Environmental DNA Assessment of Biodiversity, Abundance & Phenology of Hudson Estuary Fishes

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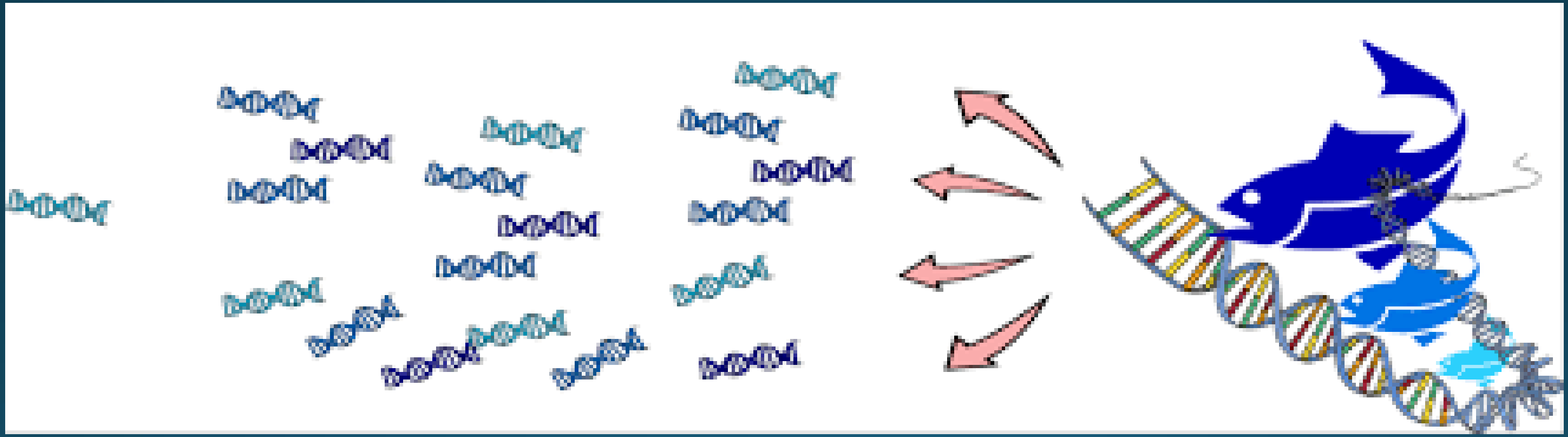
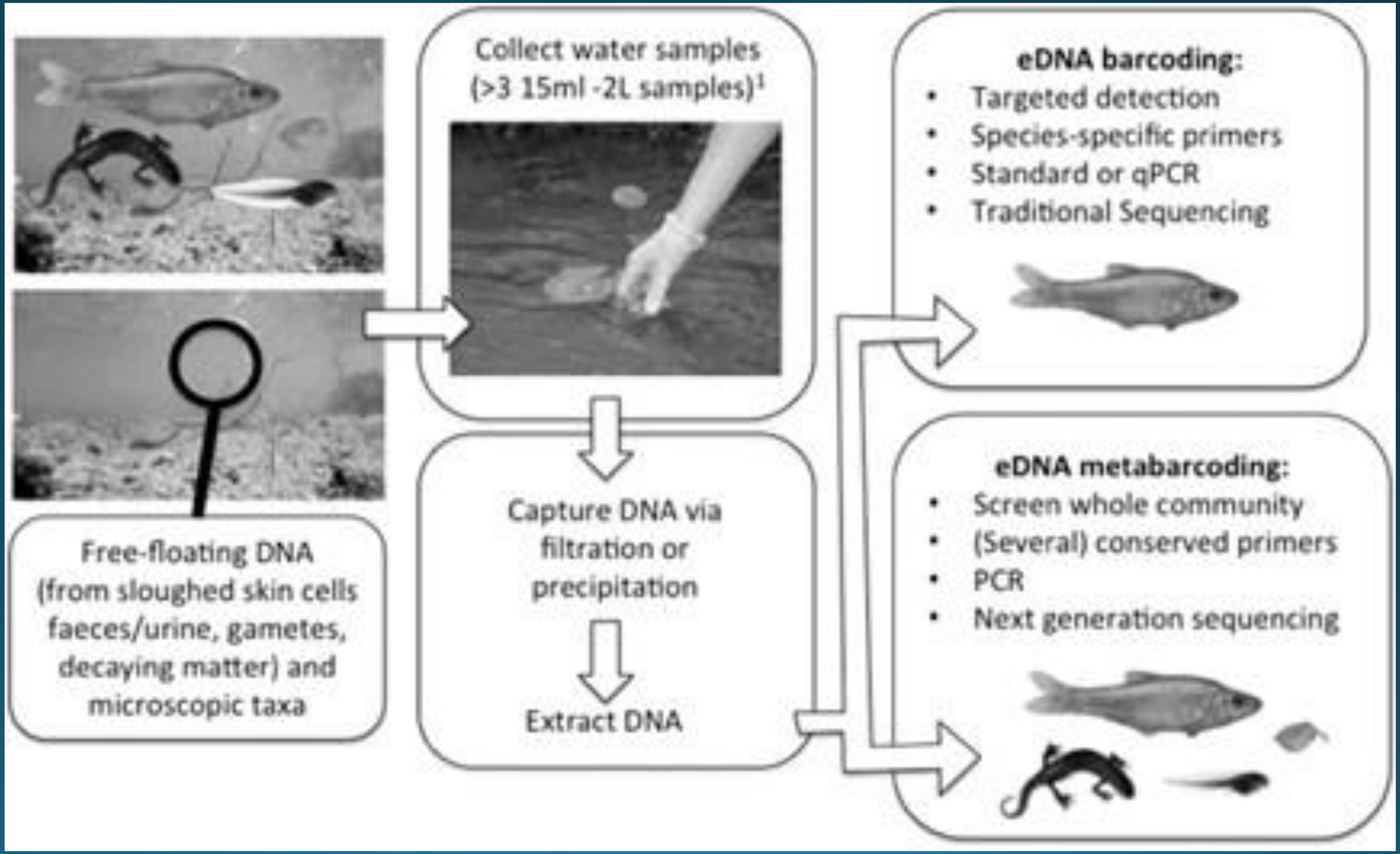
“Our waters are a soup of DNA”



Aquabiota

eDNA Analysis – Basic Processes



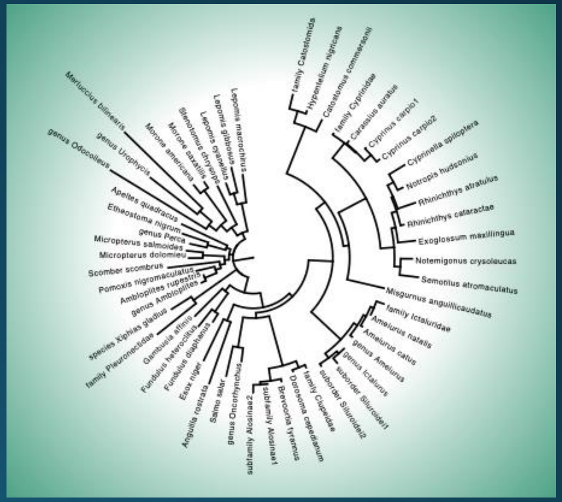
Relative importance
Of these for fish is an
interesting question

Outline

Abundance

- American Eel in the Bronx River
- *qPCR* (quantitative polymerase chain reaction)

Biodiversity

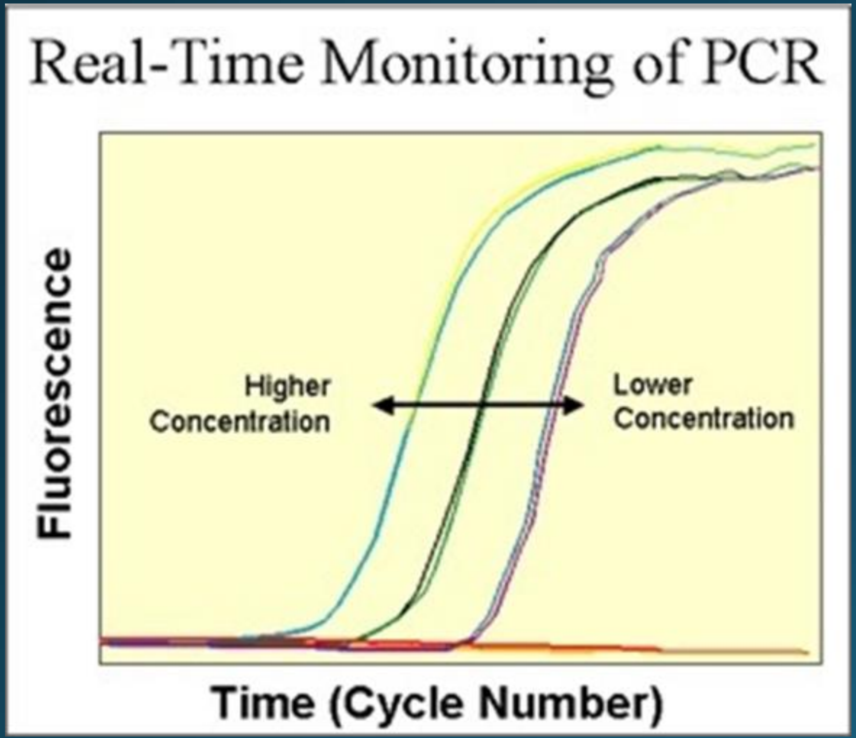


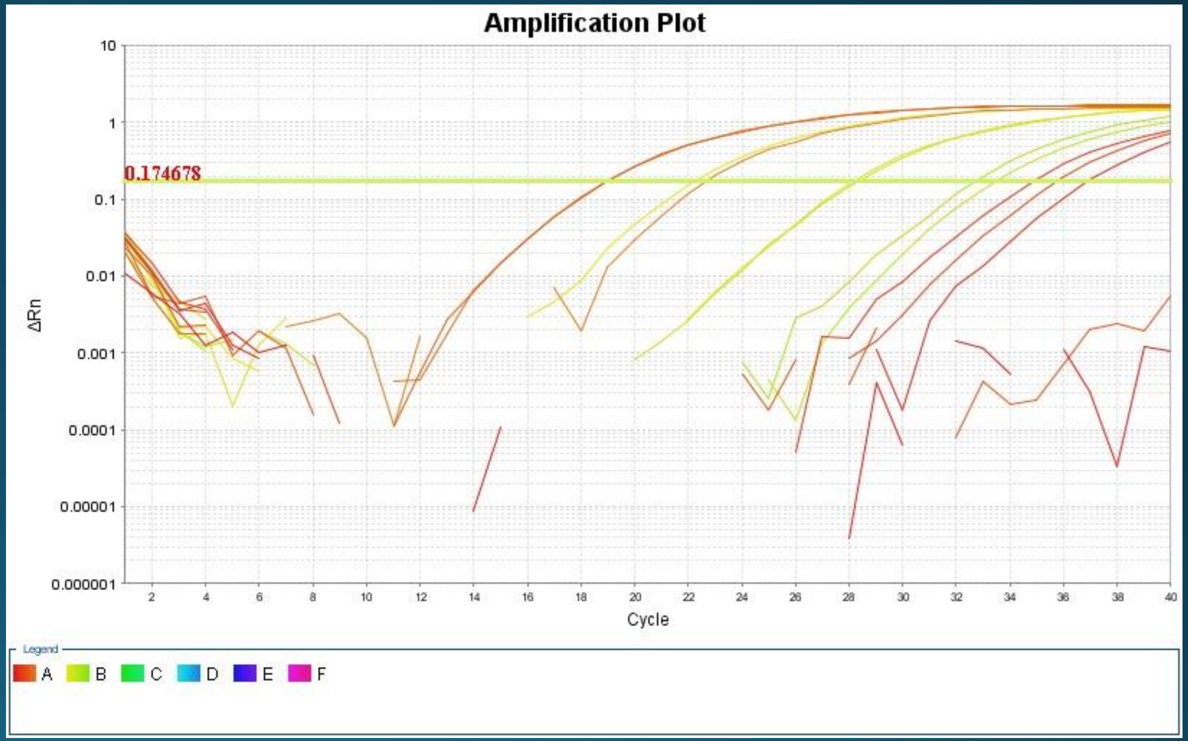
- 12 Hudson River Tributaries & 2 Mainstem Sites
- *metabarcoding* (detecting short sequences against a reference library)

Phenology

- Alewife Migrations in Bronx River - *qPCR*

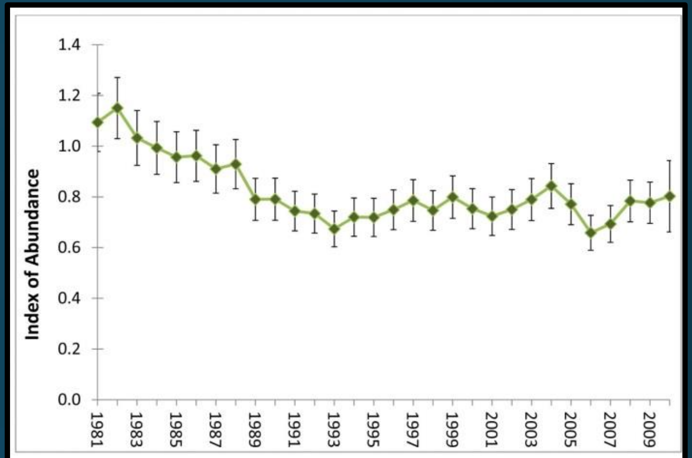
I. Assessing Abundance Using qPCR



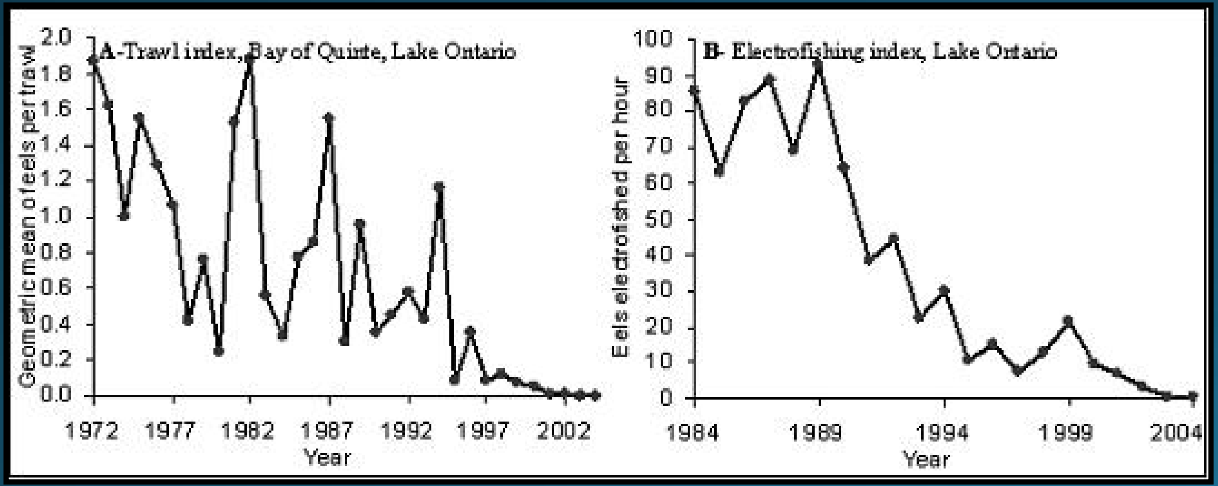


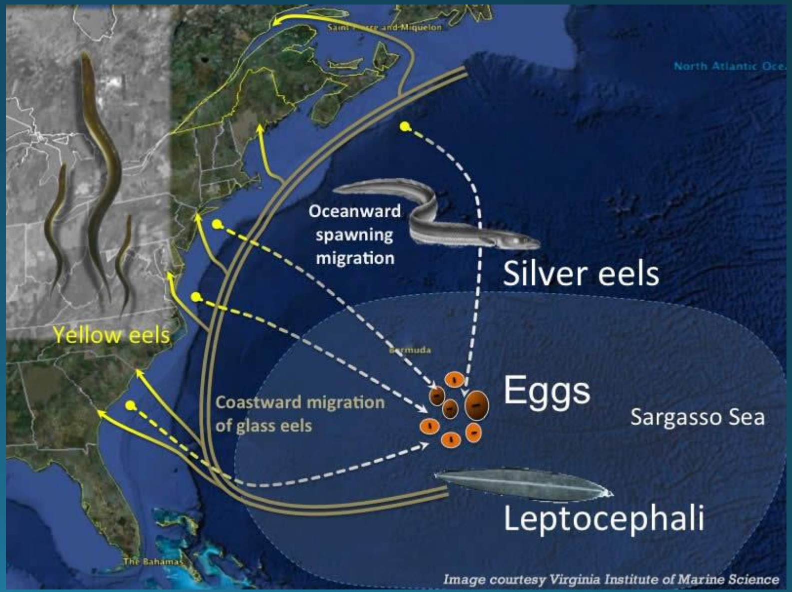
Eel Abundance in the Bronx River

American Eel
Life History
& Status



Index of Abundance for Yellow-phase American Eels along the Atlantic Coast. Source: ASMFC American Eel Benchmark Stock Assessment, 2012

