Brook trout and eDNA: General applications

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USFWS Northeast Fishery Center, Lamar, PA

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CBP STAC Understanding Genetics for Brook Trout Workshop

Environmental DNA (eDNA)

Potentially powerful tool for assessment and monitoring

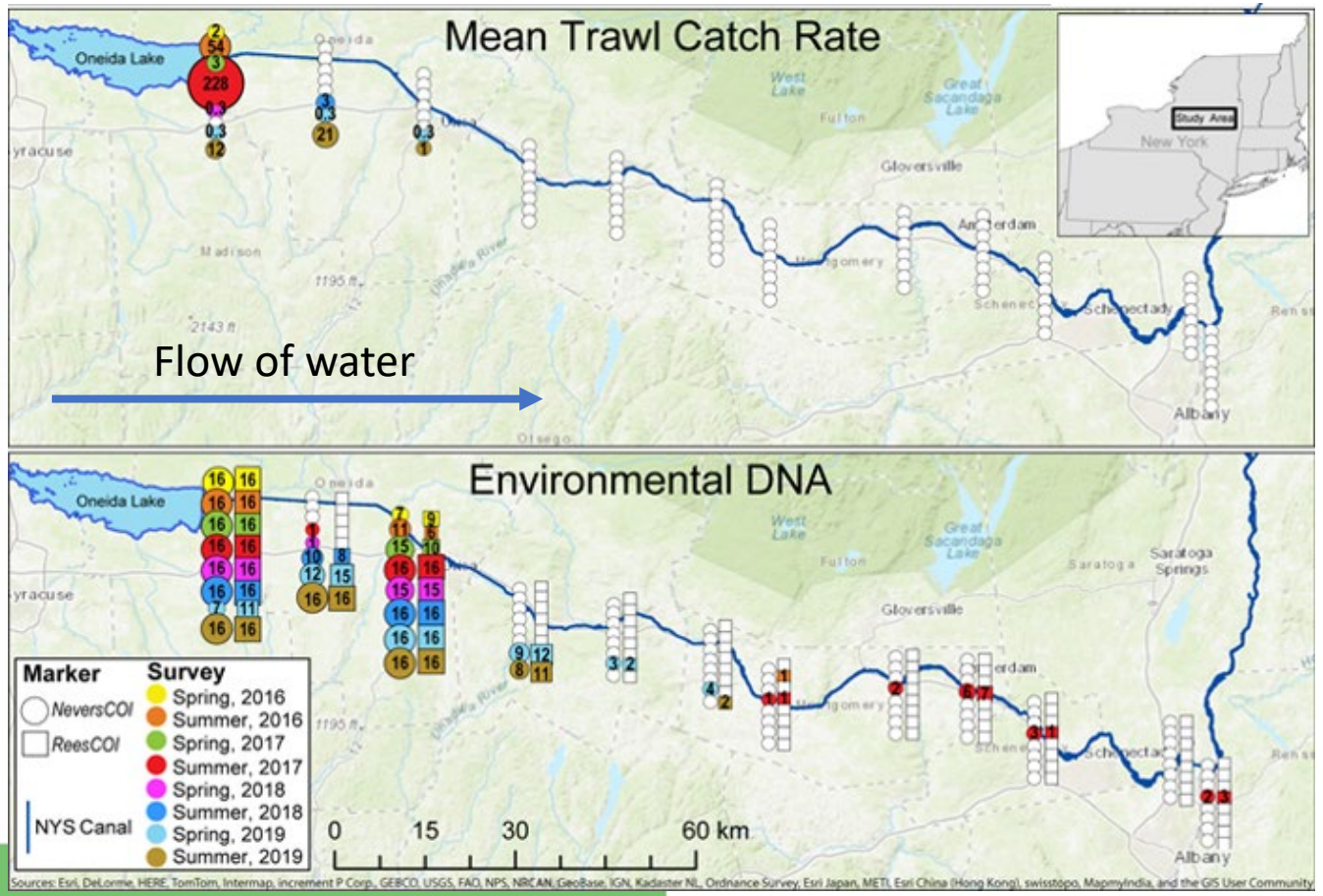
Advantages

- Increase spatial coverage to assess distribution
- Can be used to compliment field survey efforts

Challenges

- Reduce areas of uncertainty associated with sampling, lab, and data interpretation
- Identify appropriate question
  - Presence/absence
  - Quantitative
- Match technology with study goal

eDNA applications: Early detection of invasive species



Transactions of the American Fisheries Society

Article | Open Access

Eastward Expansion of Round Goby in New York: Assessment of Detection Methods and Current Range

Scott D. George, Barry P. Baldigo, Christopher B. Rees, Meredith L. Bartron, Dylan Winterhalter

First published: 12 March 2021 | https://doi.org/10.1002/tafs.10290

eDNA applications: Detection of rare or cryptic species

eDNA: Areas of uncertainty

Sampling/Study Design

When and where to sample to maximize detection?

- Seasonality
- Depth of sampling
- Distance from target

Laboratory

How can we ensure that lab methods are reliable and consistent?

- Marker specificity & sensitivity
- Inhibition & marker efficiency

Data Interpretation

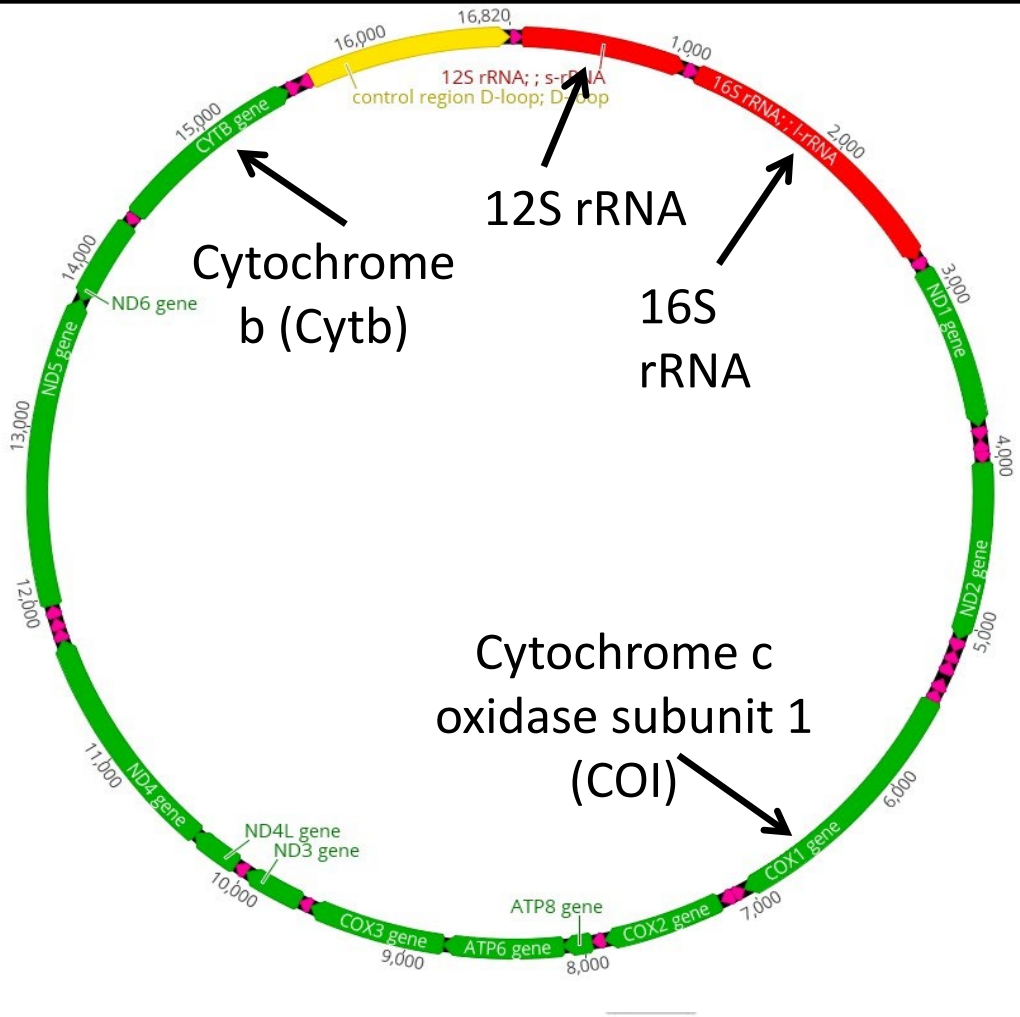
What does a positive or negative detection mean? And will it tell me how many brook trout are there?

- Strength of signal
- Quantitative associations

Mitochondrial Genome and eDNA

Why mitochondrial DNA?

- More copies in a cell
- Less variation within a species
- Variety of regions to select for marker development to provide species-level resolution



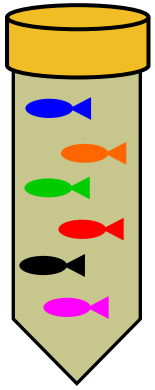
Conservation Genetics Resources
https://doi.org/10.1007/s12686-019-01111-0

METHODS AND RESOURCES ARTICLE

Fish mitochondrial genome sequencing: expanding genetic resources to support species detection and biodiversity monitoring using environmental DNA

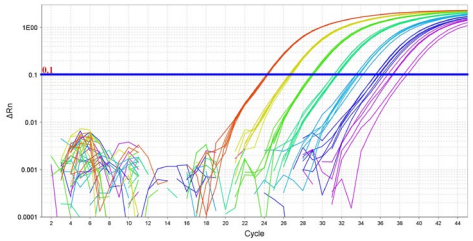
Julie C. Schroeter[1,2] · Aaron P. Maloy[1] · Christopher B. Rees[1] · Meredith L. Bartron[1]

