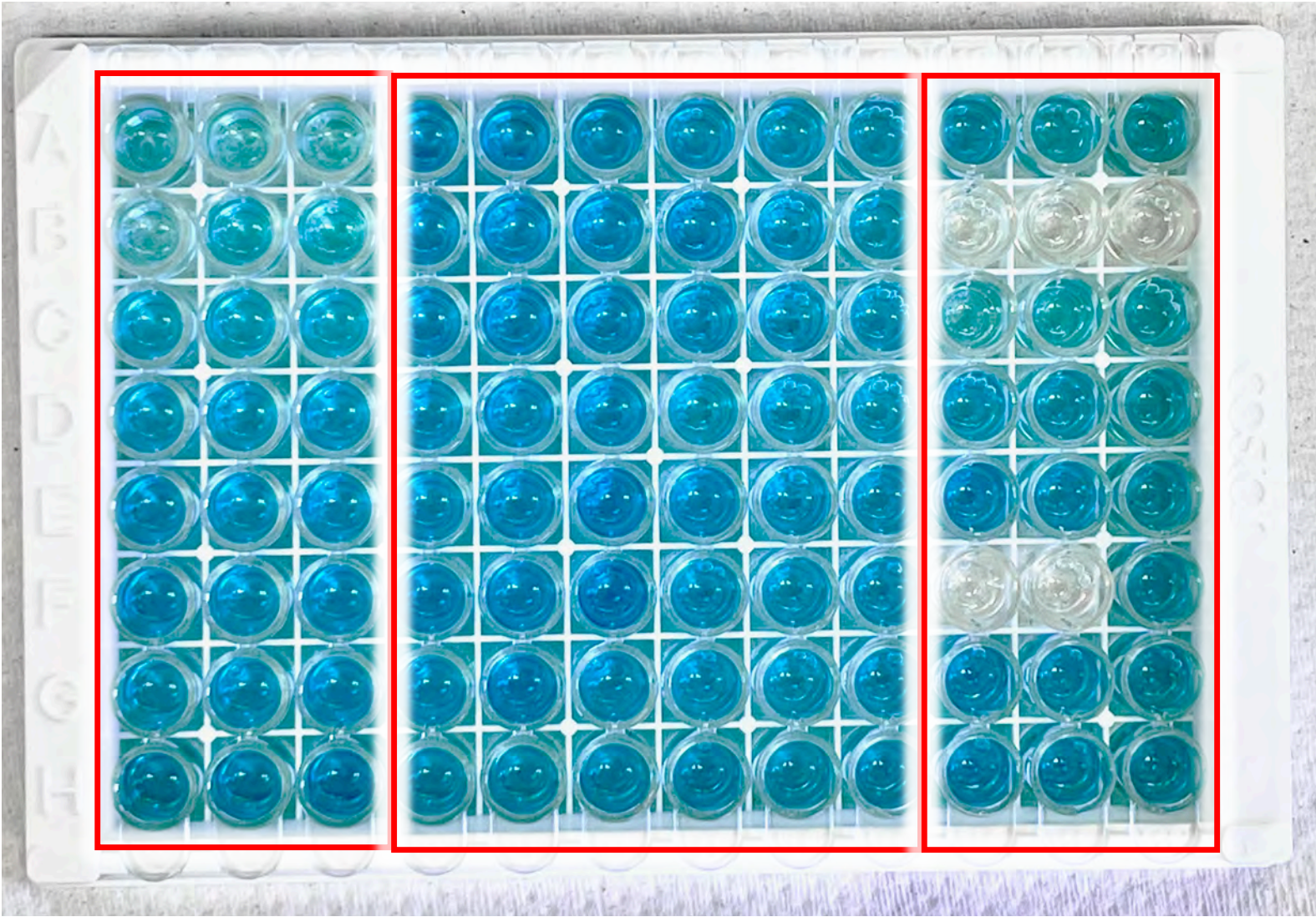


Calibration Curve

How does a (competitive) ELISA work?