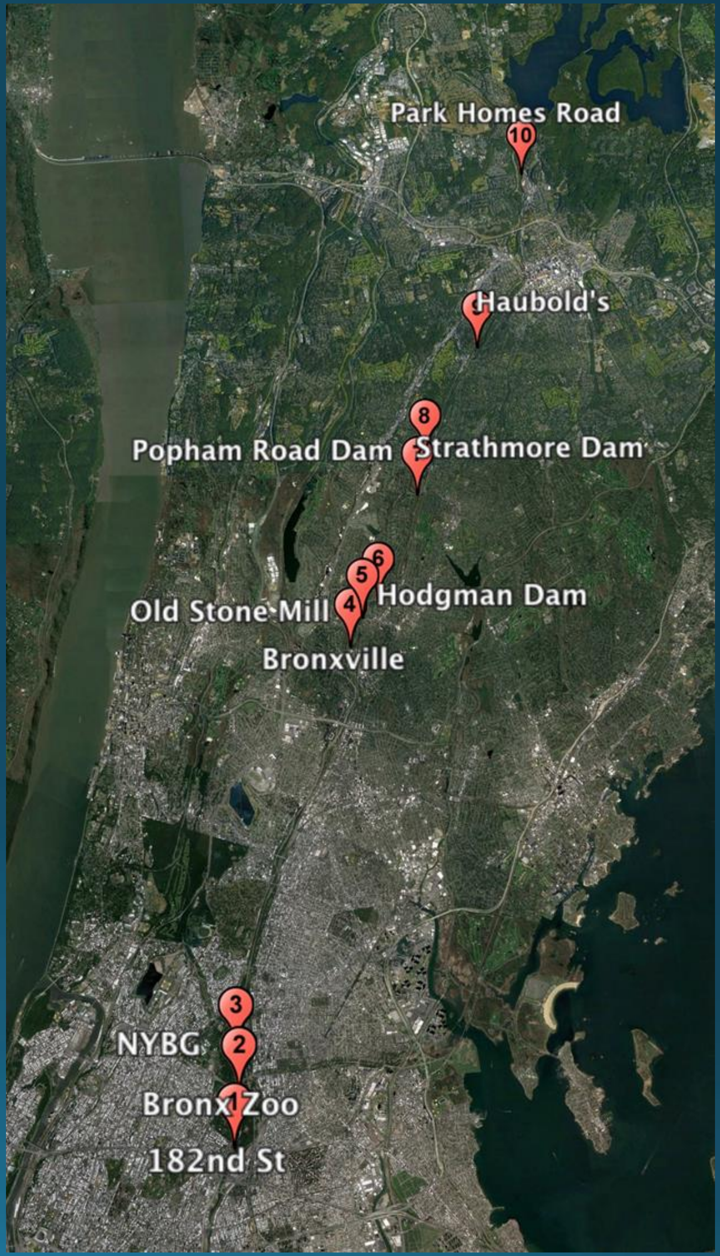




The Bronx River and Its Dams

Dam 3 - Snuff Mill Dam, Bronx Botanical Gardens

Hidden Waters Blog



A Standard Assessment Approach: Electrofishing 2014-2017

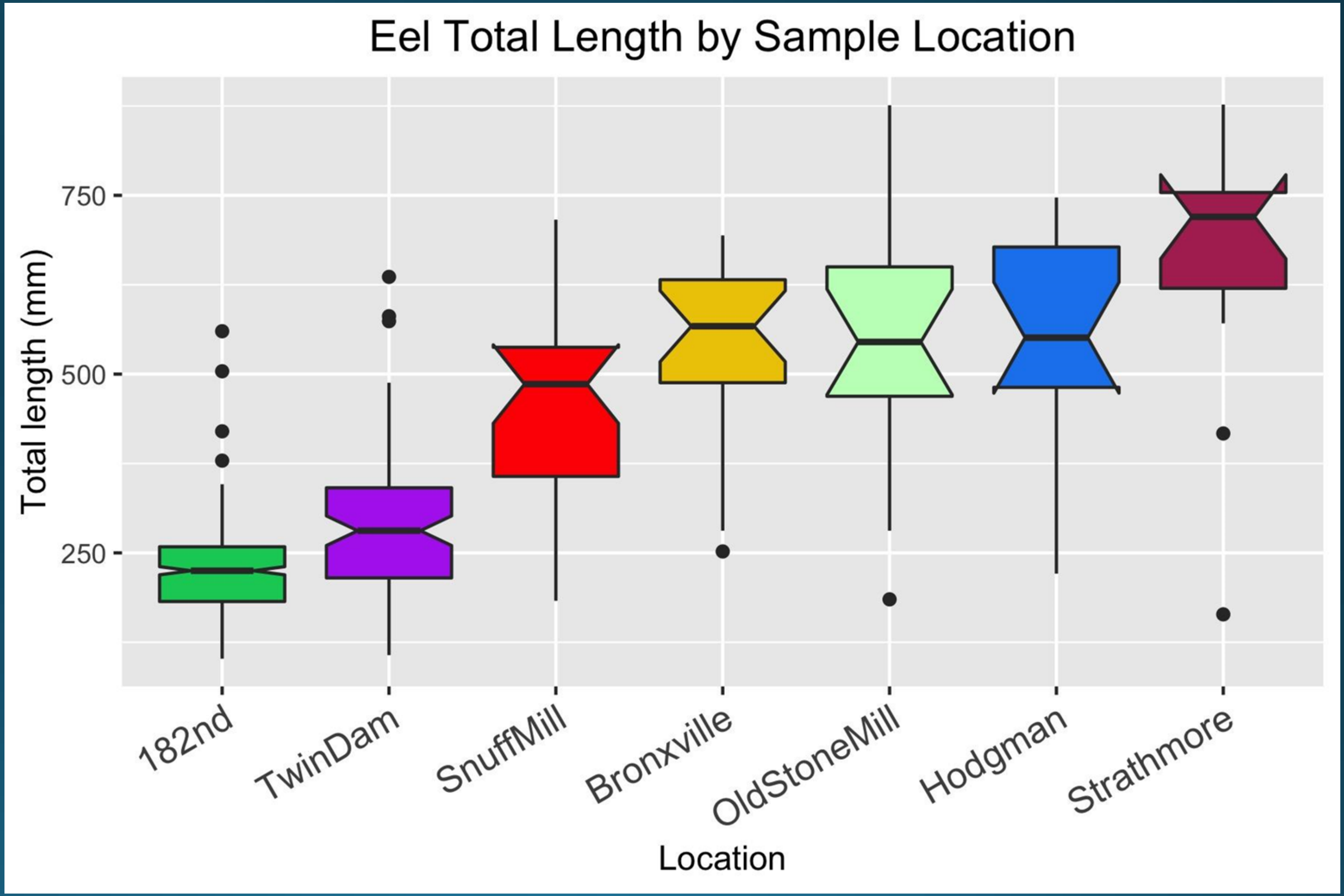
- 1023 eels captured electrofishing
- 320 tagged (length cutoff 250 mm)
- 48 recaptured -
  - High stationarity - only 1 recapture moved between river reaches

Statistical Approach for Electrofishing
(developed by Mike Bednarski, VDFG)

- 2- or 3-pass depletion
- Huggins Robust Design, by site
- Capture probability equal among occasions
- Recapture probability 0 within occasions
- Biomass = wt for mean length (Fishbase) x site abundance ÷ area
- Density = abundance ÷ area

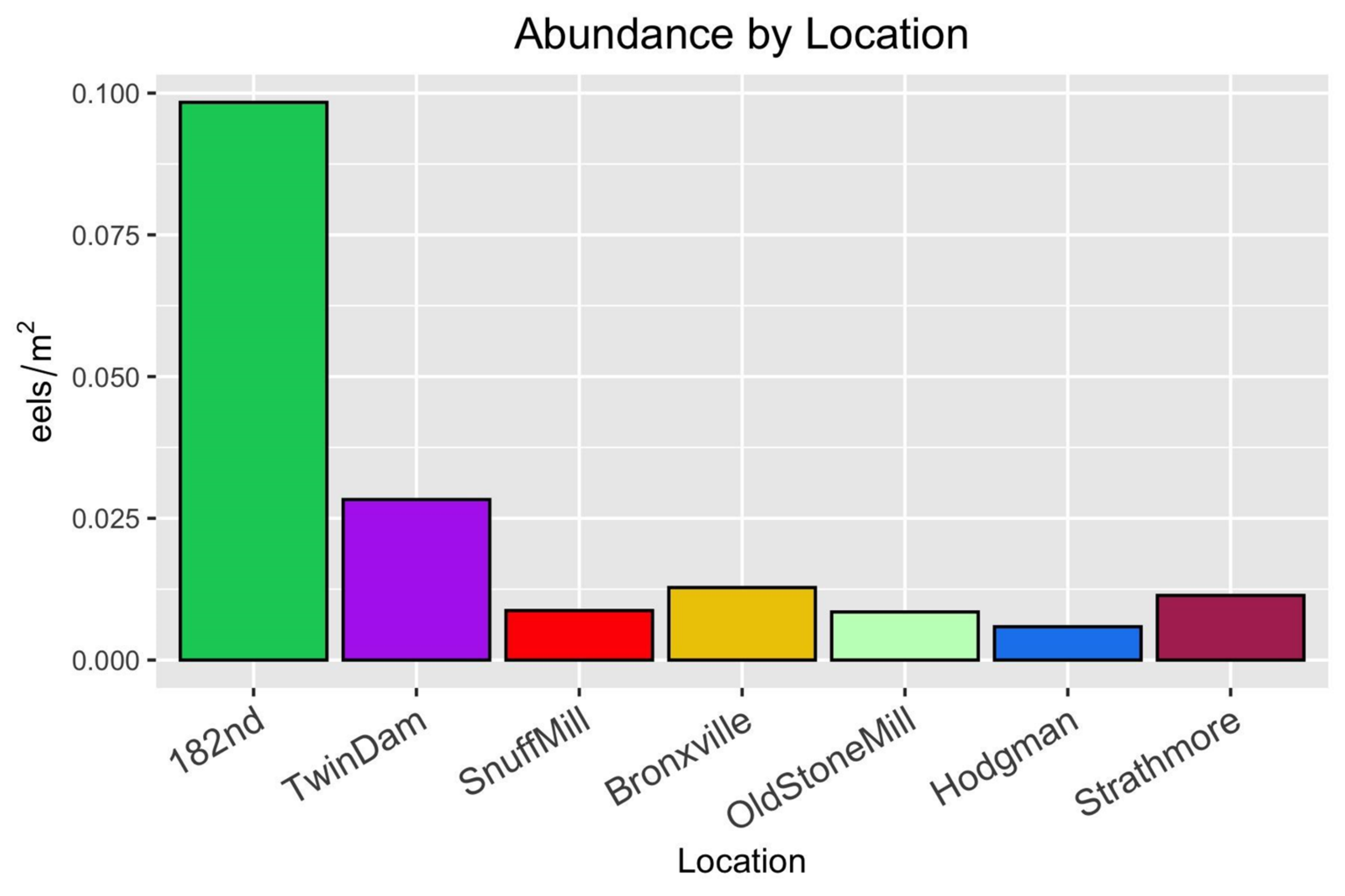
Lengths

- Eels larger upstream
- Big increase after second dam (TwinDam)