eDNA: qPCR and Metabarcoding



Mixed community sample

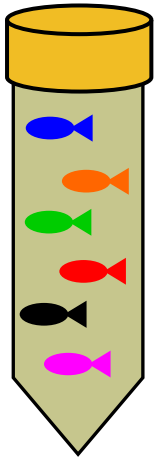
qPCR amplification



Amplification plots

Site	Detection result	
	Result	# positive
A	-	0/4
B	+	4/4
C	+	4/4

Output: Ct values, # replicates positive for target species

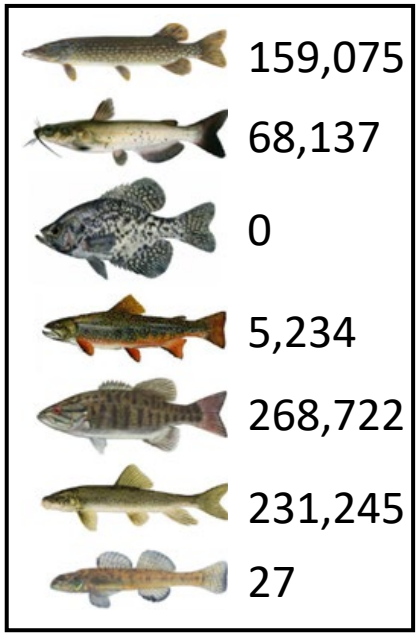


Mixed community sample

PCR and DNA sequencing

15-20 million DNA sequences

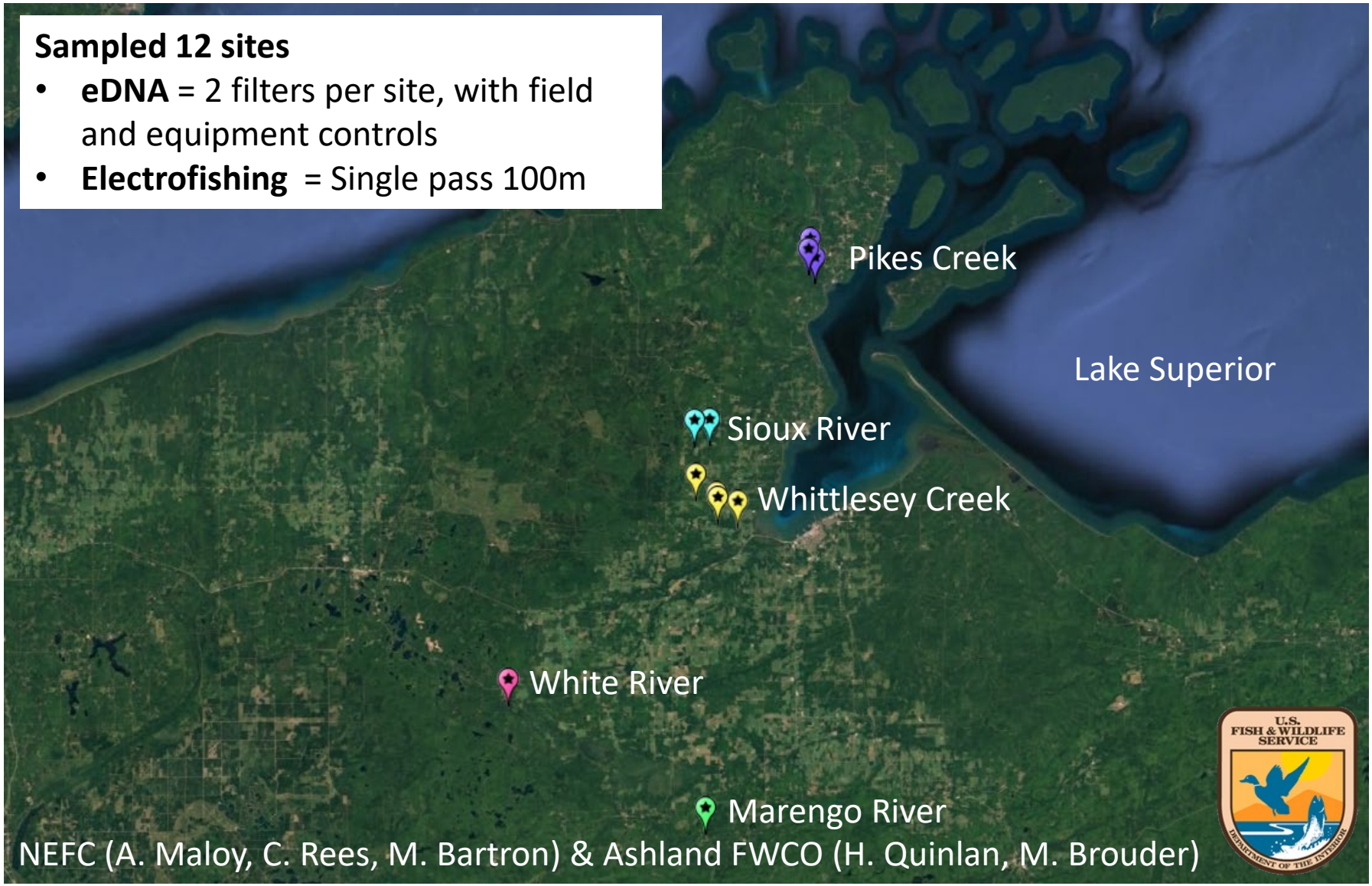
Reference database



Output: read counts

qPCR	Metabarcoding
Any sample type: eDNA, tissue, gut contents	Any sample type: eDNA, tissue, gut contents
Detects amplification of short species-specific DNA fragments	Generates up **to 25 million sequencing reads** per run
Targeted detection of **1 or 2 species per run**	Detection of **hundreds of species simultaneously** per run
Reference sequences for marker validation	**Reference sequences for species ID**
Target species must be known	**Species consideration can be broad or generic**, as well as taxa or species-specific
Sample throughput is dependent on the need for replication, **10-40 samples per run**	Each run can include up to **384 individually tagged samples (96 samples more practical**)
Sensitivity: More sensitive than traditional gear types (10 DNA copies or less)	Sensitivity: More sensitive than traditional gear types (needs further validation)

eDNA applications: Comparison of qPCR and metabarcoding



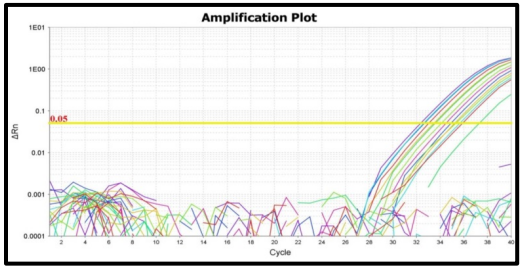
NEFC (A. Maloy, C. Rees, M. Bartron) & Ashland FWCO (H. Quinlan, M. Brouder)

eDNA applications: Comparison of qPCR and metabarcoding

Electrofishing

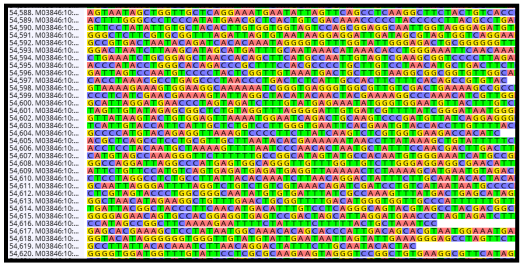
Multispecies detection
- Count of fish

Quantitative PCR



Single species detection
- DNA copies per liter

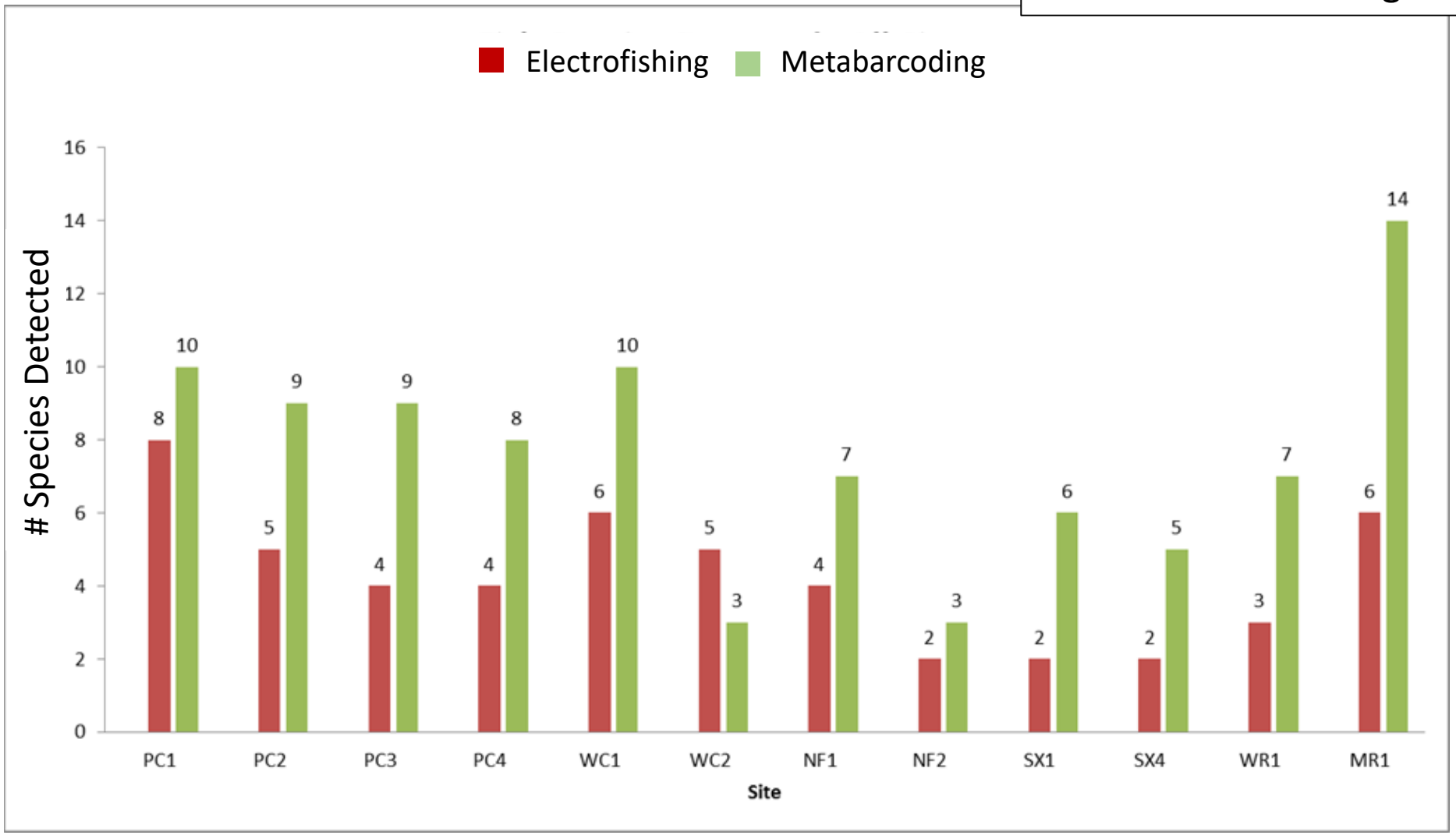
Metabarcoding



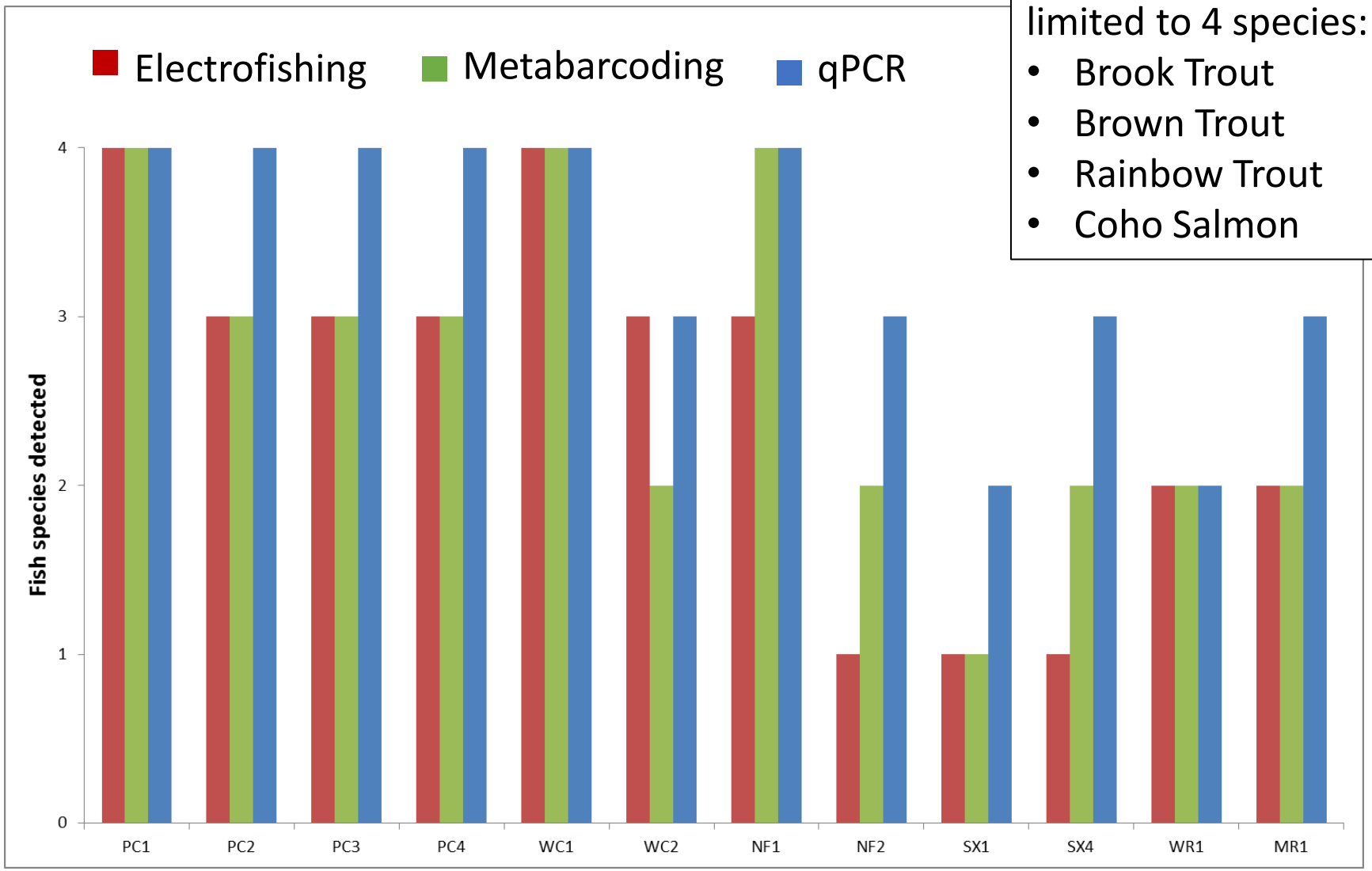
Multispecies detection
- Count of sequences