**HIGH
CAFFEINE**

**LOW
CAFFEINE**



**96-well
plate**

STANDARDS

SAMPLES

CONTROLS

Caffeine ELISA Kits

abcam

Caffeine ELISA Kit (ab285229)

\$685 /96



NEOGEN®

Caffeine/Pentoxifylline Forensic ELISA Kit (Item No. 106419)

\$286 /96

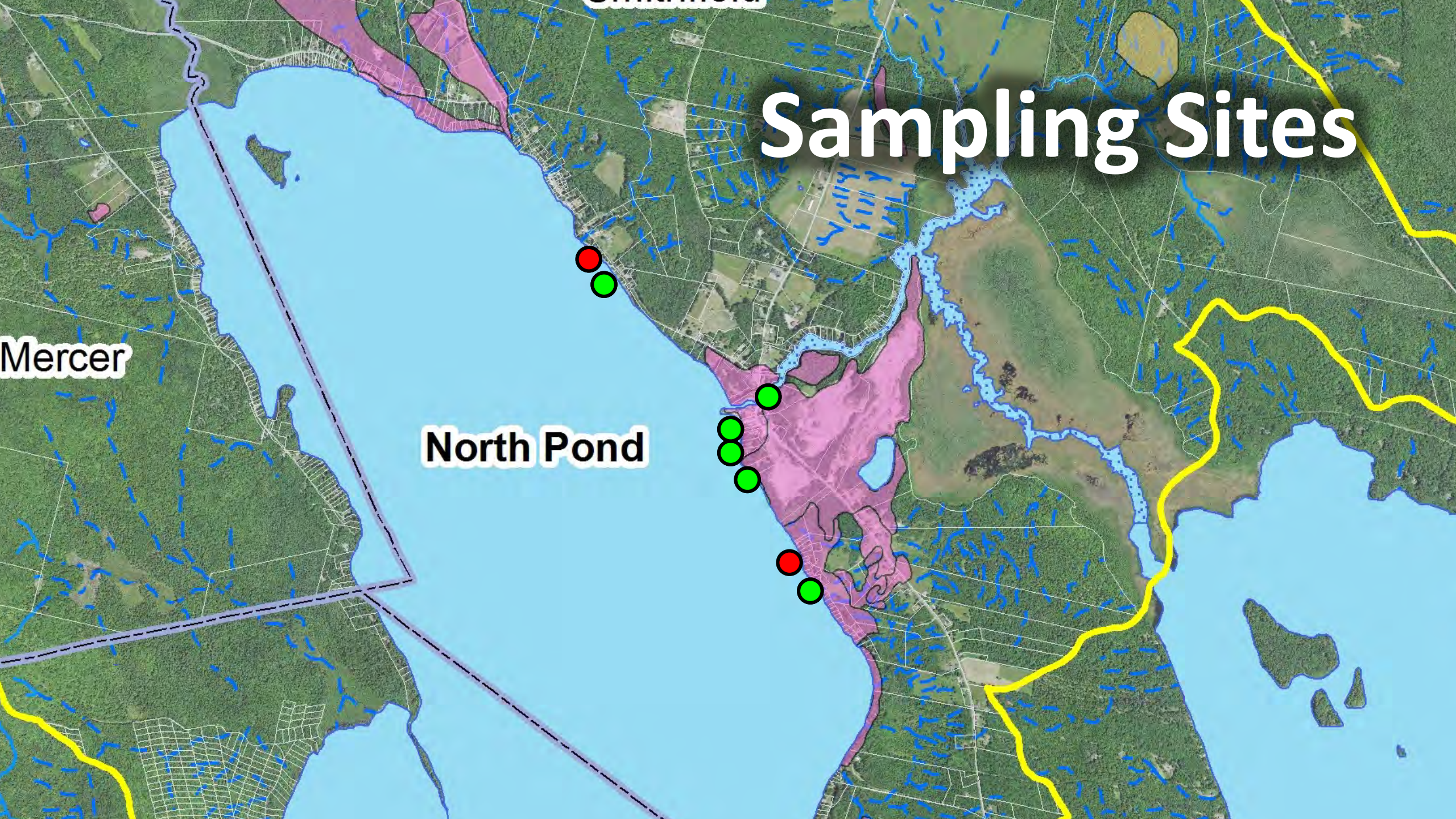
COST ANALYSIS

As few as ~24 samples if everything in triplicate
\$12 (neogen) - **\$28** (abcam) per sample

Sampling Sites

Mercer

North Pond



Expected concentrations?

Caffeine dose/person*	0.3 g/day
Population around the lake	500
Total caffeine dose	150 g/day
Volume of lake	2.05E+09 L
Lake area impacted**	10%
Effective lake volume	2.05E+08 L
Caffeine added	0.73 ppb/day
Residence time of caffeine?***	1.5 days
Expected concentration	1.1 ppb
Detection limit	~ 0.9 ppb

*3 cups of coffee/day with no treatment by septic system...

**Maybe localized plumes??

***(Lam et al 2004) microcosm study
Loss by bio/photodegradation

Questions?

Acknowledgements

Colby College

- Whitney King, Julie Millard, students Margo Kenyon and Claire Yu

7 Lakes Alliance

- Danielle Wain, Charlie Baeder