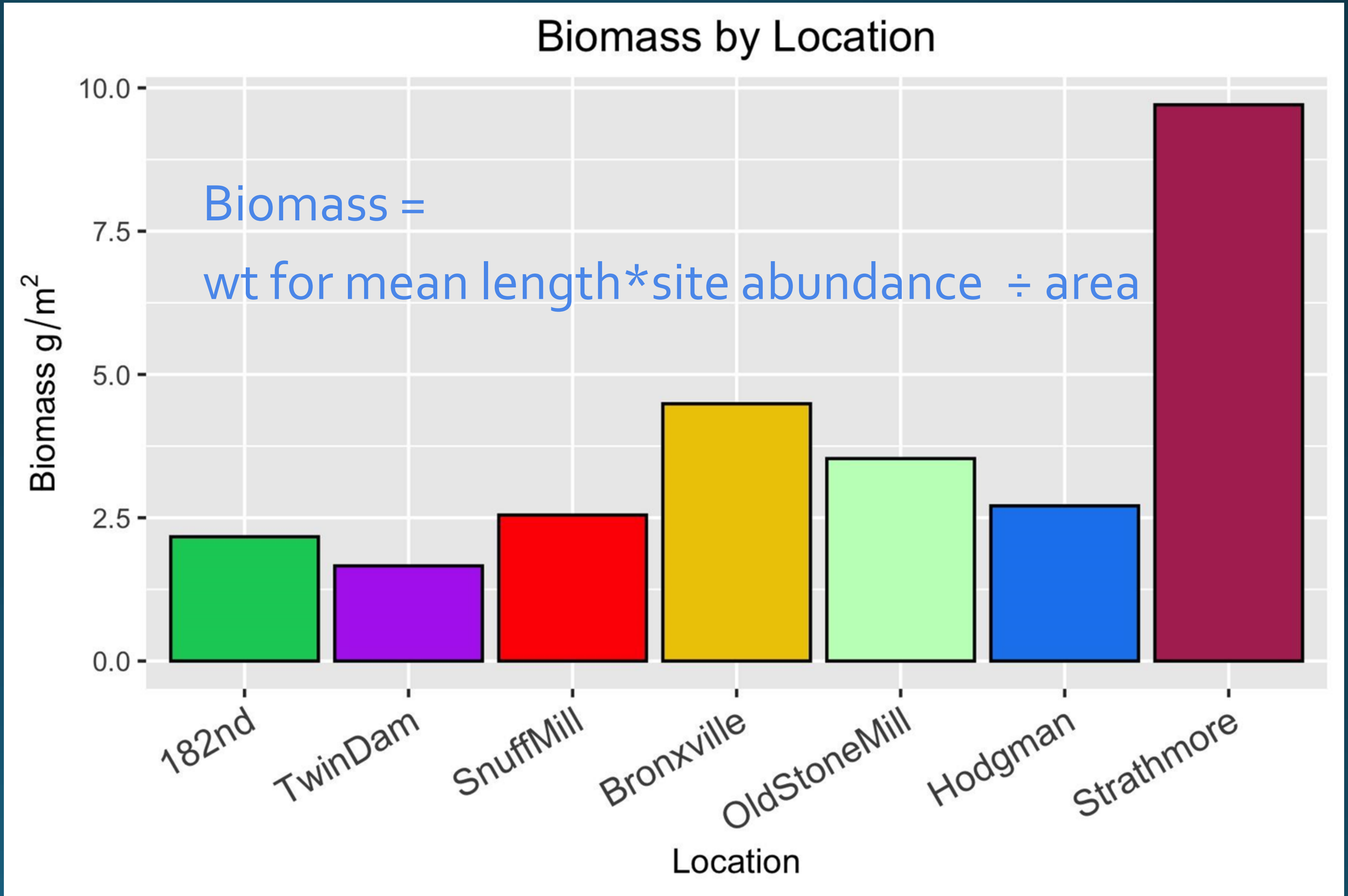
Abundance (Density)

- Abundance stabilizes after second dam



Biomass

- Biomass roughly consistent across sites



Findings that Allow for Correlation with & Interpretation of eDNA Signal

1) Eels get larger heading upstream, with a big increase past the 2nd dam

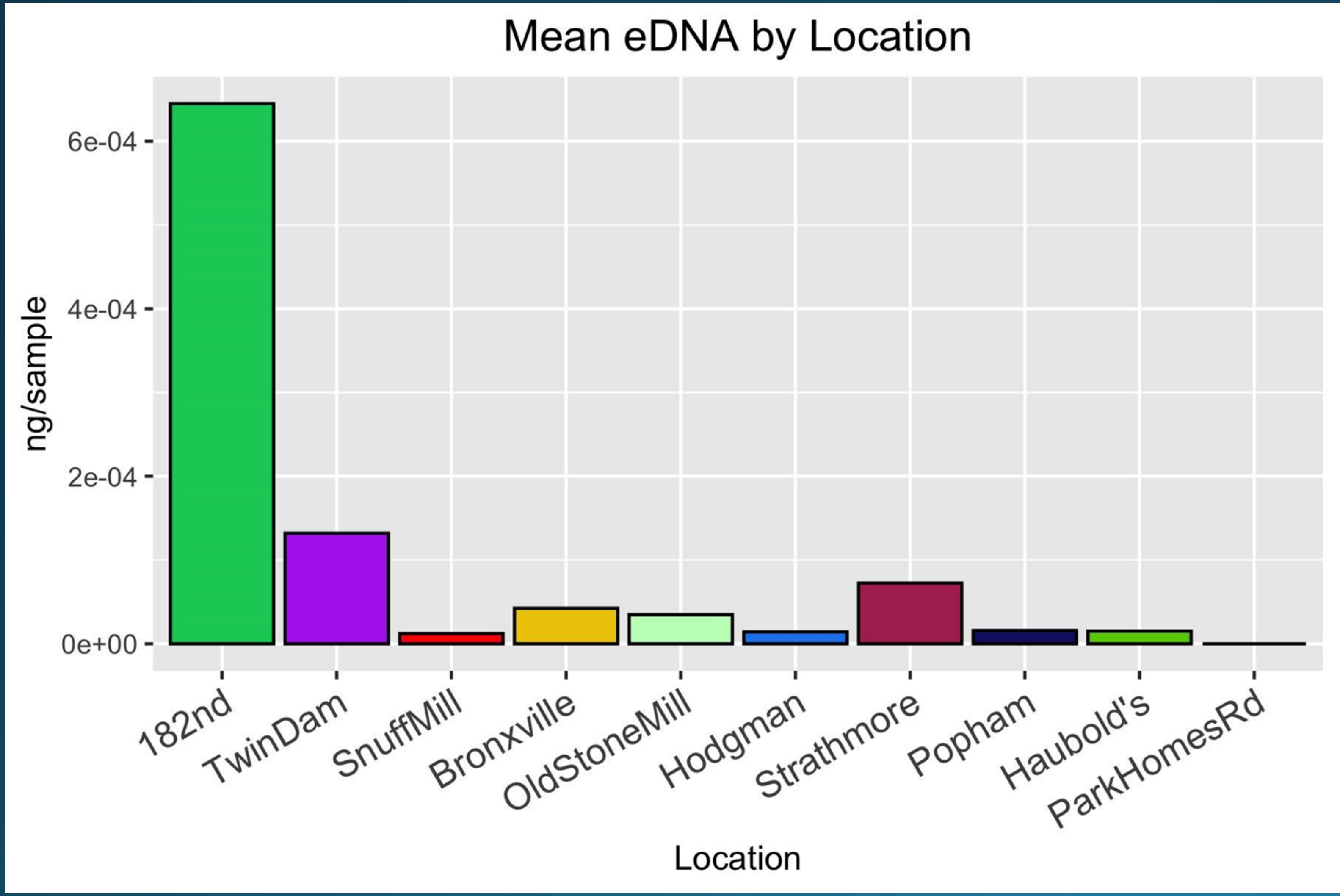
2) Eel abundance stabilizes past the 2nd dam

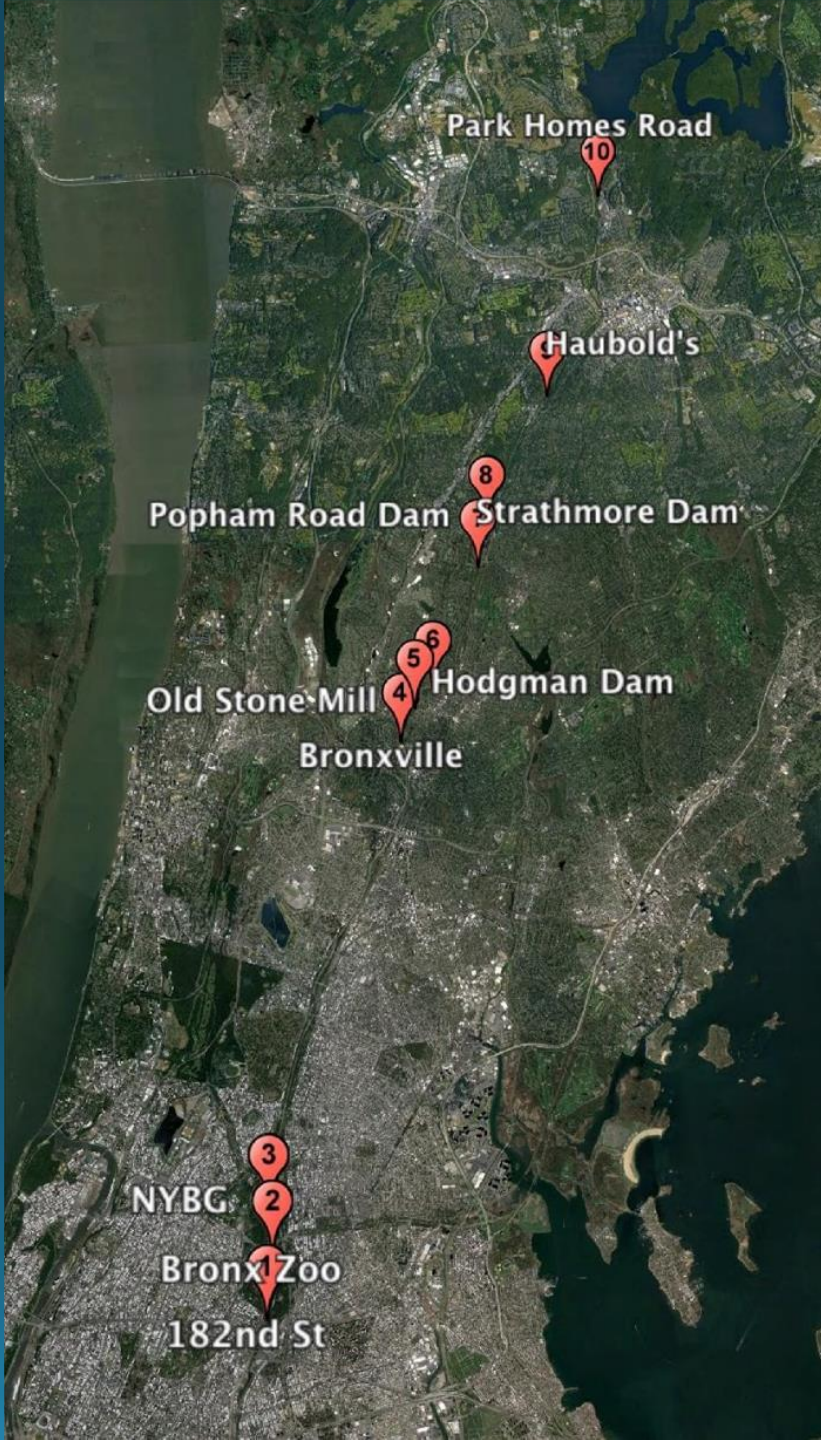
3) Biomass appears to be relatively consistent among sites

4) Dams in the Bronx River appear partially limiting

5) Tagging showed that eels appear to remain within their river segments and largely in place

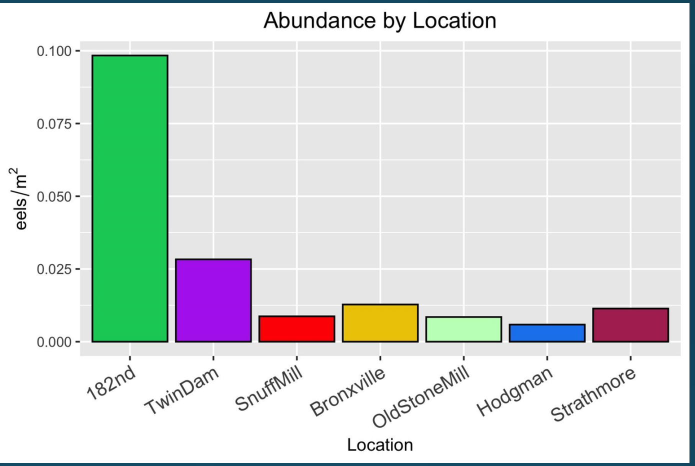
qPCR Results

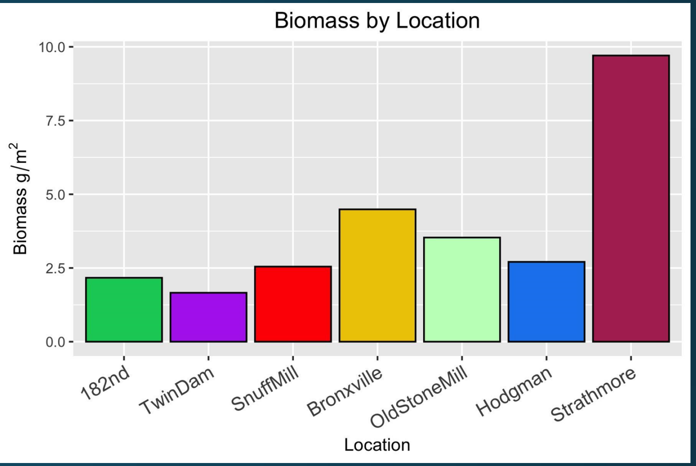


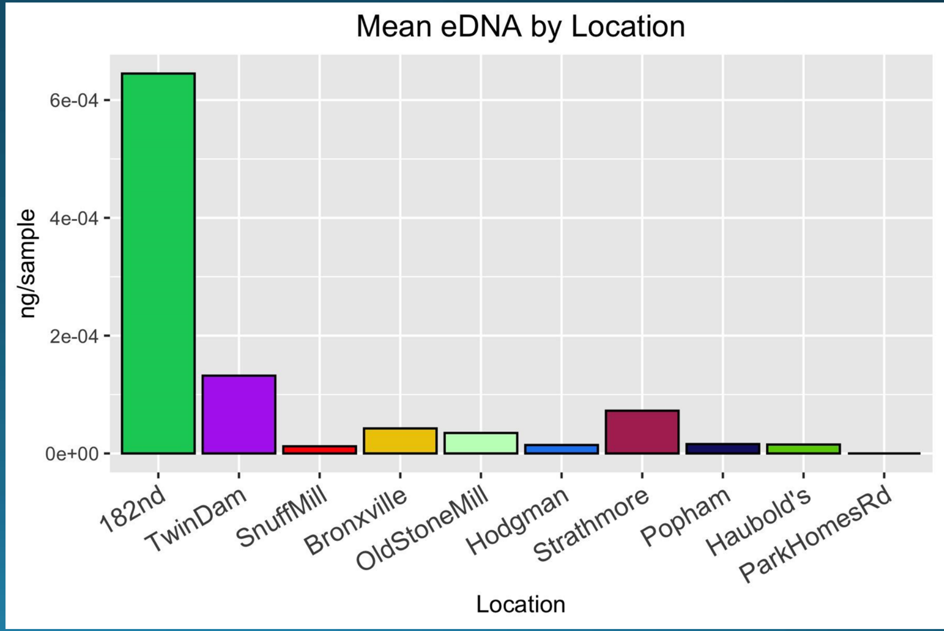


qPCR vs Electrofishing

- Abundance and biomass oppose each other
- Fewer eels upriver, but tend to be larger
- eDNA seems to align with abundance