eDNA applications: Comparison of qPCR and metabarcoding

Total species detected:
- 10 Electrofishing
- 20 Metabarcoding



eDNA applications: Comparison of qPCR and metabarcoding



This comparison limited to 4 species:

- Brook Trout
- Brown Trout
- Rainbow Trout
- Coho Salmon

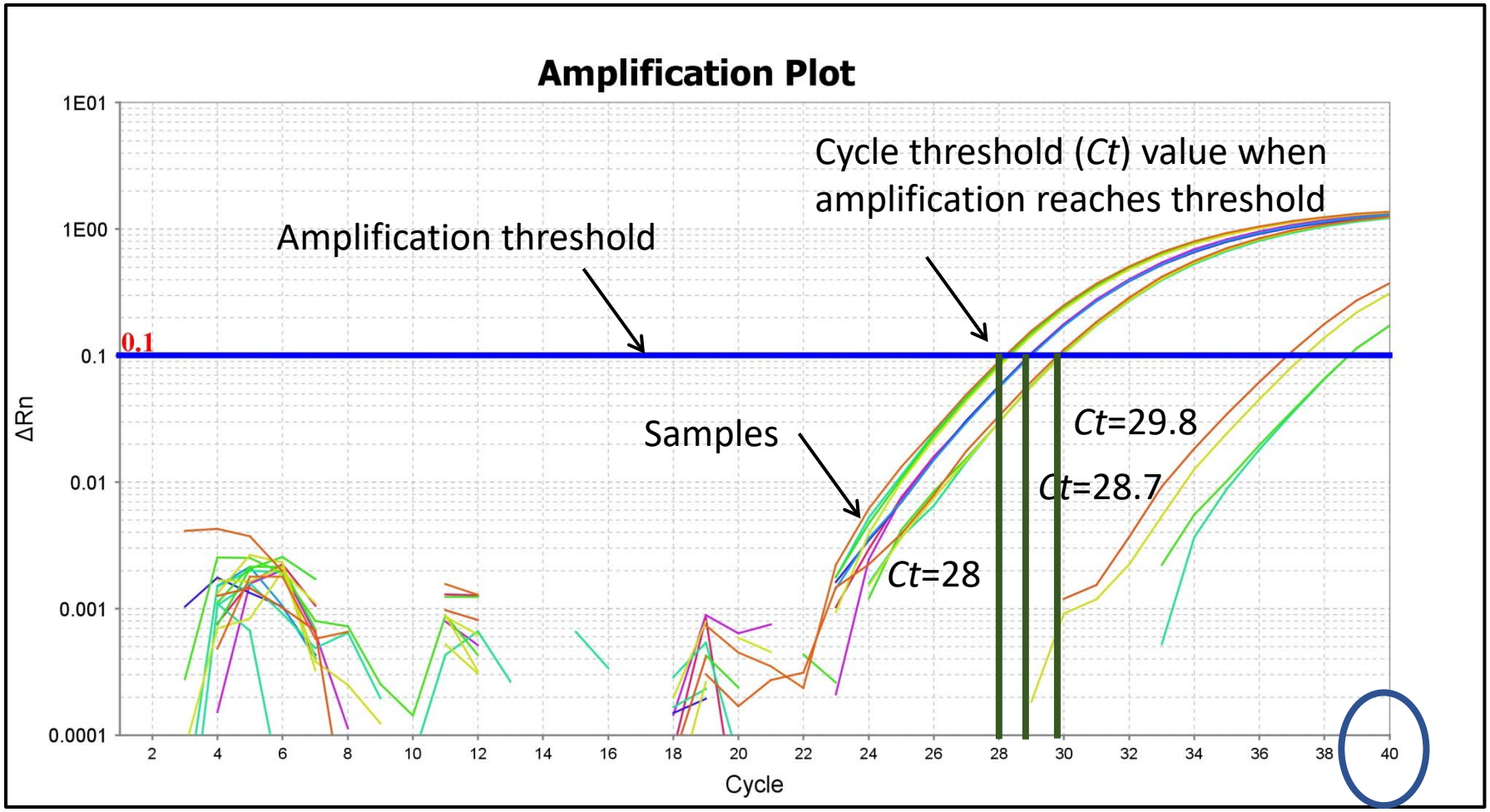
eDNA applications: Comparison of qPCR and metabarcoding

- eDNA methods more sensitive than traditional sampling gear
- Single species (qPCR) slightly more sensitive than metabarcoding
- Sampling “area” can be much different for eDNA versus electrofishing

Whittlesey Creek, WI

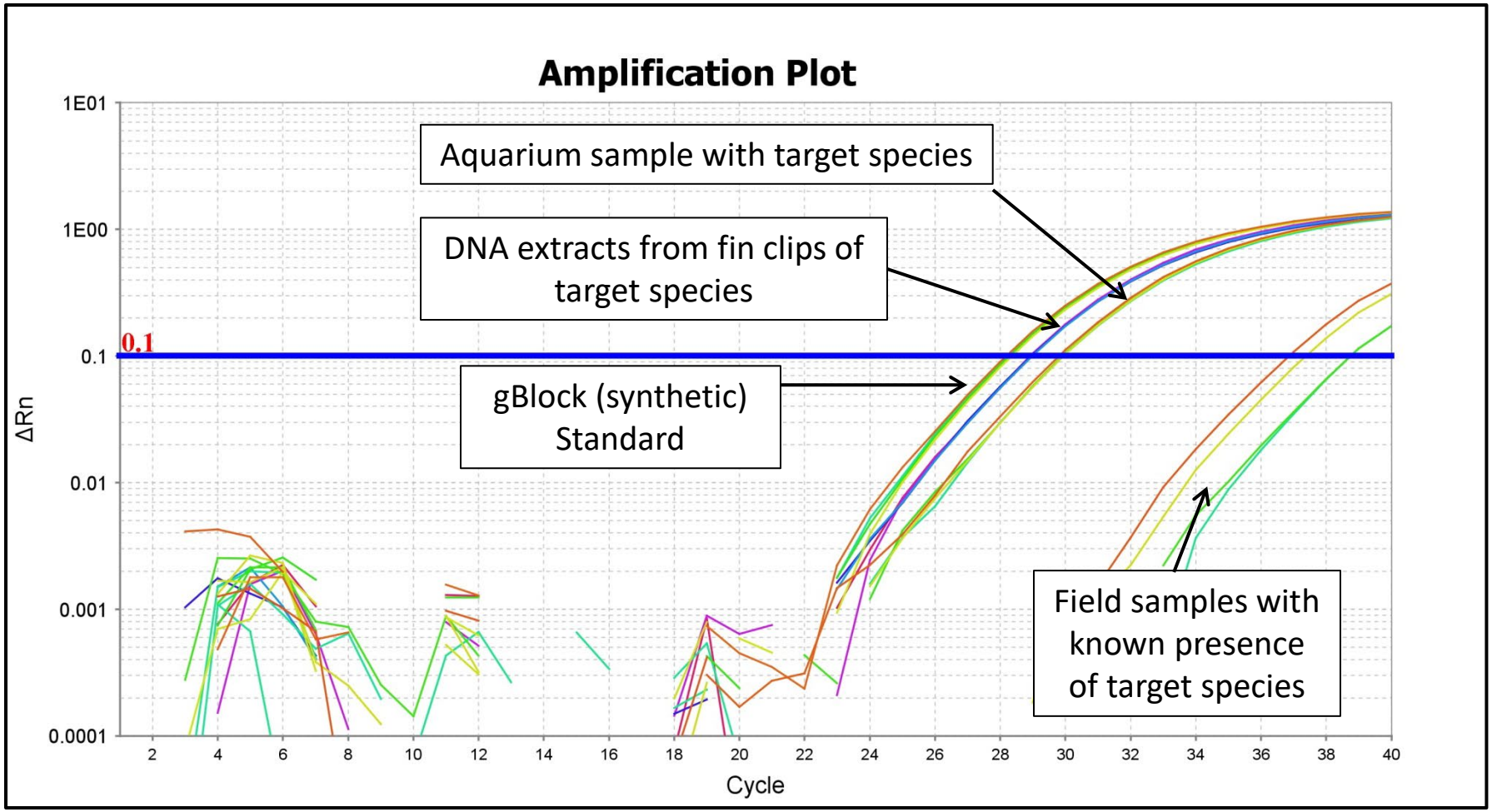
	Electrofishing	Metabarcoding
Black Bullhead		X
Blacknose Dace	X	X
Bluegill		X
Brook Stickleback	X	X
Brook Trout	X	X
Brown Trout	X	X
Central Mudminnow	X	X
Coho Salmon	X	X
Common Shiner		X
Creek Chub	X	X
Fathead Minnow		X
Lake Trout/Splake	X	X
Largemouth Bass		X
Longnose Dace		X
Redbelly Dace		X
Pumpkinseed		X
Rainbow Trout	X	X
Sculpin	X	X
Walleye		X
White Sucker		X
Total Species	**10**	**20**

qPCR background and terminology



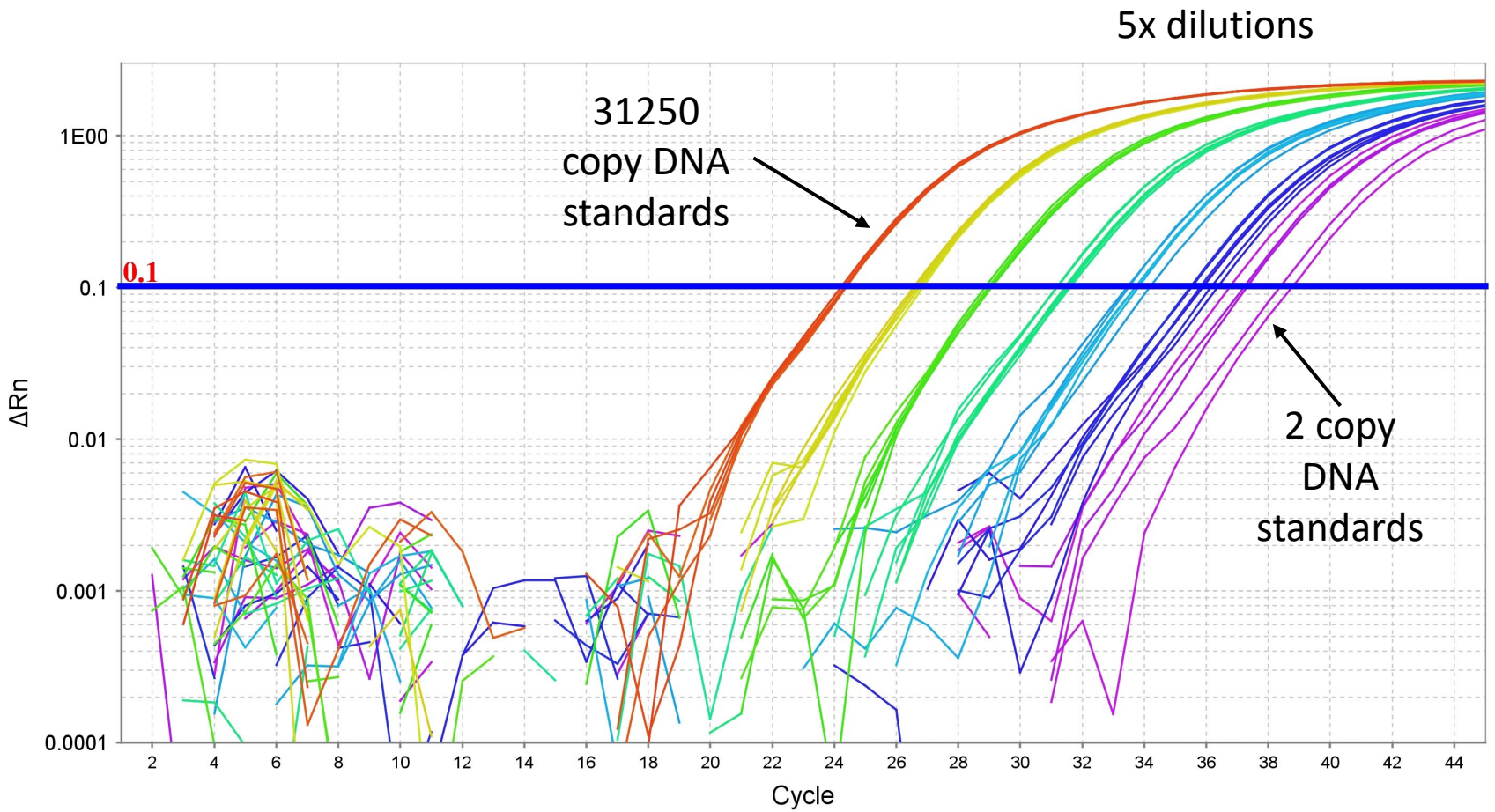
qPCR background and terminology

Understand expected amplification of DNA to evaluate marker performance, including expected behavior of field samples



qPCR background and terminology: Marker sensitivity

Standard curves used to determine marker's ability to amplify DNA of varying concentrations *helps to establish minimum concentration for positive detection*



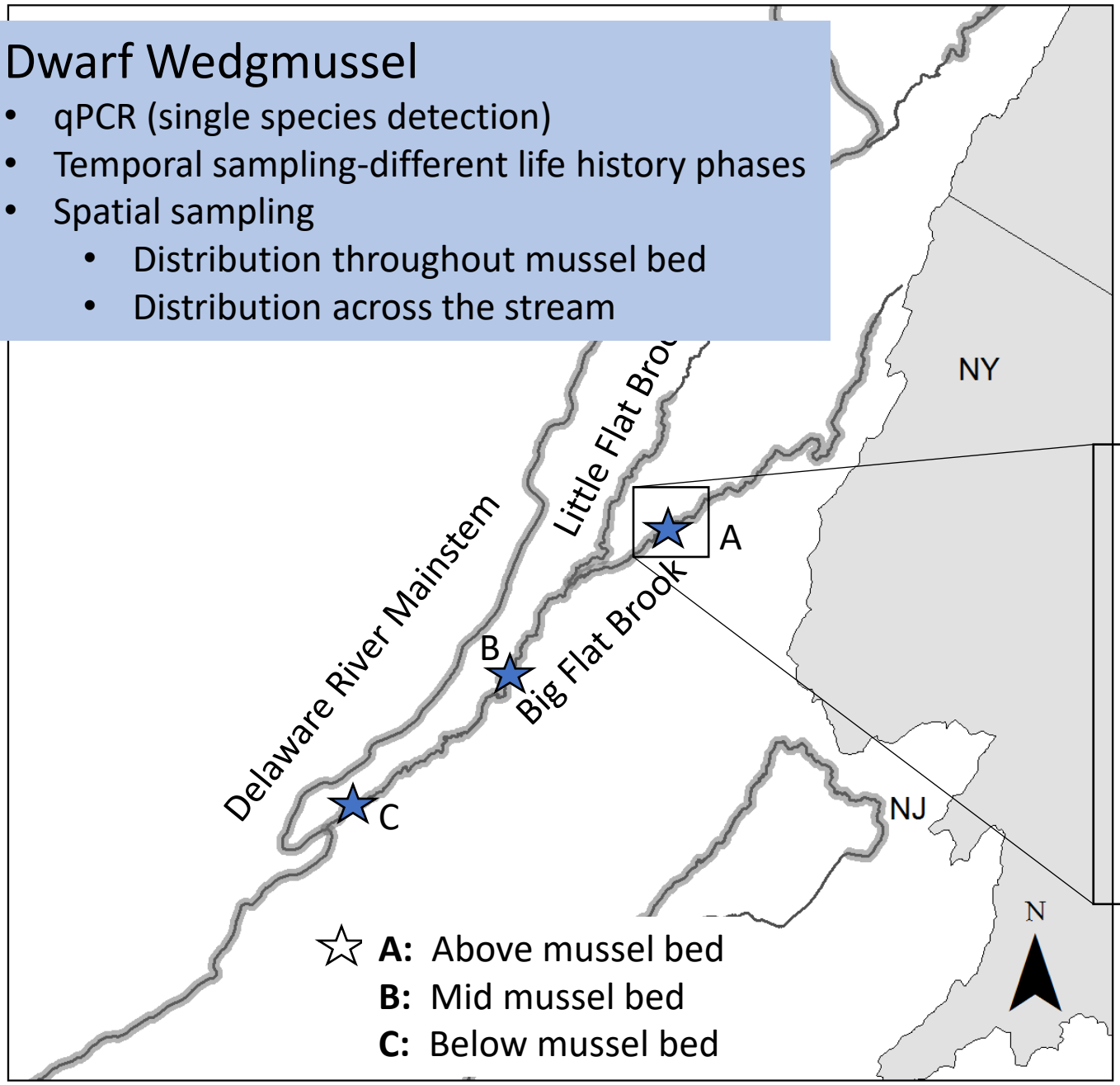
Sampling/Study Design

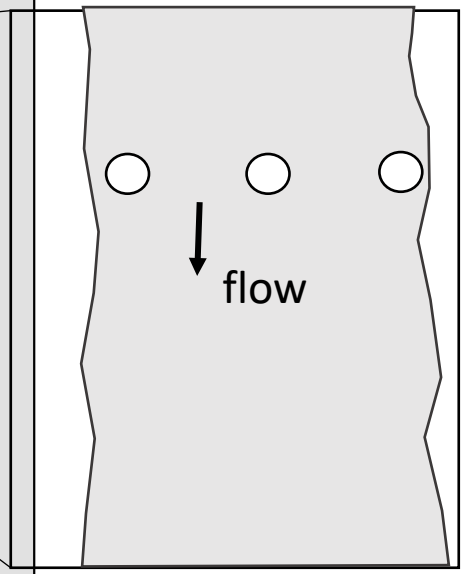
Reduce factors associated with sampling methodology that could reduce the potential for detection when species present

Sampling/Study Design: Seasonality

Dwarf Wedgmussel

- qPCR (single species detection)
- Temporal sampling-different life history phases
- Spatial sampling
  - Distribution throughout mussel bed
  - Distribution across the stream

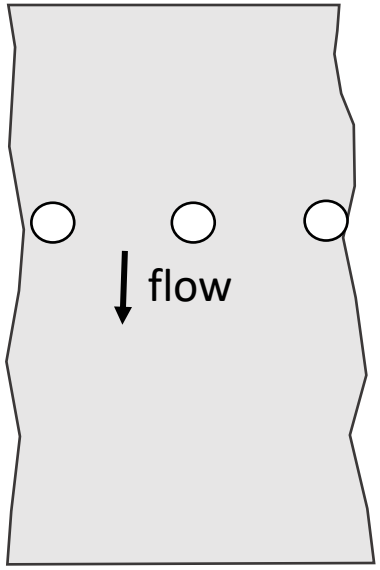




At each location, sample 3 sites, 3 times/year

Spring (April), *glochidia release*

Site	Near-bank		Mid-stream		Far-bank	
	Result	# positive	Result	# positive	Result	# positive
A	-	0/4	-	0/4	-	0/4
B	+	4/4	+	4/4	+	2/4
C	+	4/4	+	4/4	+	4/4

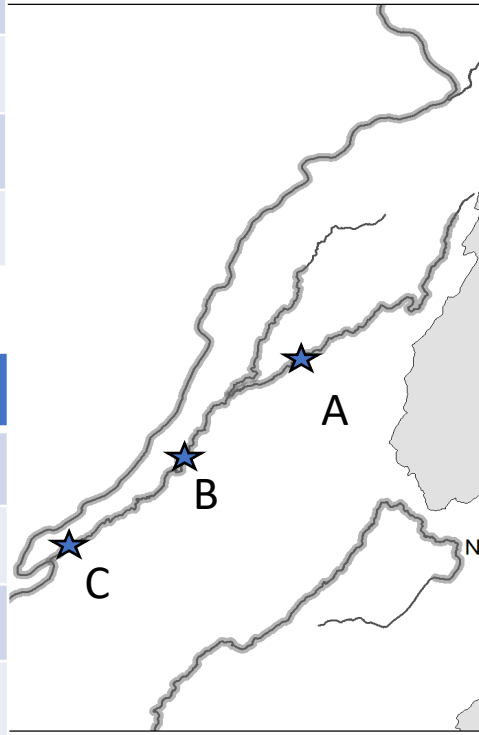


Summer (August), *gamete release*

Site	Near-bank		Mid-stream		Far-bank	
	Result	# positive	Result	# positive	Result	# positive
A	-	0/4	-	0/4	-	0/4
B	+	2/4	+	4/4	+	4/4
C	+	1/4	+	1/4	+	2/4