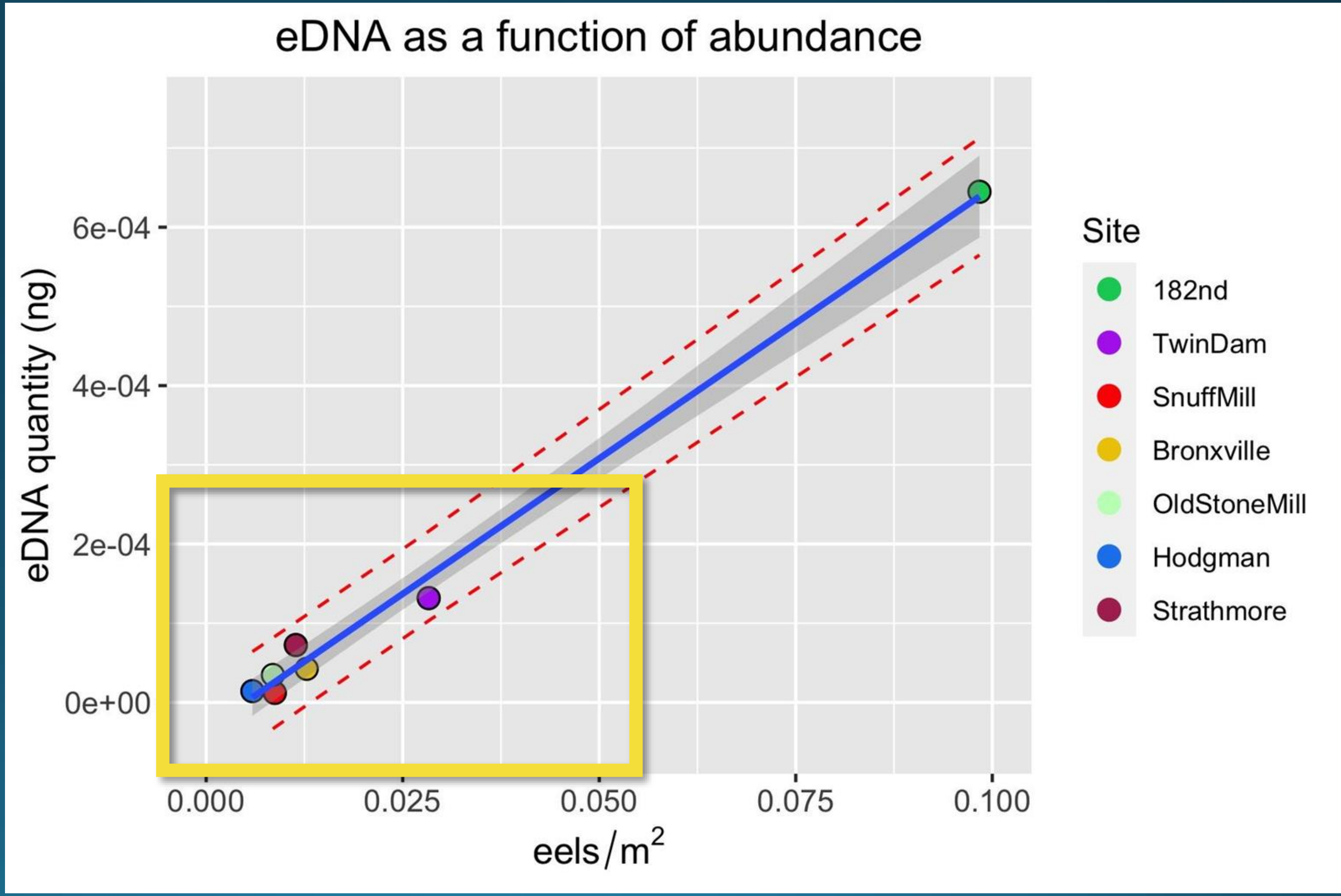


eDNA Quantity vs Eel Abundance

- R^2= 0.9919
- p= 1.28e-06

- Without 182nd St:
  - R^2=0.8477
  - p= 0.00581

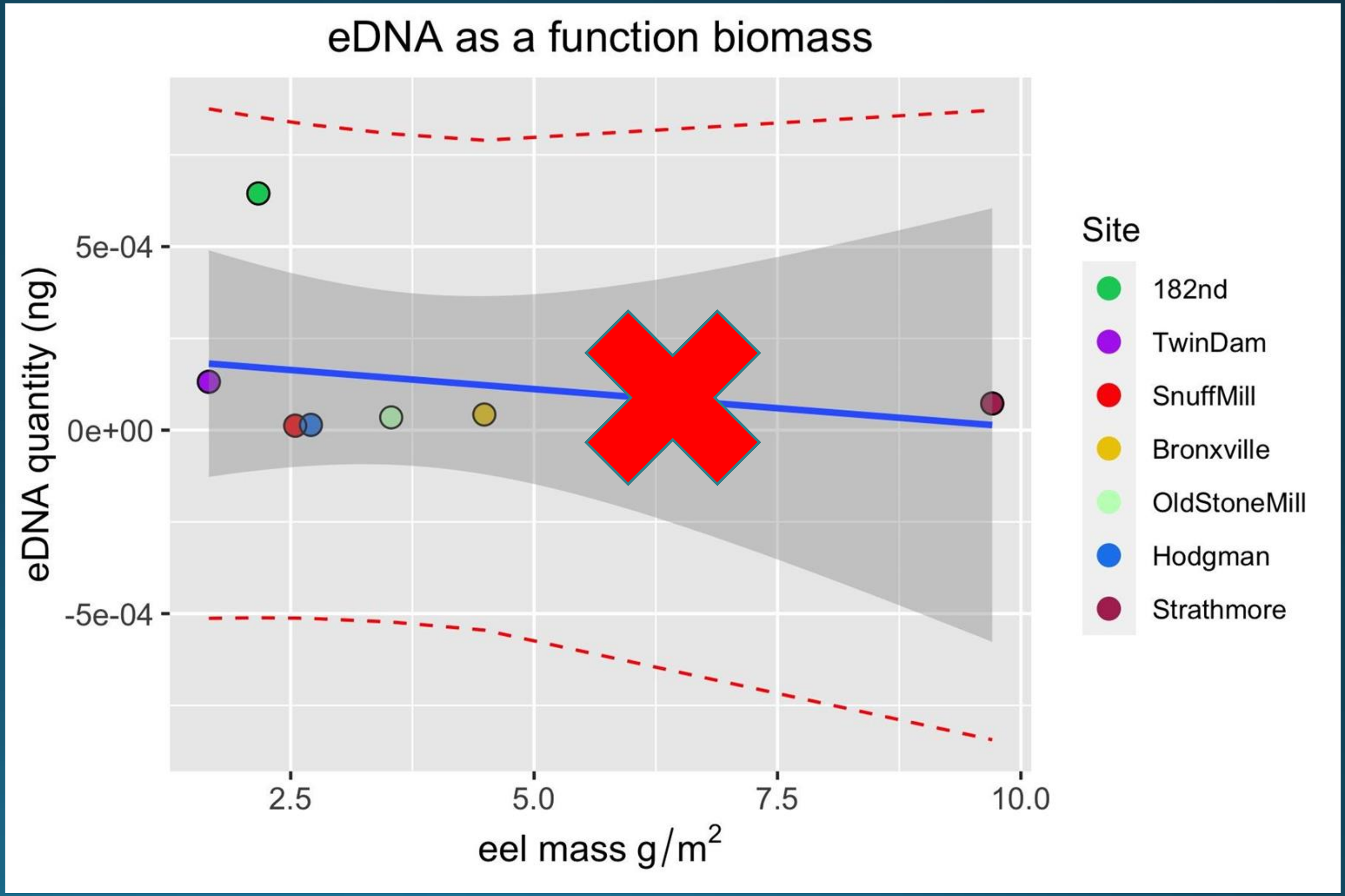
- Eel eDNA strongly associated with eel abundance



eDNA Quantity vs Eel Biomass

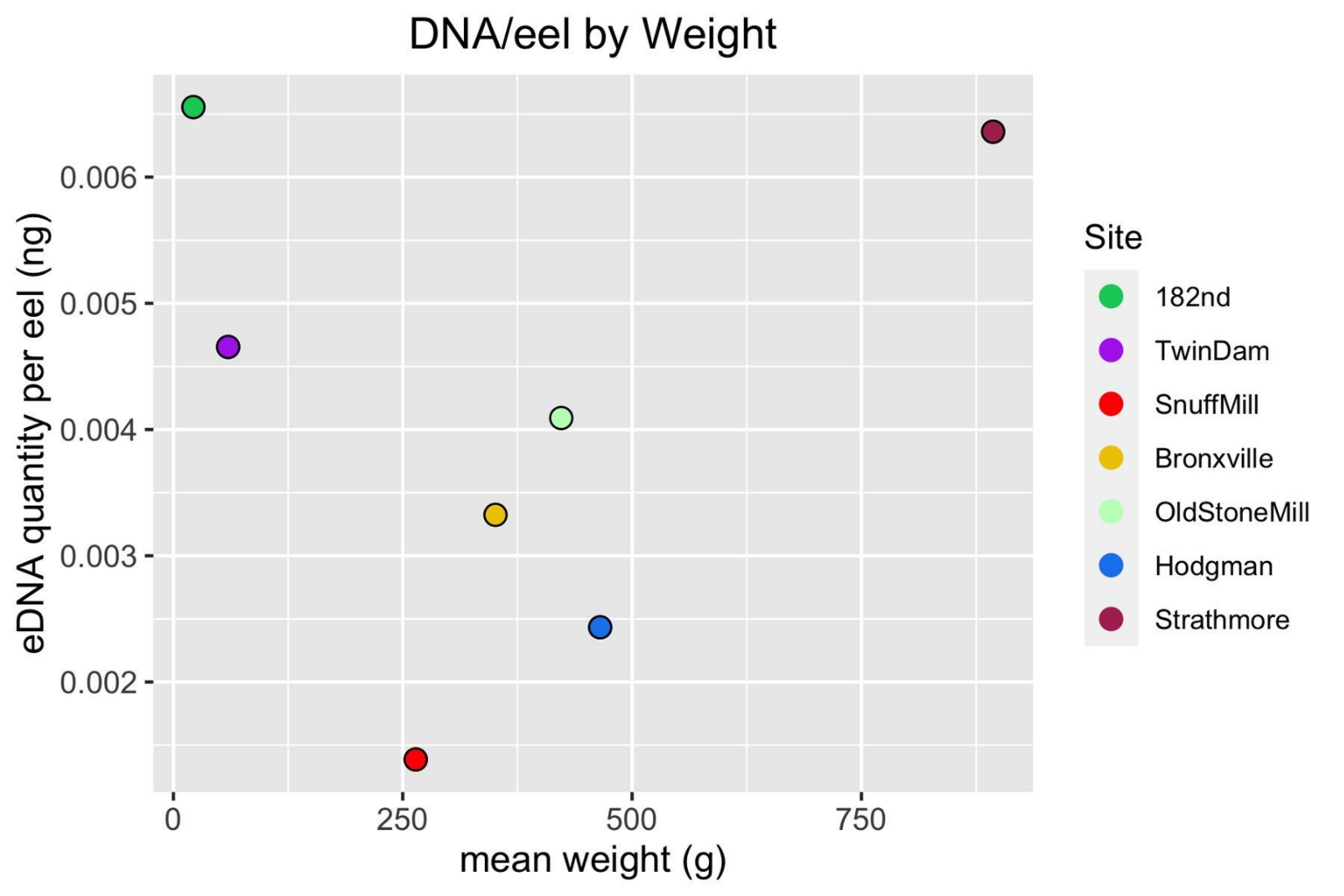
- R^2: -0.1244
- p= 0.587

- No relationship between eDNA and eel biomass



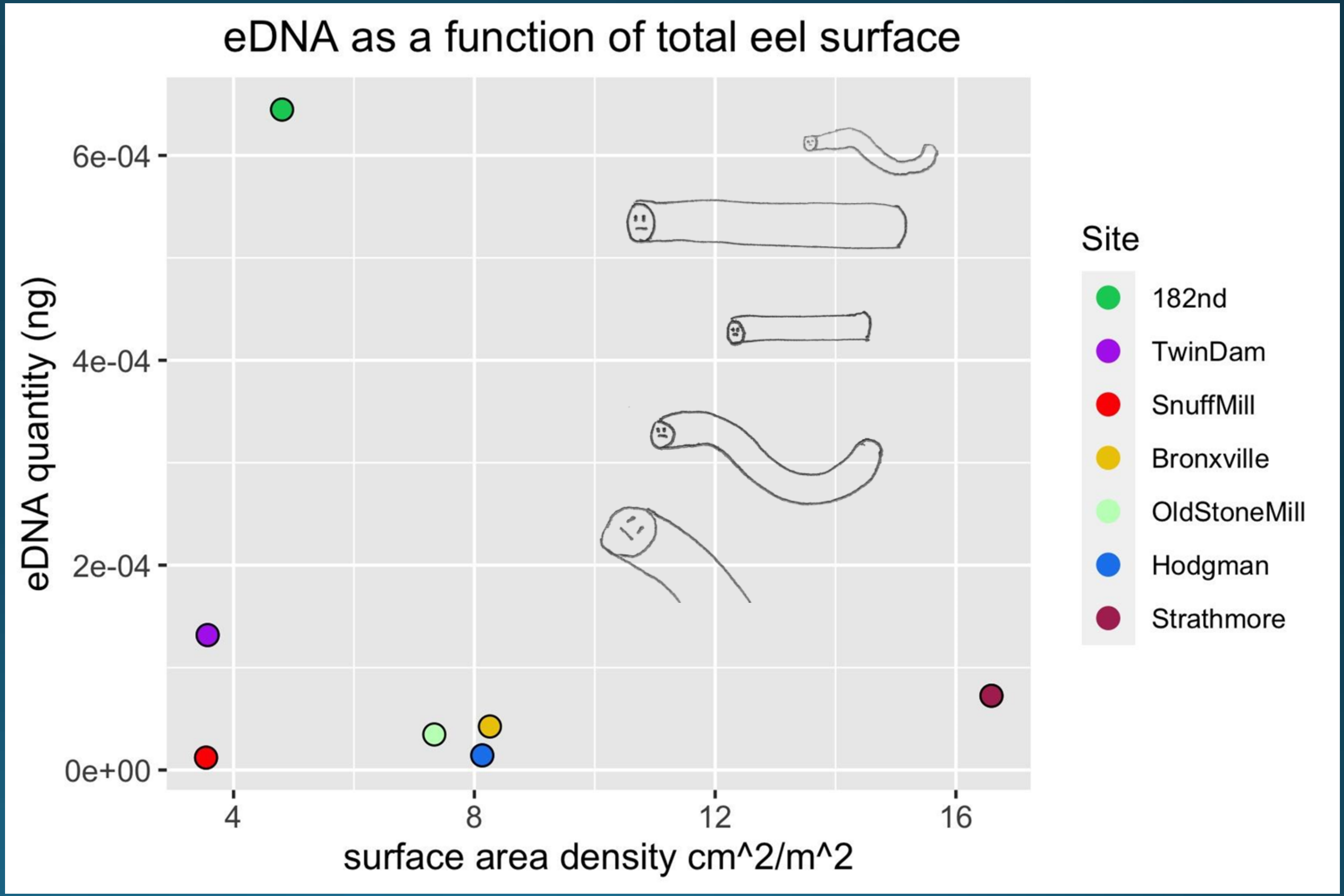
Why not biomass?