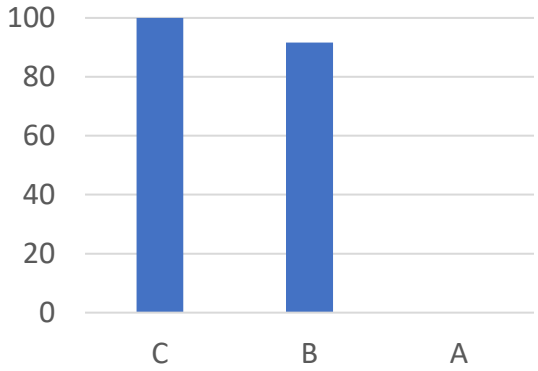
Winter (November), *dormant/sub-surface*

Site	Near-bank		Mid-stream		Far-bank	
	Result	# positive	Result	# positive	Result	# positive
A	-	0/4	-	0/4	-	0/4
B	+	2/4	-	0/4	+	1/4
C	+	3/4	+	2/4	+	1/4

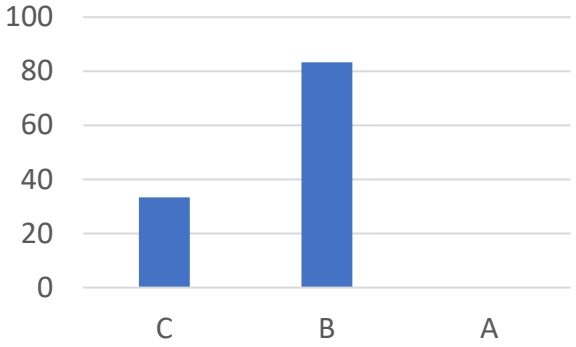


Sampling/Study Design: Seasonality of sampling

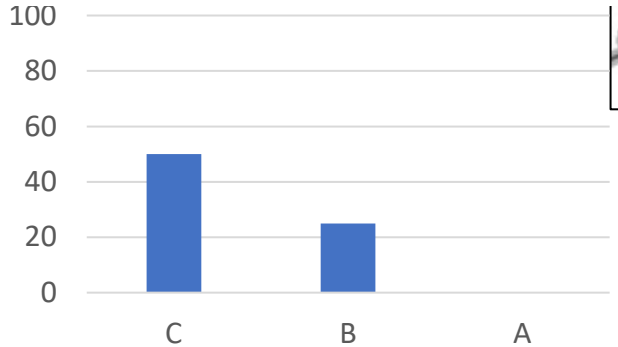
April % Detection: Glochidia release

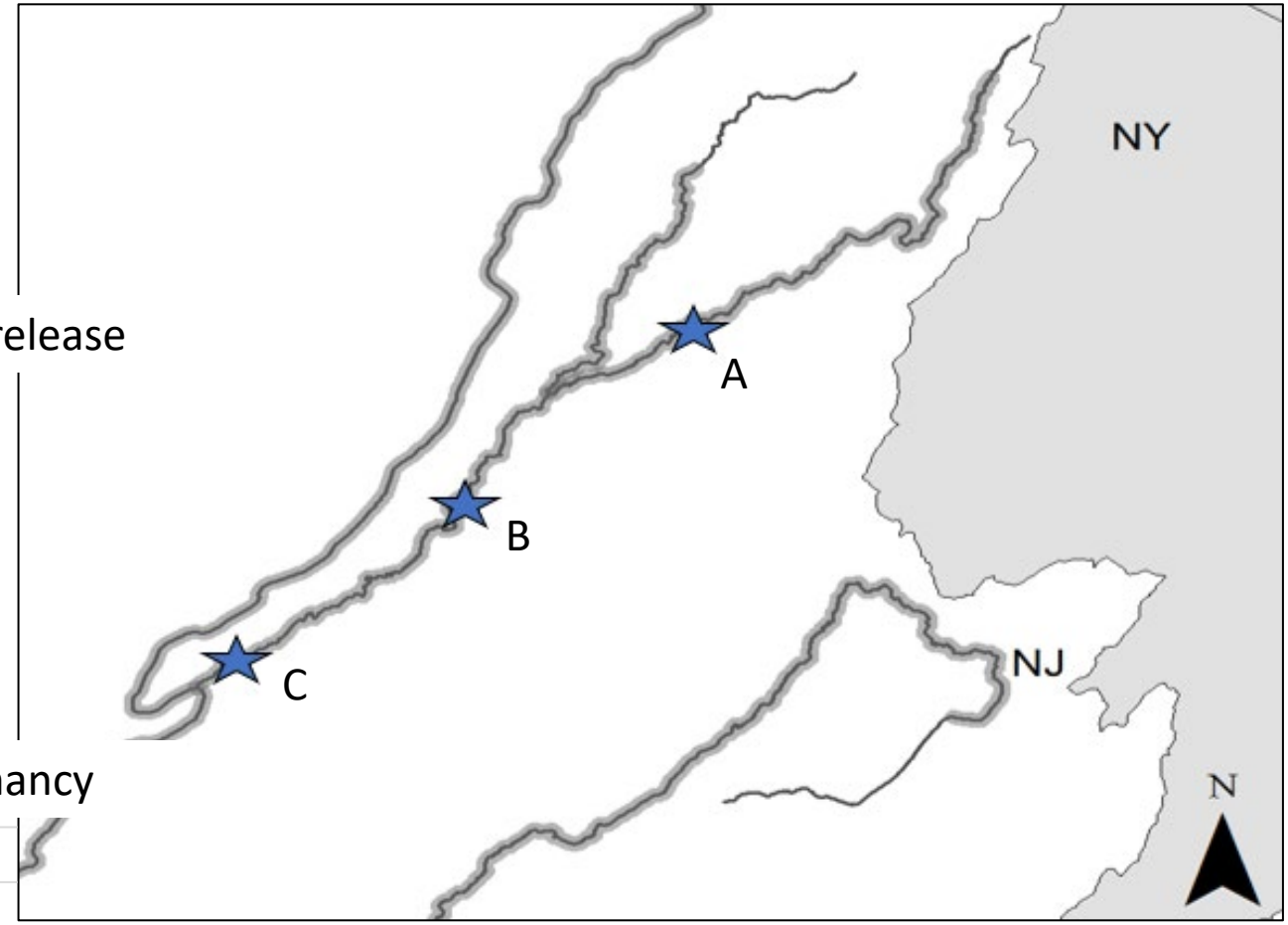


August % Detection: Gamete release

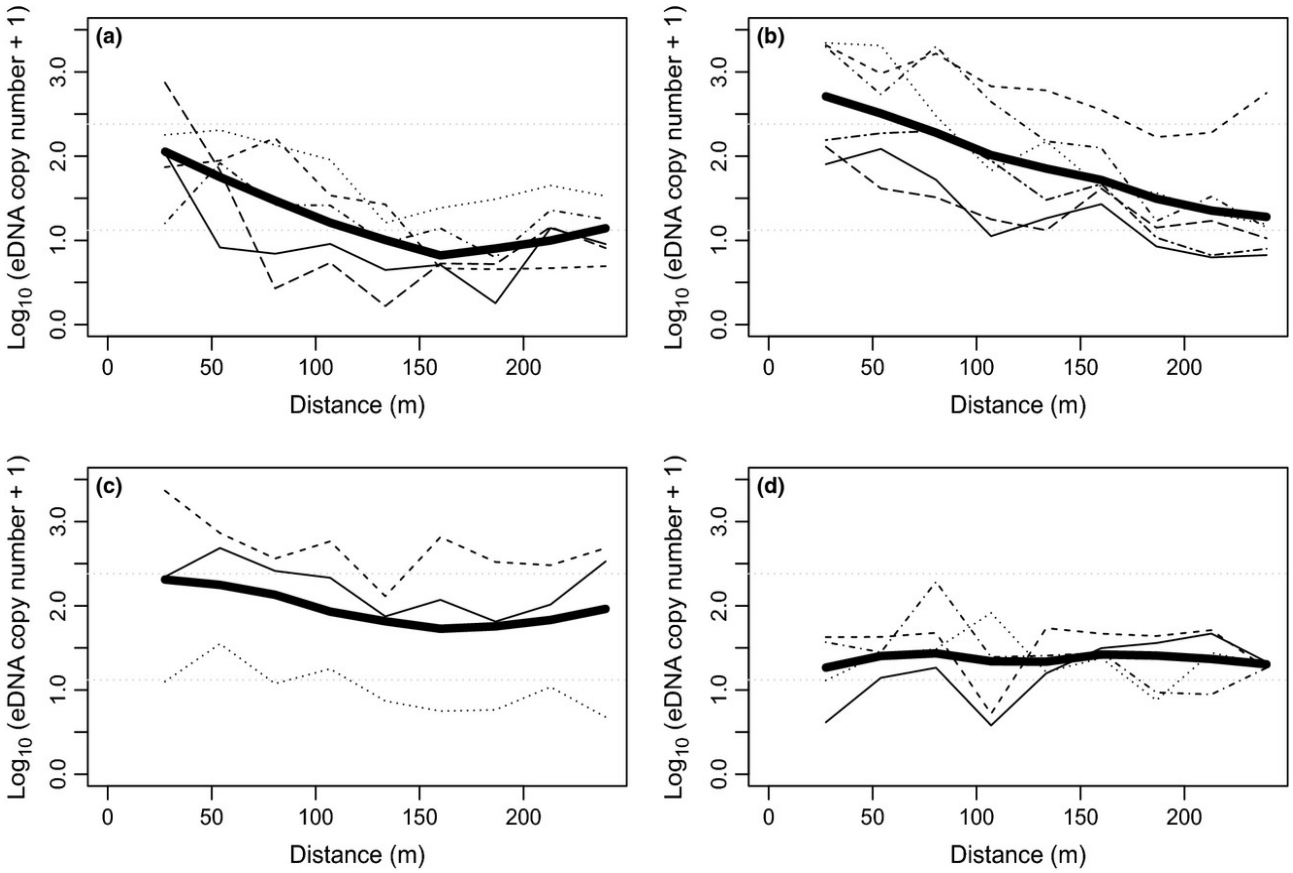


November % Detection: Dormancy





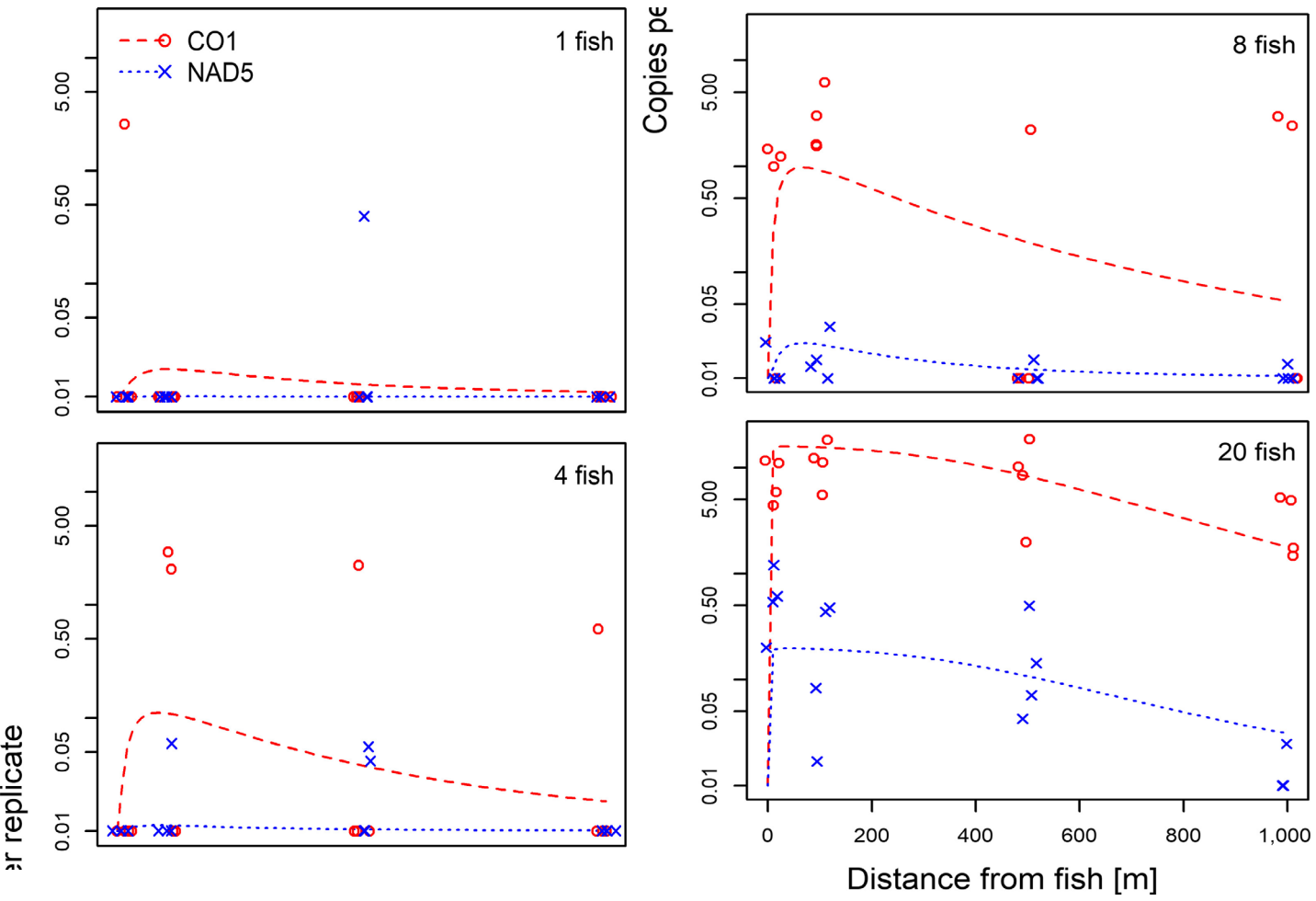
Sampling/Study Design: Distance and flow



eDNA transport and detection can vary over range of flows (very low to high)

Jane et al. 2015 Molecular Ecology Resources

Sampling/Study Design: Distance and flow



- Distance of detection ranged based on number of fish present

Wood et al. 2020 Environmental DNA

Sampling/Study Design

Biological considerations:

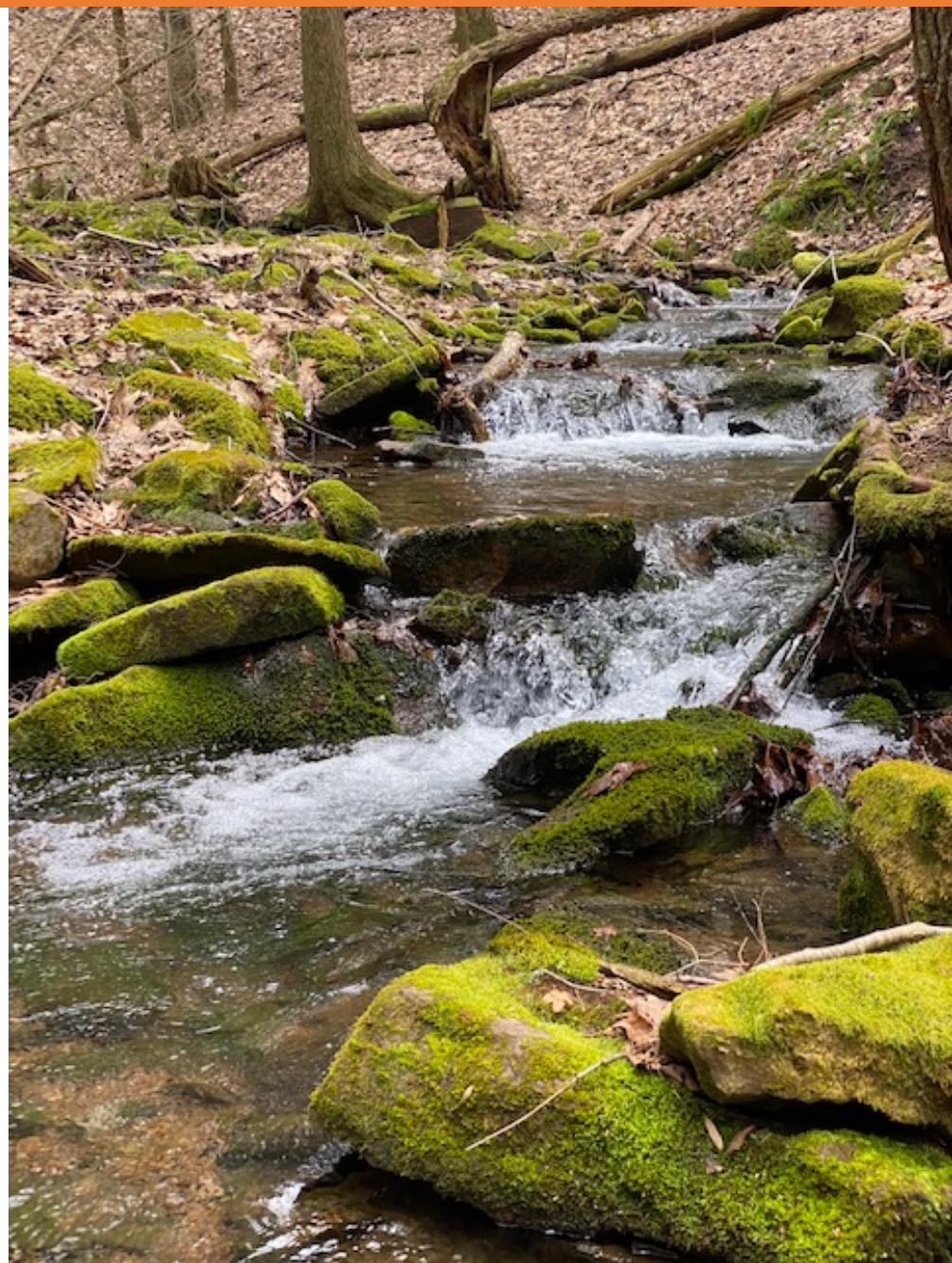
- Seasonal movements
- Distribution of target species within the habitat

Physical considerations:

- Areas where water (and DNA) has mixed throughout water column
- Stream width
- Water flows

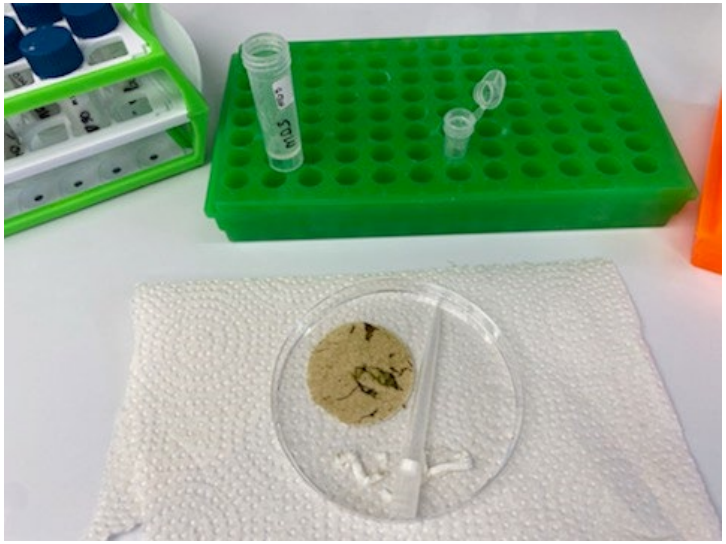
Sampling details:

- Water volume filtered
- Filter pore size
- Sample and filter controls



Laboratory

Reduce factors that would limit detection of target DNA due to laboratory processes

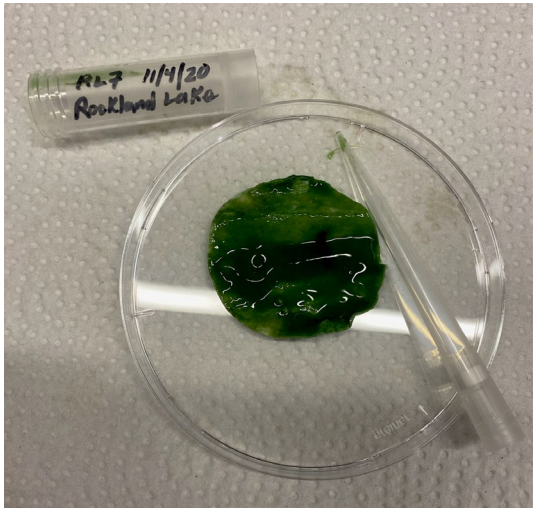


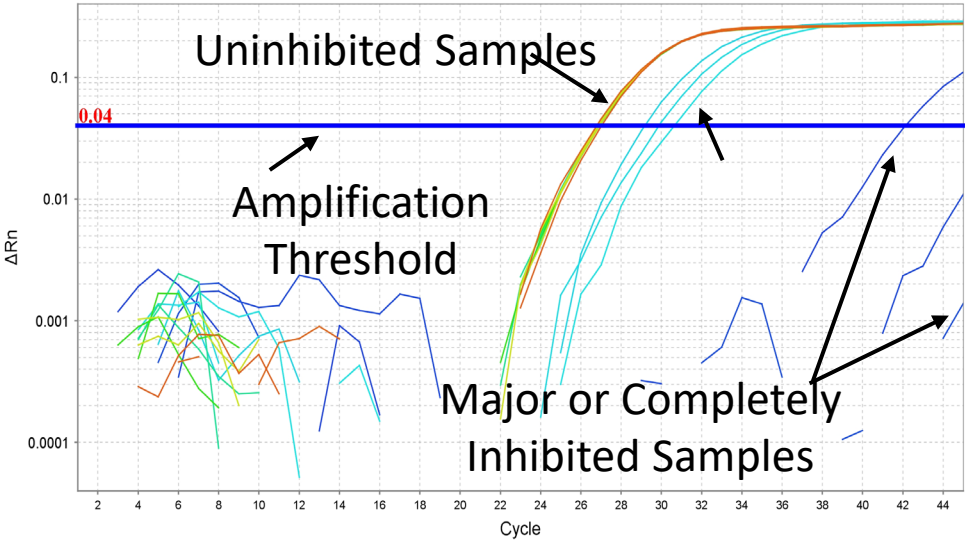
Laboratory: PCR inhibition

PCR inhibition delays amplification of target DNA amplification causing a reduction in marker efficiency = potential increase in false negative results