- No significant relationship between eel size and DNA signal
- Eels appear to contribute similar amounts of DNA at all sizes



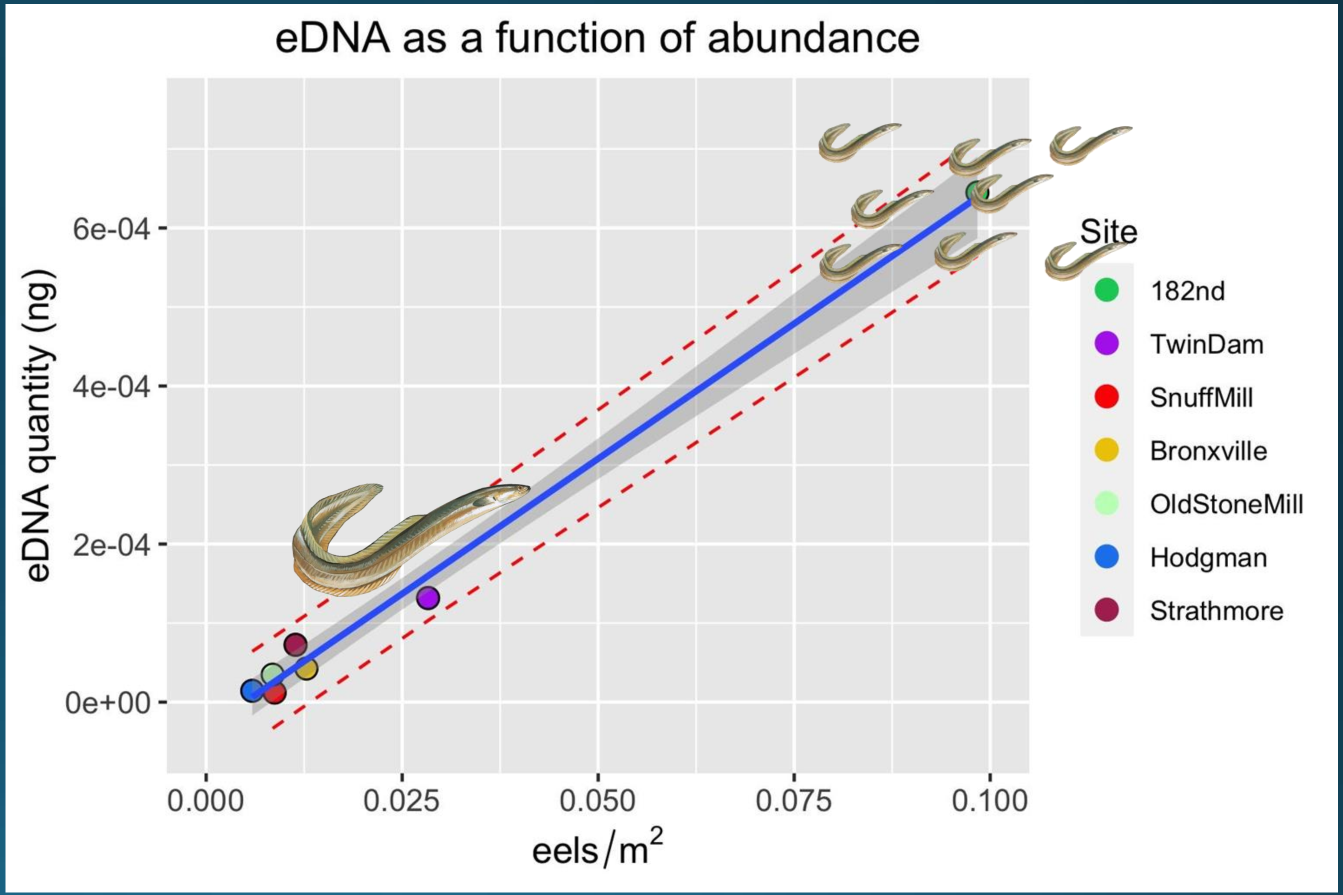
Surface area

- Skin and slime possible sources of eDNA
- Modeled eels as closed cylinders
- Total eel surface area /m² of sampling area
- No significant relationship



Bronx Eel eDNA

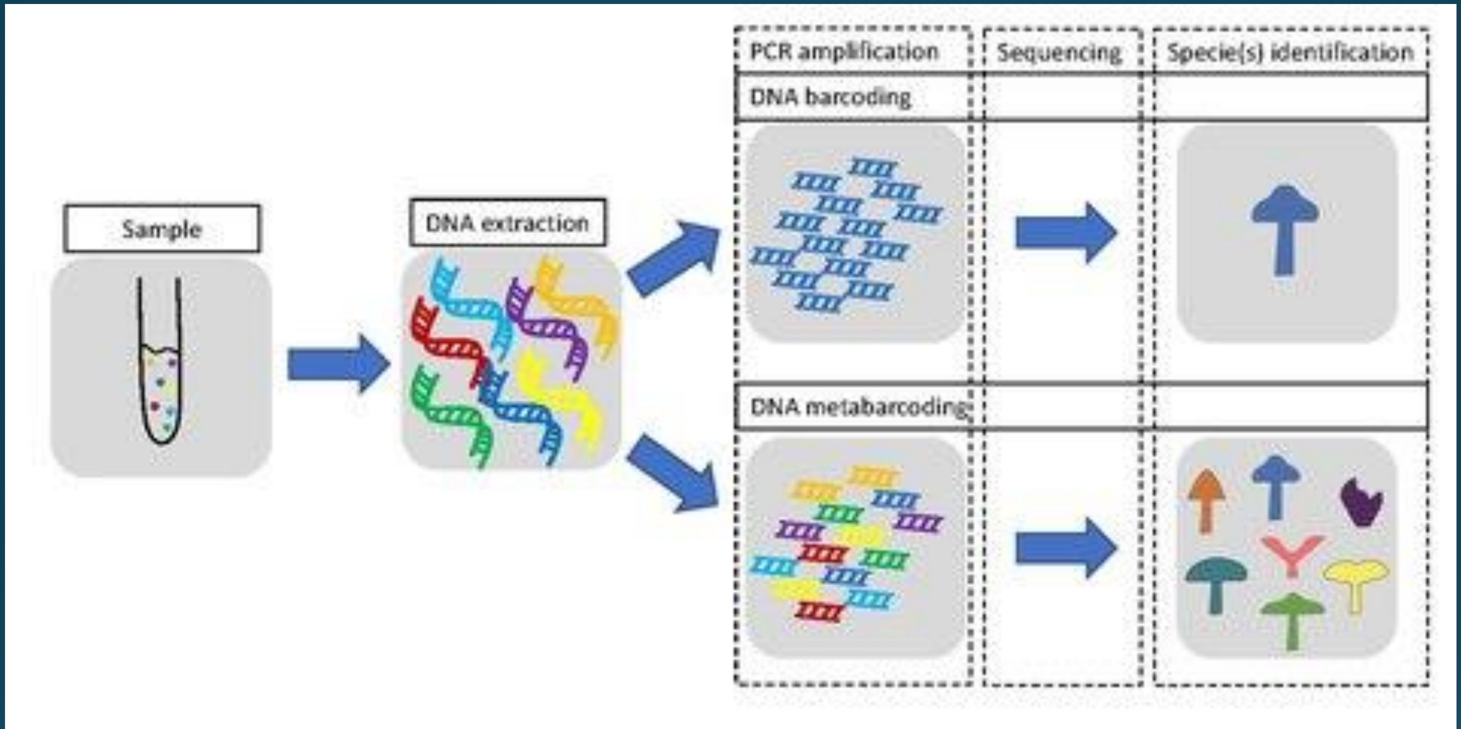
- eDNA concentration strongly associated with abundance
  - opens door for eDNA-only estimates of abundance
- Not a function of total biomass or surface area
- Mechanism of eDNA emission unknown
  - Predation, slime, skin

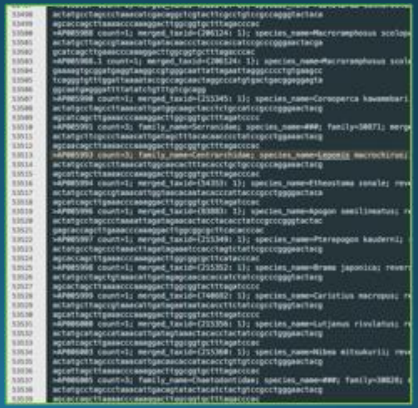


II. Fish Biodiversity – Species Richness

- 140 species – Mainstem: Federal Dam at Troy to Battery (Beebe & Savidge 1988)
- 230 species – Whole watershed south to Battery (Tom Lake 2020)
- 338 species – New York Marine Waters, New York Harbor to Battery (Briggs & Waldman 2002)

Approach - Metabarcoding





Sampling Site Types

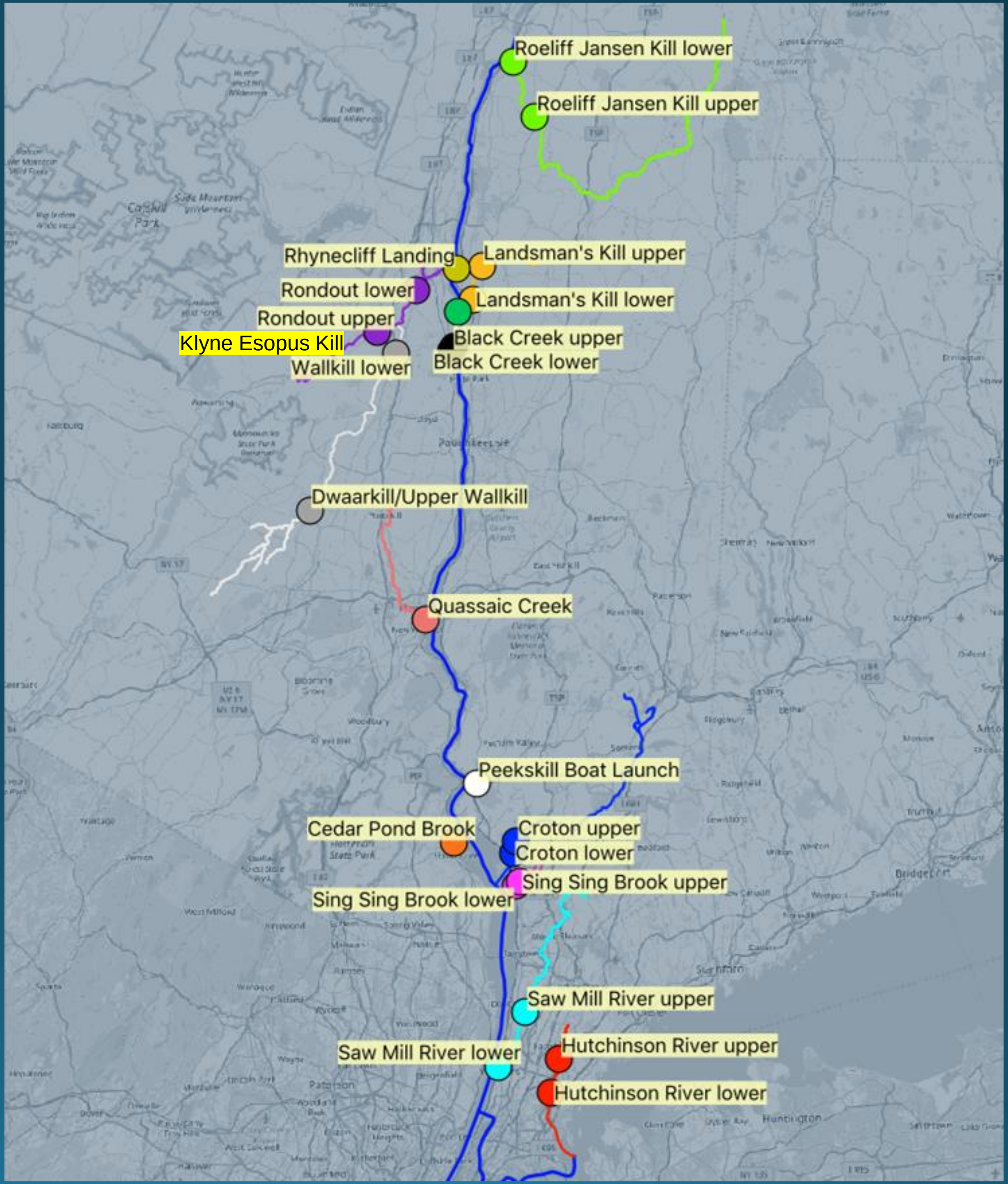
(1) At the most immediate pool above tidewater, which *might* integrate available DNA across the entire tributary's flow

(2) At a location farther upriver, nearer middle of length of river

Also, two mainstem Hudson sites

Sampling Locations

- 23 Total Sites, 21 in 13 Tributaries & 2 Mainstem Hudson
- Sampled June 14, July 11, July 18 in 2017



Error Matrix --The Minefield

Source of Detection Error	Type of Detection Error: False Positive (Type I)	Type of Detection Error: False Negative (Type II)
Method	**Problem:** Detect species when no target species eDNA is present in the sample **Sources:** (1) Incorrect detection of non-target species (i.e., insufficient assay sensitivity) or (2) DNA contamination **Solution:** Improve assay specificity and exercise care when collecting, handling, and processing samples. **Include negative controls in experimental design.**	**Problem:** Fail to detect species when target species eDNA is present in the sample **Sources:** (1) Insufficient assay sensitivity or (2) method failure during sample processing **Solution:** Improve assay specificity and exercise care when collecting, handling, and processing samples. **Include positive controls in experimental design.**
Process	**Problem:** Detect target-species eDNA when species is absent from the ecosystem **Sources:** (1) Persistence of eDNA in the environment or (2) transport of eDNA from distant sources (e.g., barge traffic, boaters, avian deposition) **Solution:** Improve knowledge of the "ecology" of eDNA in the environment	**Problem:** Fail to detect species when present in the ecosystem because viable target-species eDNA absent in sample **Sources:** (1) Failure to collect eDNA in sample or (2) eDNA degraded in sample **Solution:** Improve sample collection, handling, and processing methods.

Five Analytical Categories

(1) known species occurrences to serve as positive controls,

(2) tributaries adjacent to known occurrence-tributaries to assess possible dispersal

(3) tributaries with evidence of non-occurrence

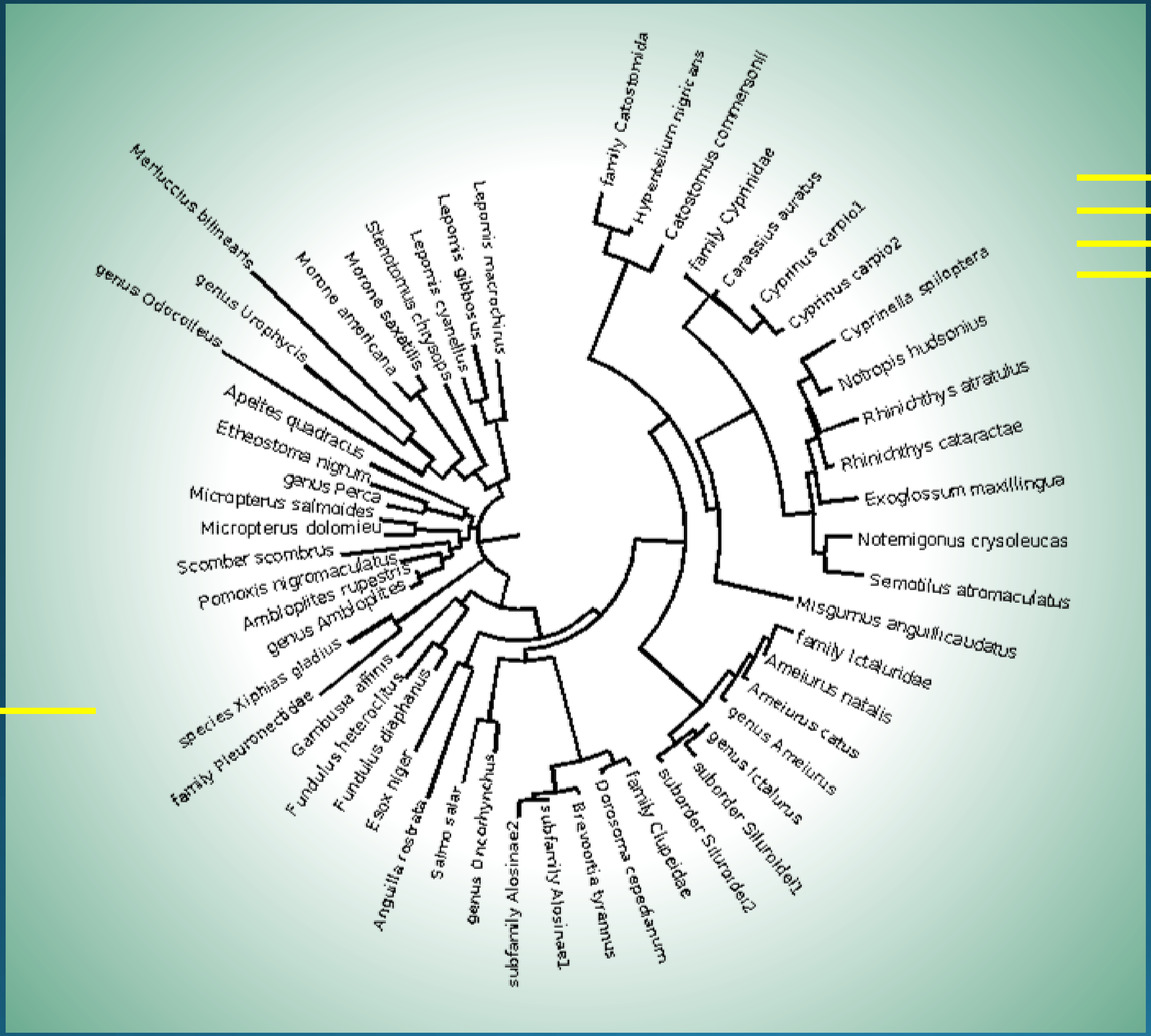
(4) tributaries with well-characterized fish communities to serve as positive controls

(5) randomly selected tributaries to assess unknown occurrences

Hudson & Tributaries – Resolved Fish Taxa

37 species: 24 native, 8 alien, 5 false positives; 11 Higher-level taxa

Species	Common Name	Status
Ambloplites rupestris	Rock bass	Native
Ameiurus catus	White bullhead	Native
Ameiurus natalis	Yellow bullhead	Native
Anguilla rostrata	American eel	Native
Apeltes quadracus	fourspine stickleback	Native
Brevoortia tyrannus	Menhaden	Native
Catostomus commersonii	White sucker	Native
Cyprinella spiloptera	Spotfin shiner	Native
Dorosoma cepedianum	Gizzard shad	Native
Esox niger	Chain pickerel	Native
Etheostoma nigrum	Johnny darter	Native
Fundulus diaphanus	Banded killifish	Native
Fundulus heteroclitus	Mummichog	Native
Gambusia affinis	Western mosquitofish	Alien
Hypentelium nigricans	Northern hogsucker	Native
Lepomis cyanellus	Green sunfish	Alien
Lepomis gibbosus	Pumpkinseed	Native
Lepomis macrochirus	Bluegill	Alien
Merluccius bilinearis	Silver hake	FalsePos
Micropterus dolomieu	Smallmouth bass	Alien
Micropterus salmoides	Largemouth bass	Alien
Misgurnus anguillicaudatus	Oriental weatherfish	Alien
Morone americana	White perch	Native
Morone saxatilis	Striped bass	Native
Notemigonus crysoleucas	Golden shiner	Native
Pomoxis nigromaculatus	black crappie	Alien



Species	Common Name	Status
Rhinichthys atratulus	eastern blacknose dace	Native
Rhinichthys cataractae	Longnose dace	Native
Salmo salar	Atlantic salmon	FalsePos
Scomber scombrus	Atlantic mackerel	FalsePos
Stenotomus chrysops	Scup	FalsePos
Xiphias gladius	Swordfish	FalsePos
Cyprinus carpio	Common carp	Alien
Carassius auratus	Goldfish	Alien
Semotilus atromaculatus	Creek chub	Native
Notropis hudsonius	Spottail shiner	Native
Exoglossum maxillingua	Cutlips minnow	Native
Genus		
Ambloplites	Sunfish	
Ameiurus	Bullhead	
Ictalurus	Catfish	
Perca	Perch	
Subfamily		
Alosinae	Shads	
Family		
Catostomidae	Suckers	
Clupeidae	Herrings	
Cyprinidae	Carp	
Ictaluridae	Catfish	
Pleuronectidae	Flounders	
Suborder		
Siluroidei	Catfishes	

Roeliff-Jansen Kill

Category (1): positive control for sea lamprey ammoceotes found in 2013; Evans and Limburg (2015)

Result: 2 sites - 17 species identified

No sea lamprey signal -- primer problem

Sea Lamprey

Klyne Esopus Kill

- Category (1): positive control for Oriental weatherfish found in 2009 by Schmidt and Schmidt (2014)
- 1 site: 10 species identified
- *No signal from Oriental weatherfish*
- *Positive signal from green sunfish*

Green Sunfish

Landsman Kill

- Category (2): possible colonization by Oriental weatherfish
- 1 site -- 14 species detected
- *Positive signal for Oriental weatherfish* (proximal to Klyne Esopus Kill)
- *Positive signal for Pleuronectidae*

Oriental Weatherfish

Rondout Creek

- (Category 1): positive control for sea lamprey ammoceotes found in 2013; Evans and Limburg (2015)
- 2 sites -- 7 species detected
- *No sea lamprey signal; positive signal for green sunfish*

Wallkill

- Category (1): positive control for Oriental weatherfish found in 2009 by Schmidt and Schmidt (2014); also provides notes on fish community, including presence of exotic green sunfish and distributionally uncommon eastern mudminnow. Wallkill also be assessed for ongoing presence of northern snakehead
- 2 sites -- 12 species detected
- *No signal from weatherfish, mudminnow or snakehead; positive signal for green sunfish*

Eastern mudminnow

Northern snakehead

Black Creek

- Category (3): negative control for sea lamprey ammoceotes not found in 2013; Evans and Limburg (2015)
- 2 sites -- 10 species detected
- *No sea lamprey signal*
- *No Alosinae signal (despite run sizes of about 400,000 alewives)*
- *Positive on Pleuronectidae*

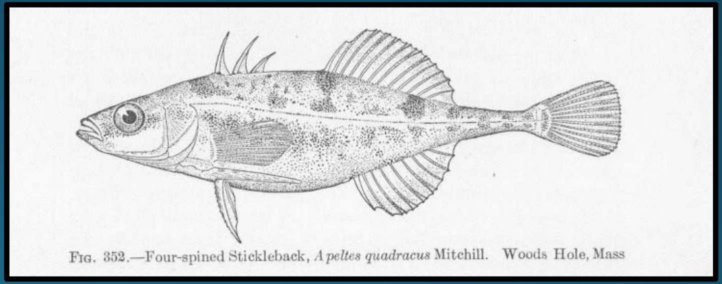
Quassaic Creek

- Category (5): randomly selected tributaries to assess unknown occurrences
- 1 site -- 12 species detected
- *No surprises*

Cedar Pond Brook

- Category (1): positive control for sea lamprey ammoceotes found in 2013; Evans and Limburg (2015)
- 1 site -- 18 species detected
- *No sea lamprey signal; positive signal from four-spined stickleback and green sunfish*

Four-spined
Stickleback



Croton River

- Category (3): negative control for sea lamprey ammocoetes not found in 2013; Evans and Limburg (2015)
- 2 sites -- 18 species identified
- *No sea lamprey signal detected*
- *Strong Alosinae signal*

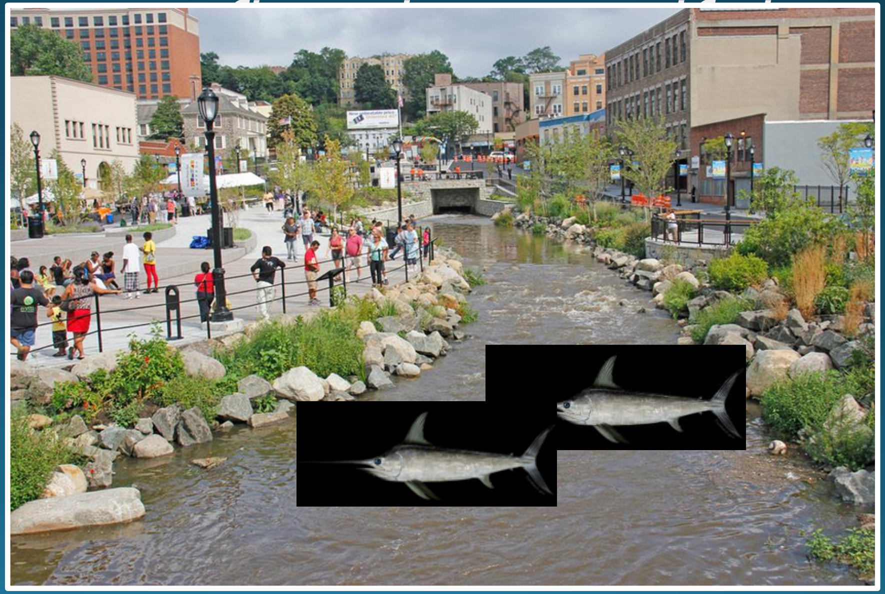
Alewife

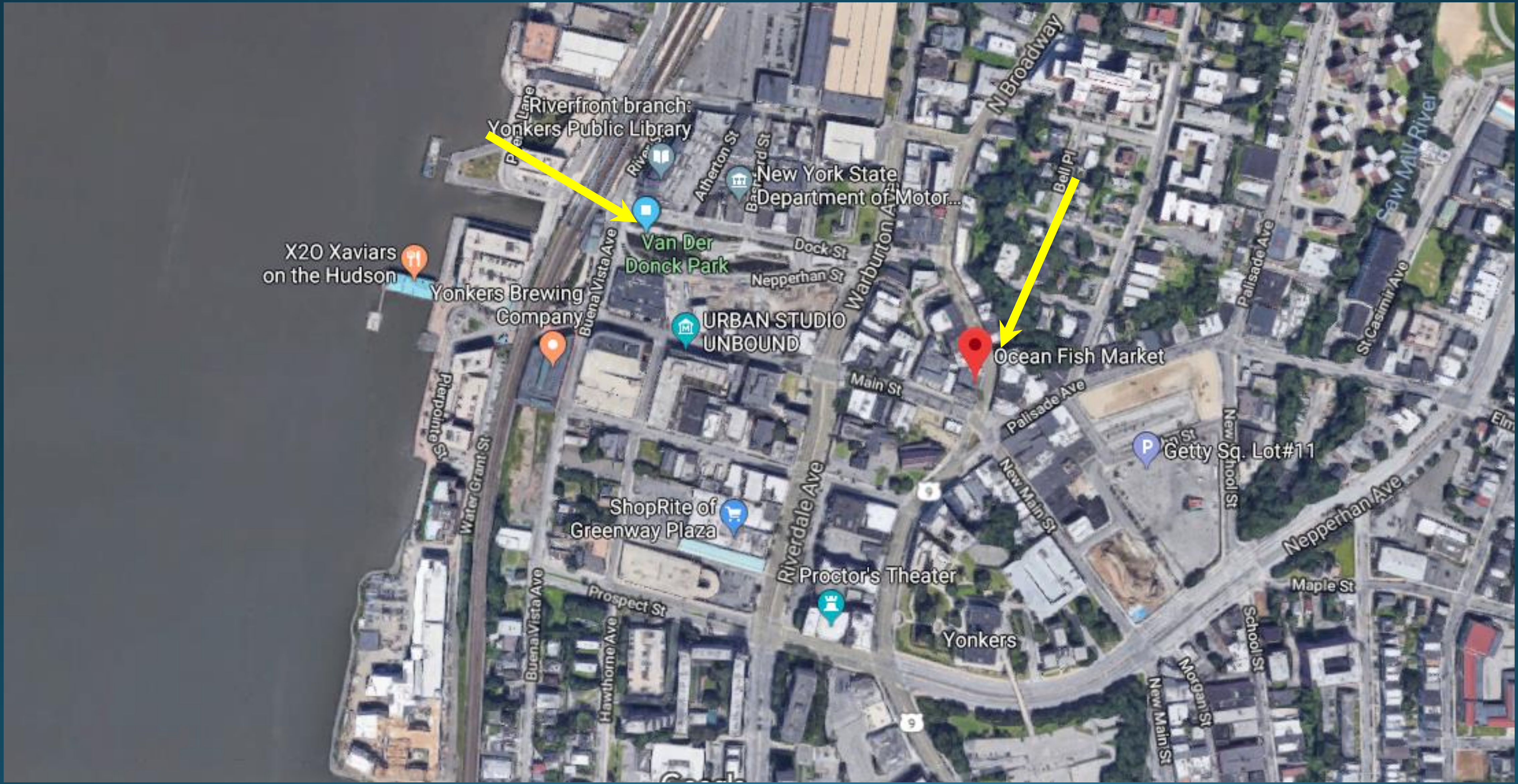
Sing Sing Brook

- Category (4): positive control for fish community surveyed by electrofishing by Waldman in (2016)
- 2 sites -- 12 species identified
- *No surprises*

Saw Mill River

- Category (4): positive control for fish community described by Rachlin and Warkentine (2012)
- 2 sites -- 16 (+6) species detected
- *Downstream site positive for green sunfish, plus Atlantic salmon, silver hake, Atlantic mackerel,*