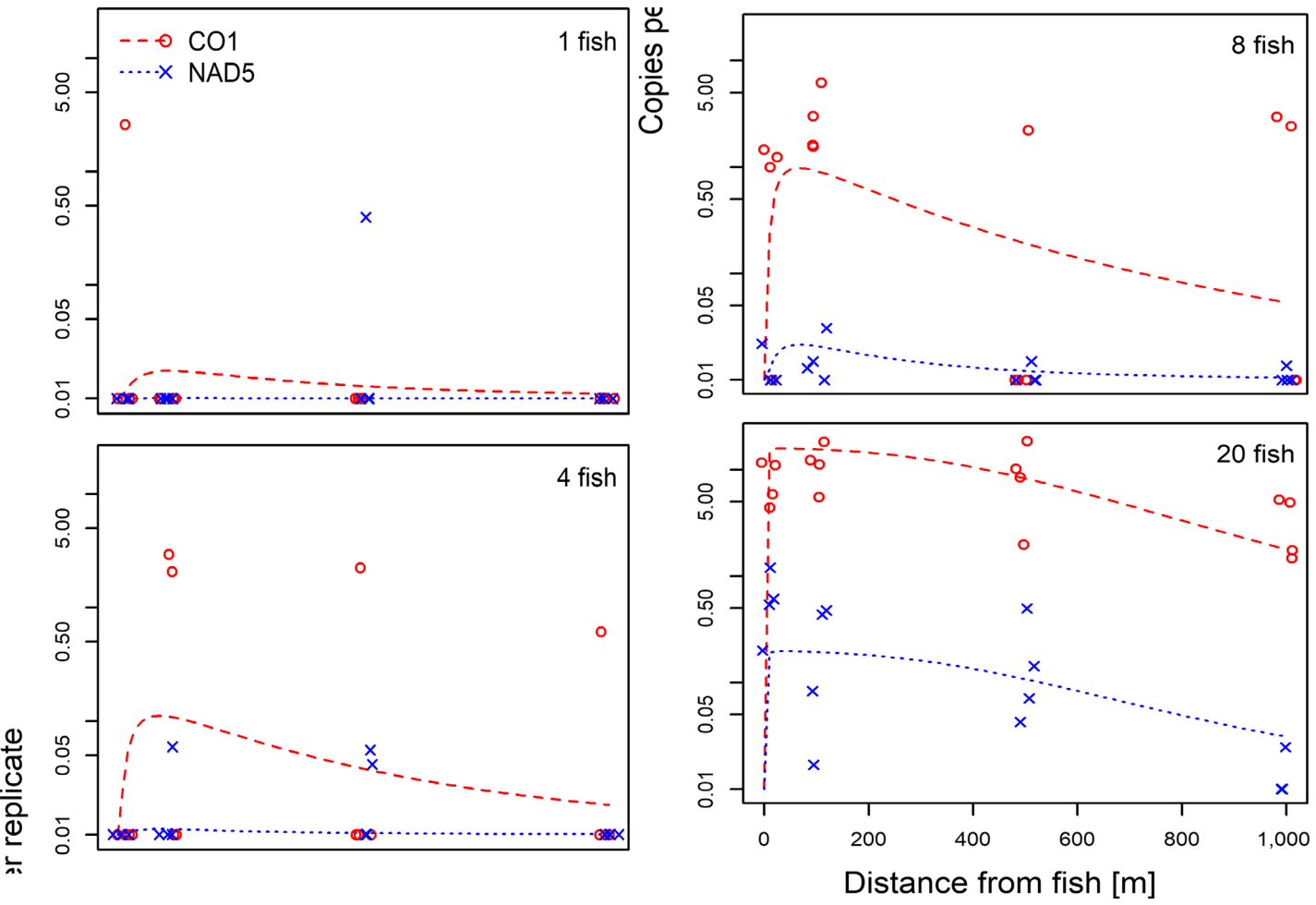
<u>Sources of inhibition for field samples:</u>

- Algae
- Sediment & decomposing plant materials: humic acid, tannic acid, fulvic acid, phytic acid…
- Phenols/Polyphenols: decomposing berries and plant materials
- Other sources of DNA





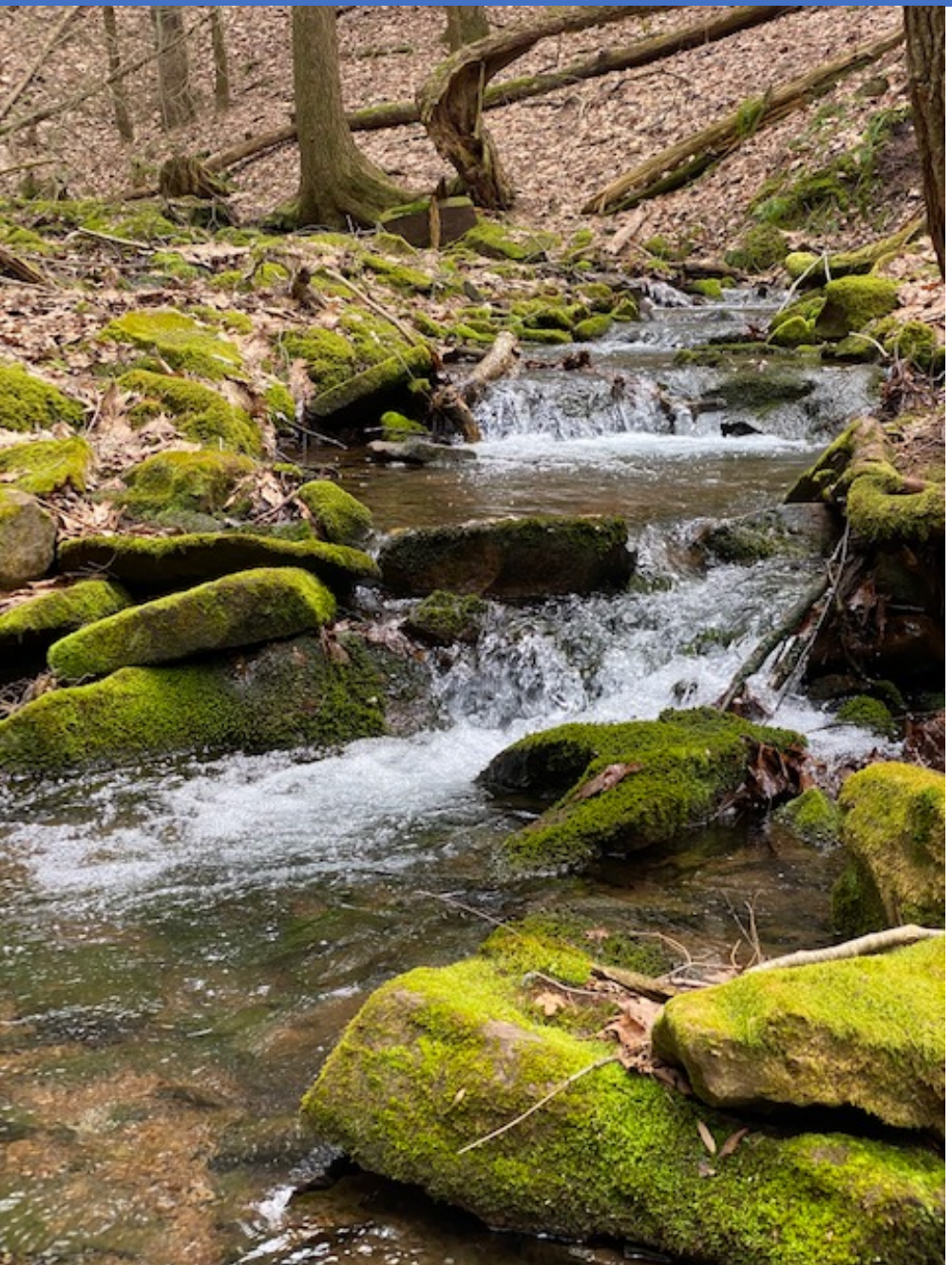
Laboratory: Marker specificity and sensitivity



- Difference in detection based on marker
- Variation in marker performance can impact consideration of “positive detection”

Wood et al. 2020 Environmental DNA

Laboratory



Marker evaluation:

- Quantify sensitivity
- Confirm specificity to target species
- Understand expected amplification of field samples

Inhibition:

- Adjust sampling to minimize
- Test for influence of inhibition on amplification

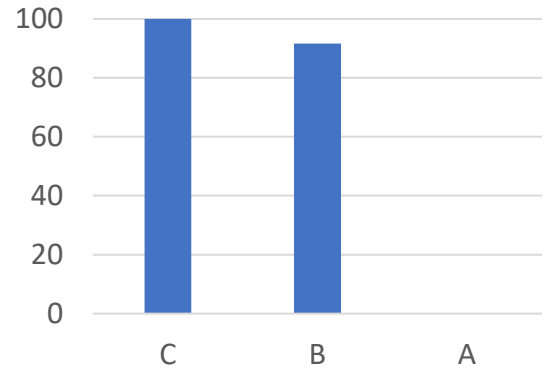
Data Interpretation

Confidence that negative and positive detections reflect the true state of presence

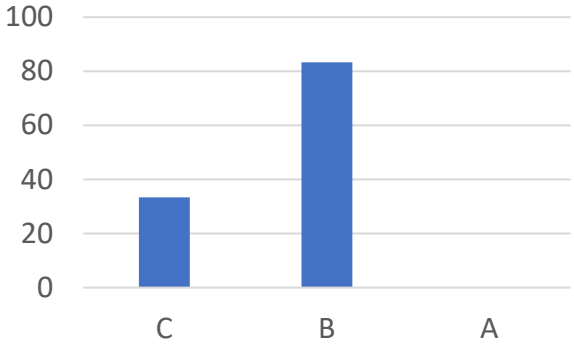
- Reduce uncertainty with sampling processes
- Reduce uncertainty with laboratory processes
- Clarify expectation of what detection means, that a positive detection indicates presence of DNA
  - Consider alternate sources of DNA
- Management action should require high level of confidence in results