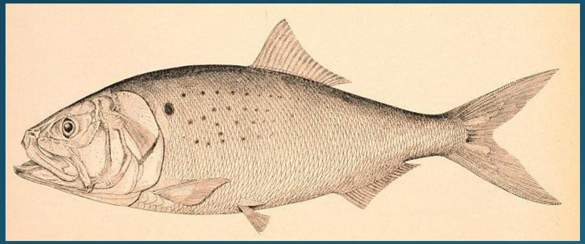


Riverfront branch: Yonkers Public Library
New York State Department of Motor...
Van Der Donck Park
X2O Xaviars on the Hudson
Yonkers Brewing Company
URBAN STUDIO UNBOUND
Ocean Fish Market
Getty Sq. Lot#11
ShopRite of Greenway Plaza
Proctor's Theater
Yonkers
N Broadway
Bell Pl
Dock St
Nepperhan St
Warburton Ave
Main St
Palisade Ave
Buena Vista Ave
Atherton St
Pierpointe St
Water Grant St
Riverdale Ave
New Main St
New School St
Nepperhan Ave
Maple St
School St
Morgan St
Prospect St
Hawthorne Ave
St Casimir Ave
Saw Mill River

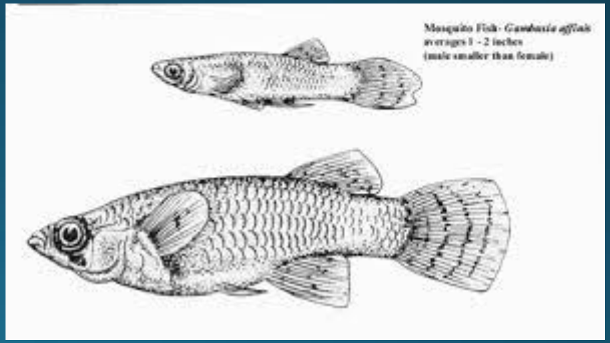
Hutchinson River

- Category (4): tributaries with well characterized fish communities to serve as positive controls
- 2 sites -- 16 (+2) species detected
- *Positive but weak signal for menhaden, plus western mosquitofish, porgy & Atlantic salmon*

Menhaden



Western Mosquitofish



Mainstem Hudson

Rhinecliff (oligohaline)

13 species detected

Strong Alosinae signal

Peekskill (mesohaline)

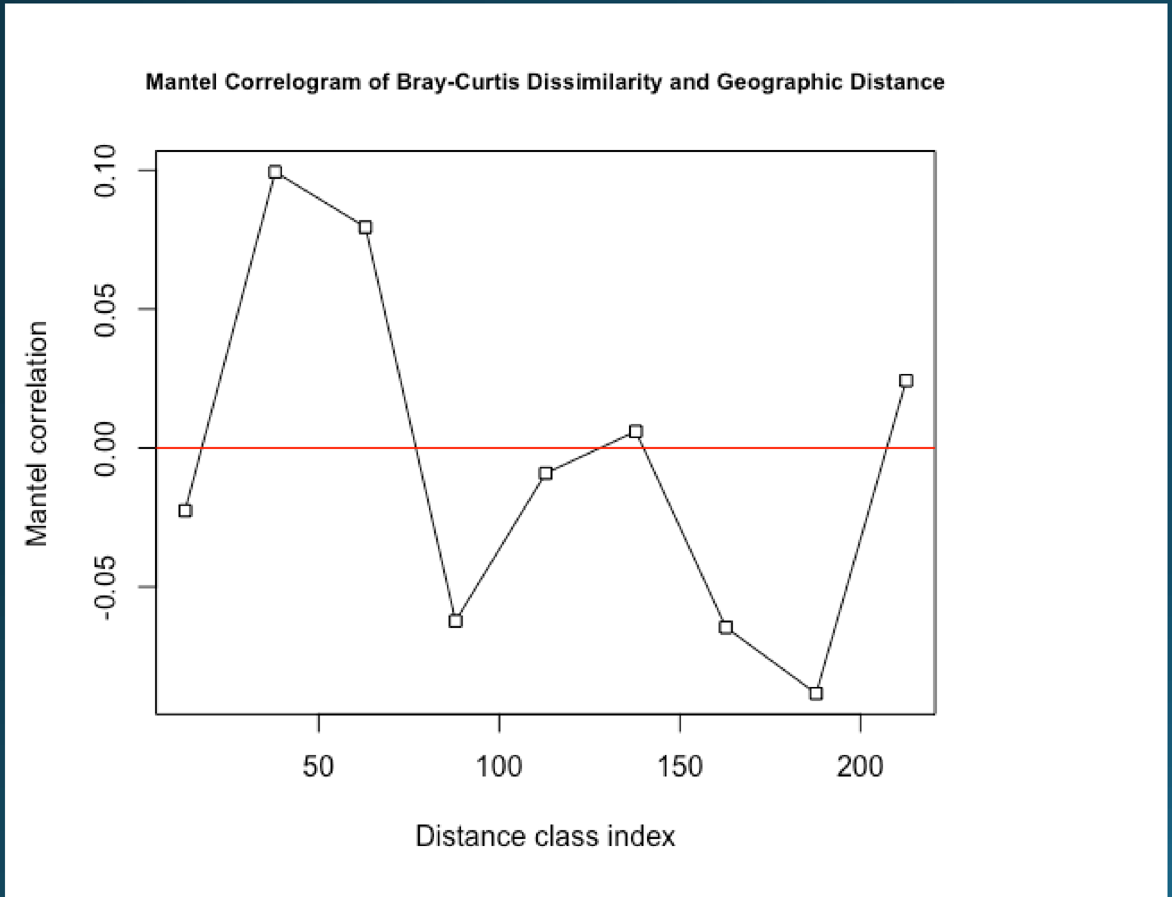
8 species detected

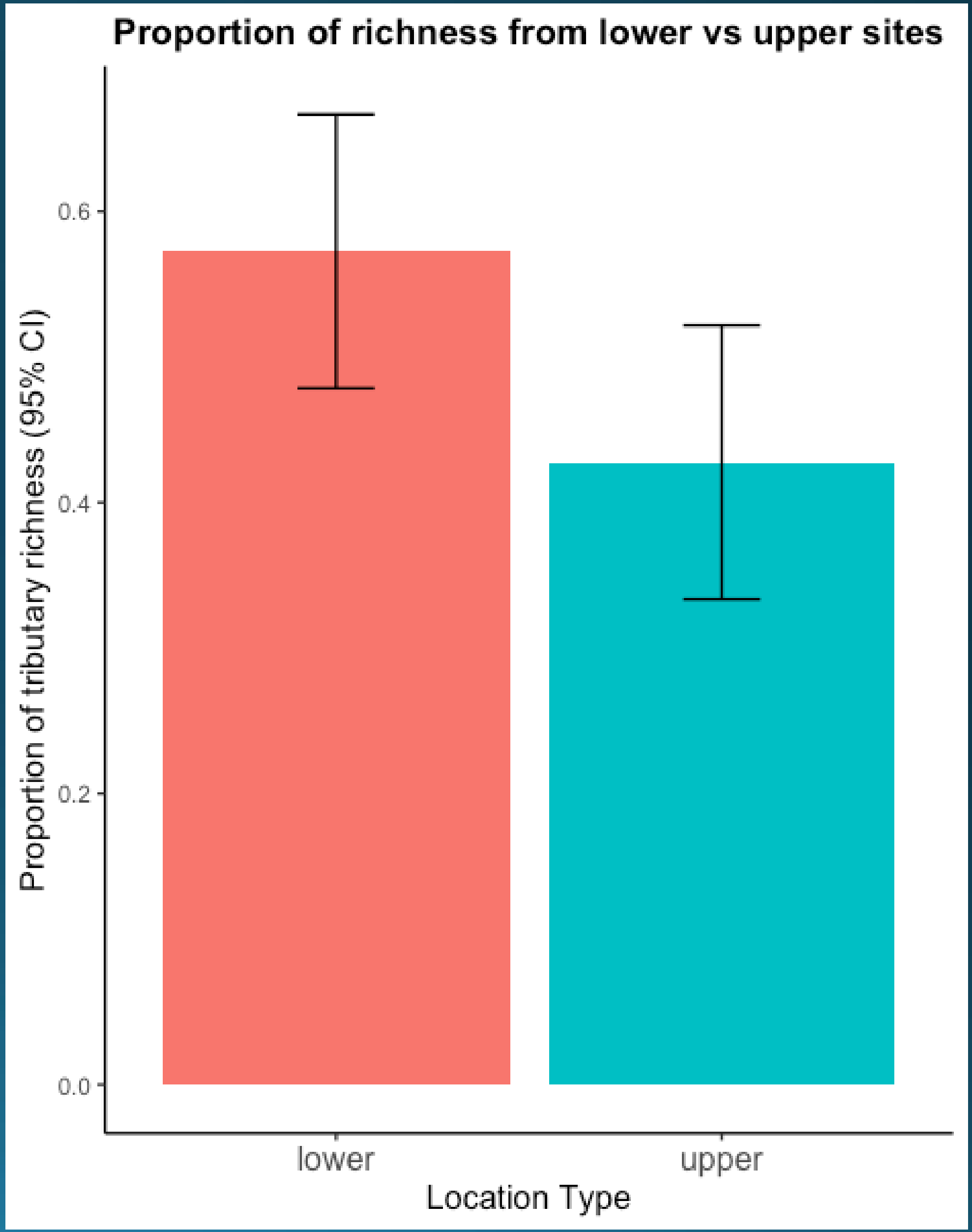
Weak Alosinae signal

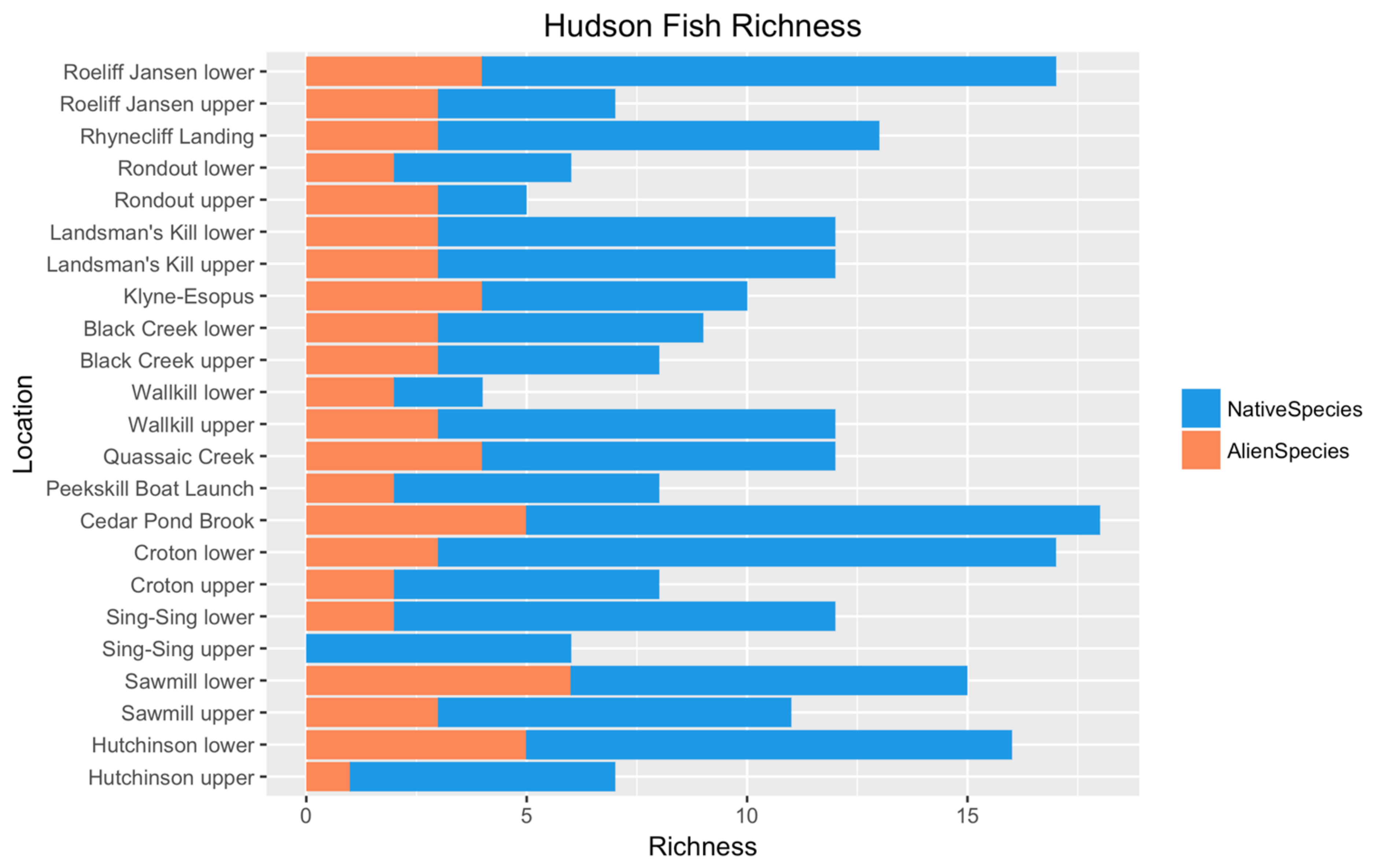
Total found in both: 16

Occurrences by Number of Sites

- American eel 23/23
- Pumpkinseed sunfish 23/23
- Common carp 20/23
- Mummichog 20/23
- Johnny darter 17/23
- Oriental weatherfish 1/23
- Western mosquitofish 1/23
- Northern hogsucker 1/23
- Menhaden 2/23


Mantel Correlogram of Bray-Curtis Dissimilarity and Geographic Distance
Mantel correlation
0.10
0.05
0.00
-0.05
50
100
150
200
Distance class index


Proportion of richness from lower vs upper sites
Proportion of tributary richness (95% CI)
0.6
0.4
0.2
0.0
lower
upper
Location Type



Hudson Fish Richness
Location
Roeliff Jansen lower
Roeliff Jansen upper
Rhynecliff Landing
Rondout lower
Rondout upper
Landsman's Kill lower
Landsman's Kill upper
Klyne-Esopus
Black Creek lower
Black Creek upper
Wallkill lower
Wallkill upper
Quassaic Creek
Peekskill Boat Launch
Cedar Pond Brook
Croton lower
Croton upper
Sing-Sing lower
Sing-Sing upper
Sawmill lower
Sawmill upper
Hutchinson lower
Hutchinson upper
0
5
10
15
Richness
NativeSpecies
AlienSpecies

Some Notes

- Species detected were mostly common ones seen with other gear
- eDNA provides identifications at higher taxonomic levels, e.g., genus. Some may be same as species resolved, some may not be.
- In total, of 32 "real" species detections, 24 Native vs. 8 non-native
- Green sunfish becoming more prominent in Hudson tribs

  (8/23 sites, 6 watersheds)
- Weatherfish do not appear to have spread widely yet
- It may have been too late in season to detect alewives in lower trib sites

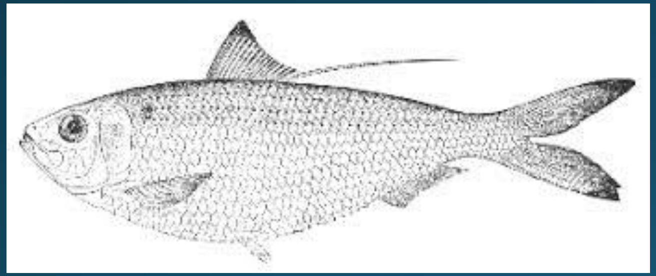
Some Notes

- Mainstem species richness detected was low; 13 in Rhinecliff & 8 in Peekskill
- Inshore vs. offshore signal?
- In common at two mainstem Hudson sites:

  --white sucker, johnny darter, mummichog, pumpkinseed, carp
- Despite mid-June sampling, no striped bass detected in either mainstem site! (But some elsewhere)
- Cross sectional complexity? Should we consider that each individual has a DNA "plume" of dispersion and decay?