Data Interpretation: Seasonal variation influence on detection

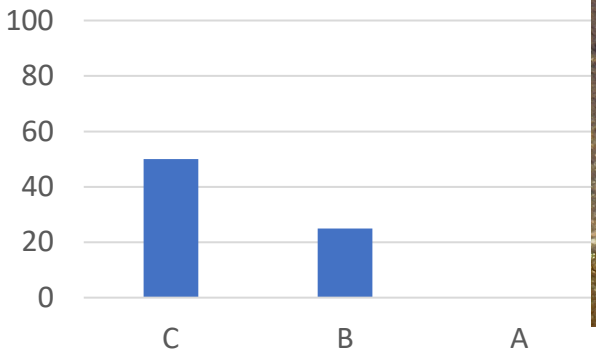
April % Detection



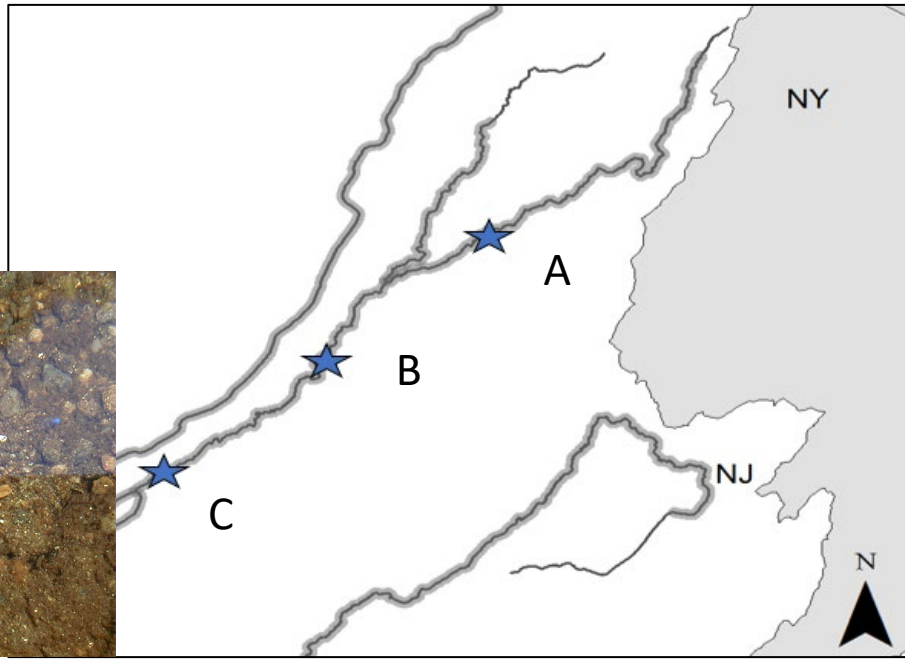
August % Detection



November % Detection

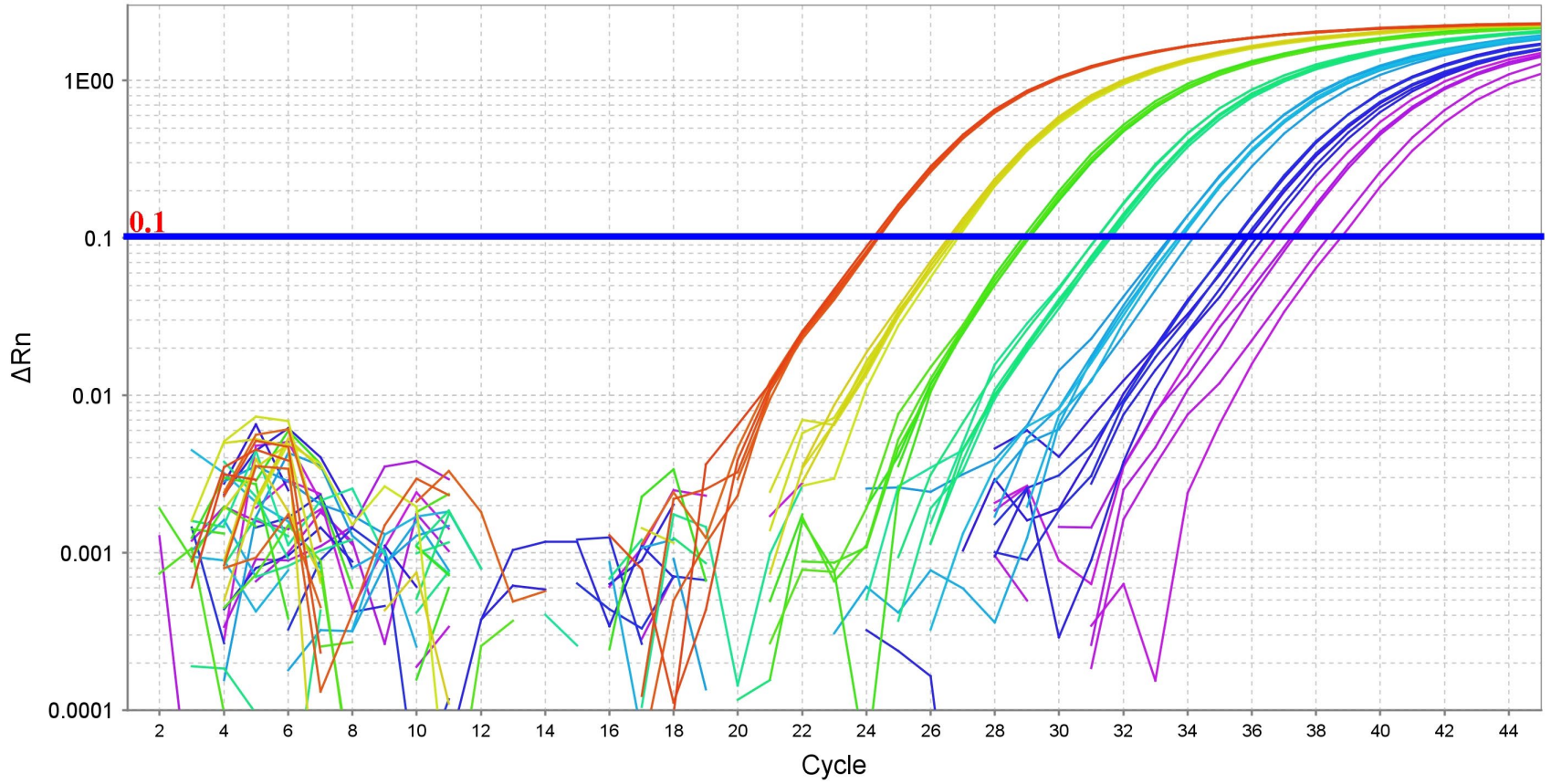


- If seasonal movement significant, repeated sampling over season and year

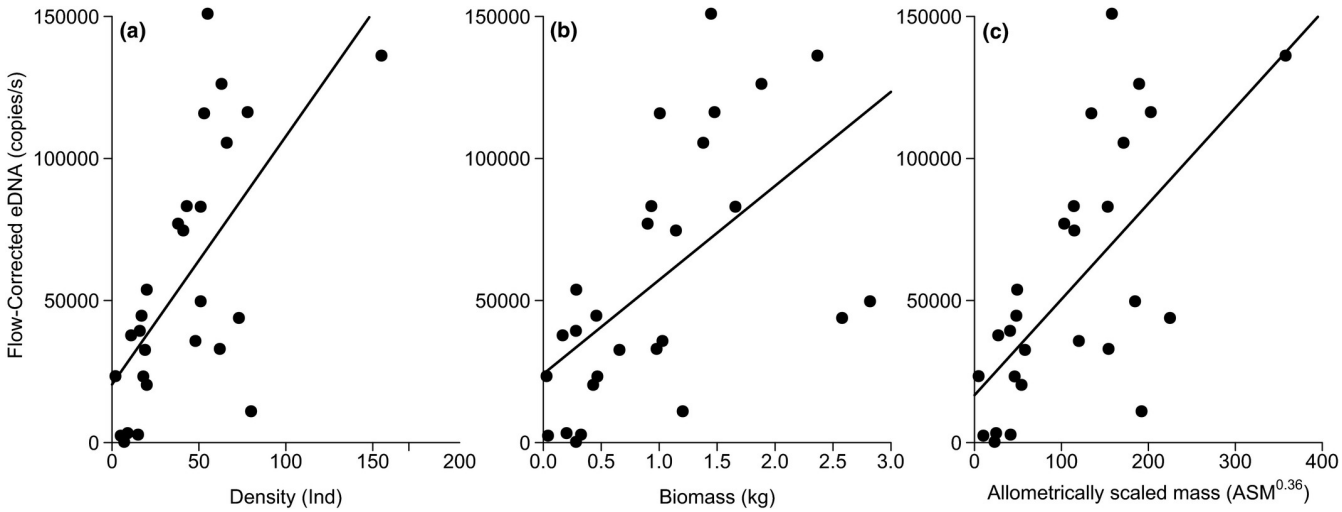


Data Interpretation: Quantitative association of results

Can we correlate eDNA data to predict biomass?



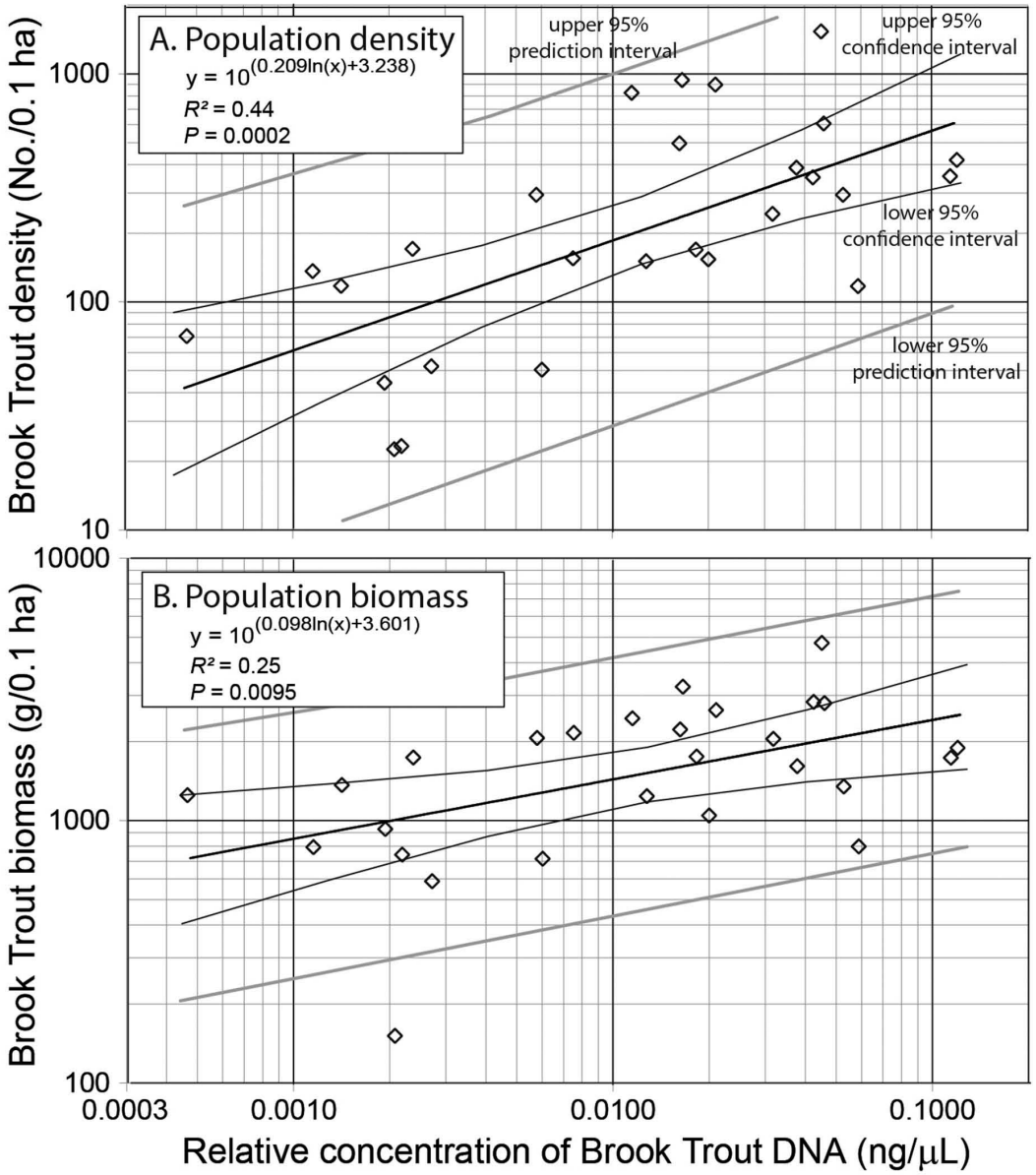
Data Interpretation: Quantitative association of results



- eDNA production did not scale linearly with biomass, but better correlated with density
- 43% of the variation in eDNA concentration is explained by ASM
- Predictive ability would have high uncertainty
- Consideration of lentic vs. lotic system also important

Yates et al. 2020 Environmental DNA

Data Interpretation: Quantitative association of results



- eDNA results correctly predicted presence/confirmed absence at 85 to 92.5% sites
- eDNA explained 44% of the variability in Brook Trout population density and 24% variability in biomass

Baldigo et al. 2016 TAFS

Data Interpretation: Quantitative association of results

Can we correlate eDNA data to predict biomass?

More correlational studies needed to link eDNA and population survey data using traditional assessment methods

Predictive ability is low, at least currently (for both biomass and density)

Consider the species diversity, habitat quality, and system and their impact on sample quality

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