What sort of species richness results should we expect from eDNA in a big river?

Richness may be misleading; evenness may be more important



Thread Herring

Gray Triggerfish

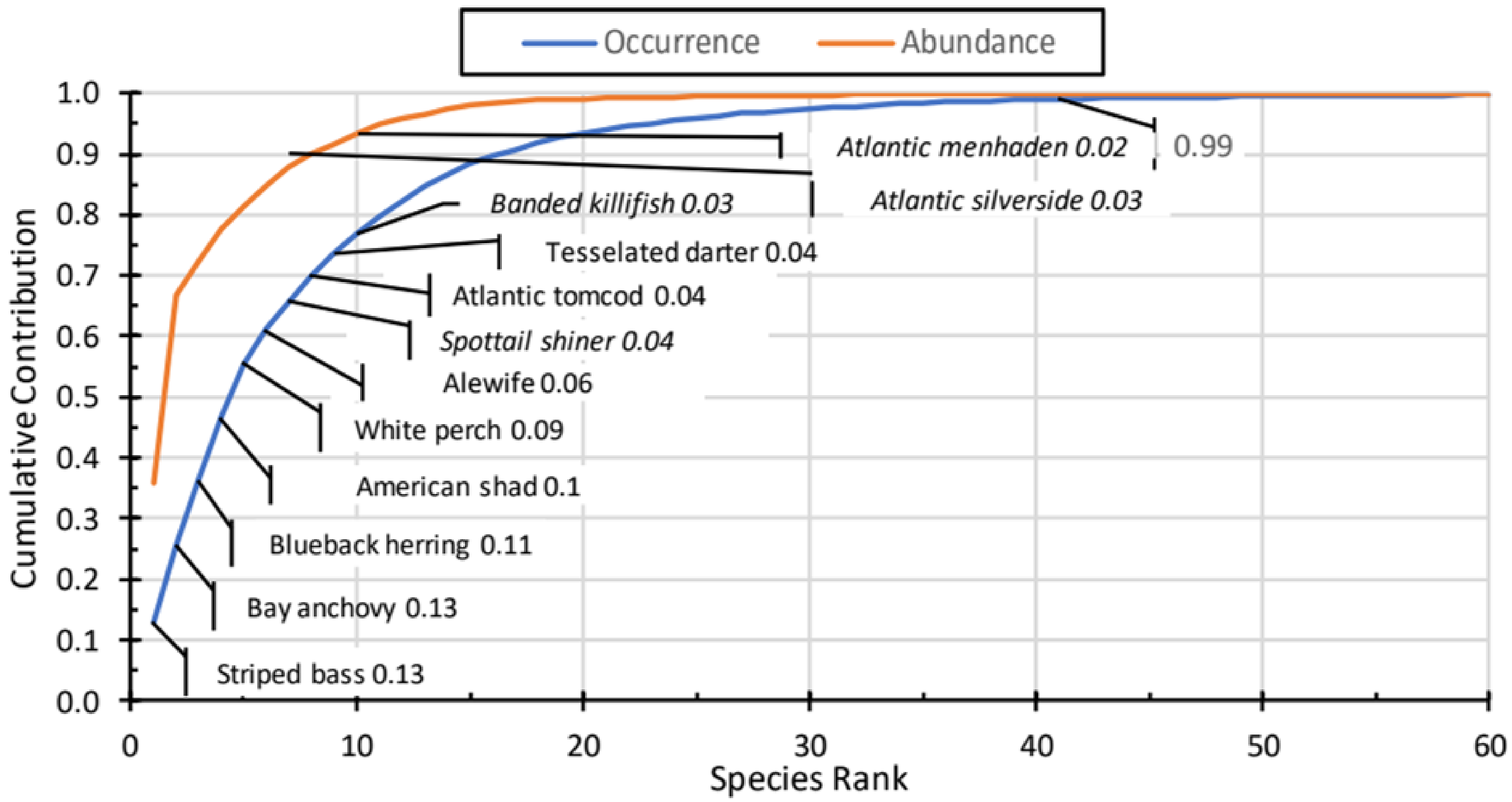


Figure 3-4 Cumulative occurrence and abundance curves for fish species collected as juveniles in BSS and FSS sampling, 1974-2017, with the 10 most frequent species. Eight of these species were also among the 10 most abundant. Species in italic font were only in the top 10 for curve indicated.

Example – Category 4

Fish species identified in Bronx River sites using traditional sampling of 9 sites over two years (Rachlin et al. 2007) versus eDNA sampling of 6 sites in 2016

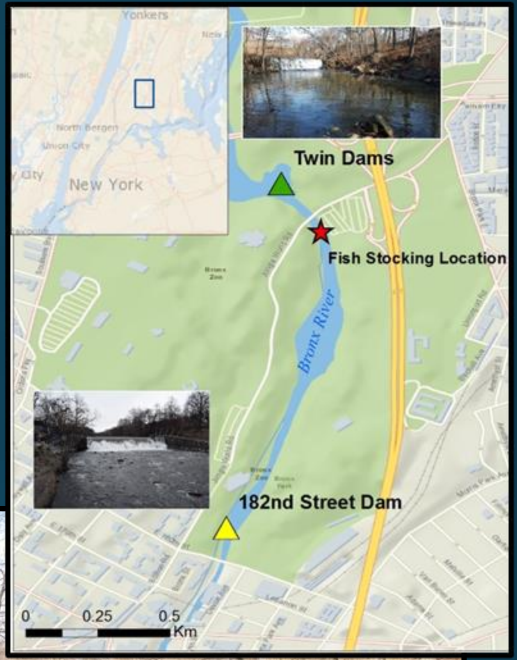
Tributaries are far more easily characterized

?

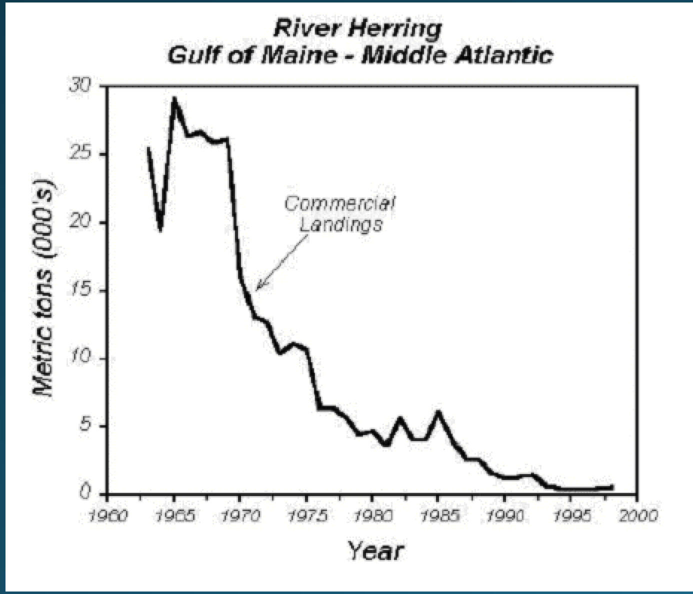
Species	Common name	2007 survey	eDNA
Ameirus natalis	Yellow bullhead		x
Ameirus nebulosus	Brown bullhead	x	x
Anguilla rostrata	American eel	x	x
Apeltes quadracus	Fourspine stickleback	x	x
Carassius auratus	goldfish	x	x
Catastomus commersonii	white sucker	x	x
Cyprinus carpio	common carp	x	x
Esox sp.	pickerel	x	x
Etheostoma sp.	darter	x	x
Fundulus diaphanus	banded killifish	x	x
Fundulus heteroclitus	mummichog	x	x
Gambusia affinis	mosquitofish	x	x
Ictalurus sp.	catfish		x
Lepomis auritus	redbreast sunfish	x	x
Lepomis gibbosus	pumpkinseed	x	x
Lepomis macrochirus	bluegill	x	x
Luxilis cornutus	common shiner	x	x
Micropterus dolomieu	smallmouth bass	x	x
Micropterus salmoides	largemouth bass	x	x
Morone americana	white perch		x
Morone chrysops	white bass		x
Moxostoma sp.	redhorse		x
Notemigonus crysoleucas	golden shiner	x	x
Notropis hudsonius	spottail shiner		x
Perca flavescens	yellow perch	x	x
Pimephales promelas	fathead minnow	x	x
Rhinichthys atratulatus	blacknose dace	x	x
Salmo trutta	brown trout	x	x
Semotilus atromaculatus	creek chub	x	x

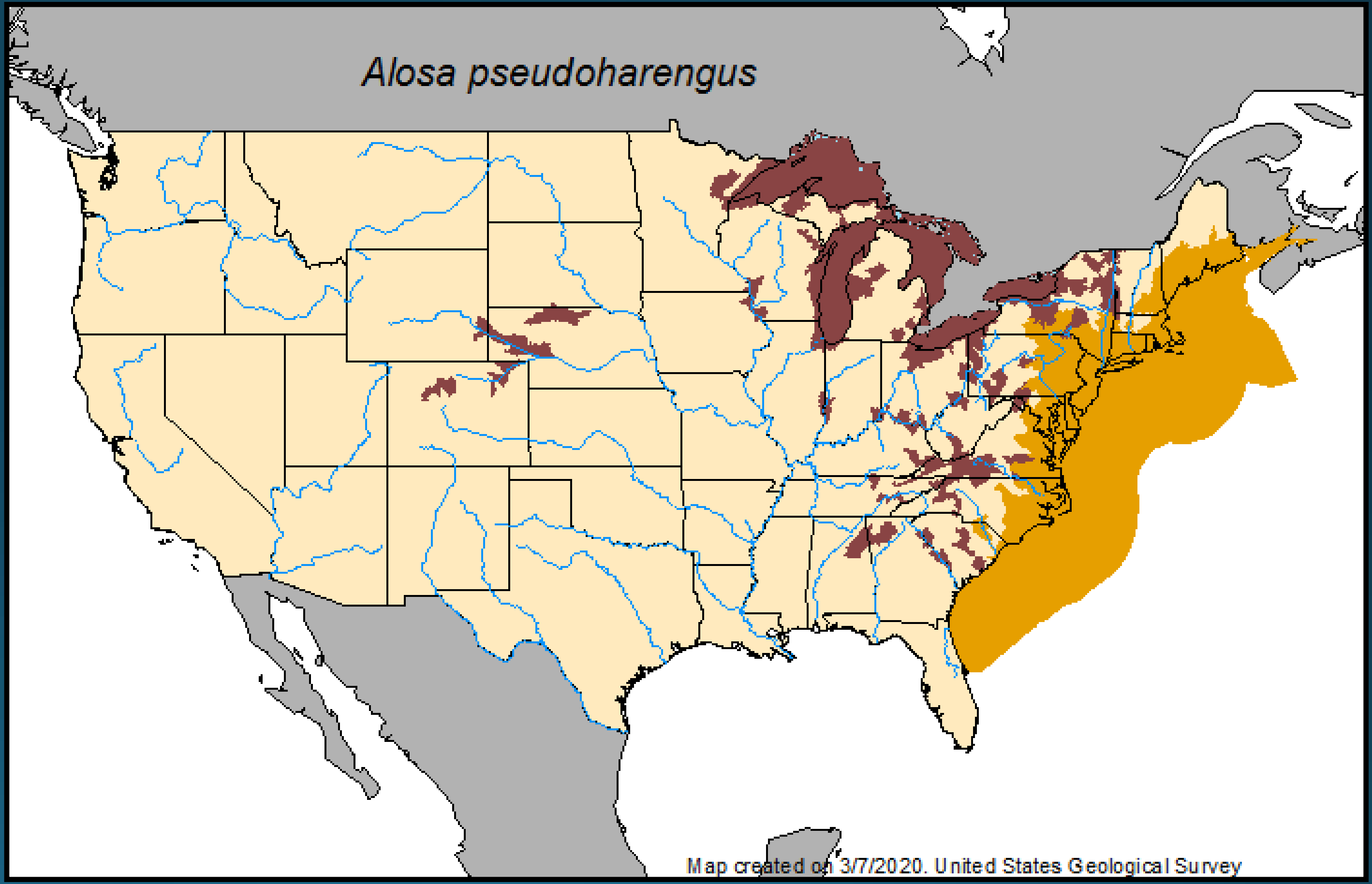
eDNA Phenology of a Fish Species
Tracking the eDNA Signal

Curbed New York



Alewife *Alosa pseudoharengus*

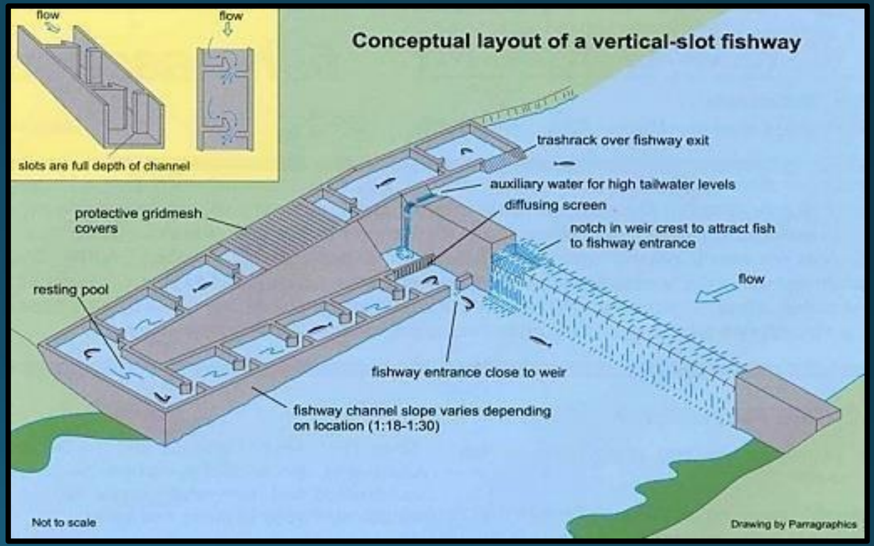




Alewife in the Bronx River – A Recent History

Trapping Returning Migrants

Fish Ladder at 182nd Street Dam Inaugurated 2015



Two Sources -- Stocking & Volitional Passage

Stocking

2006 - 200

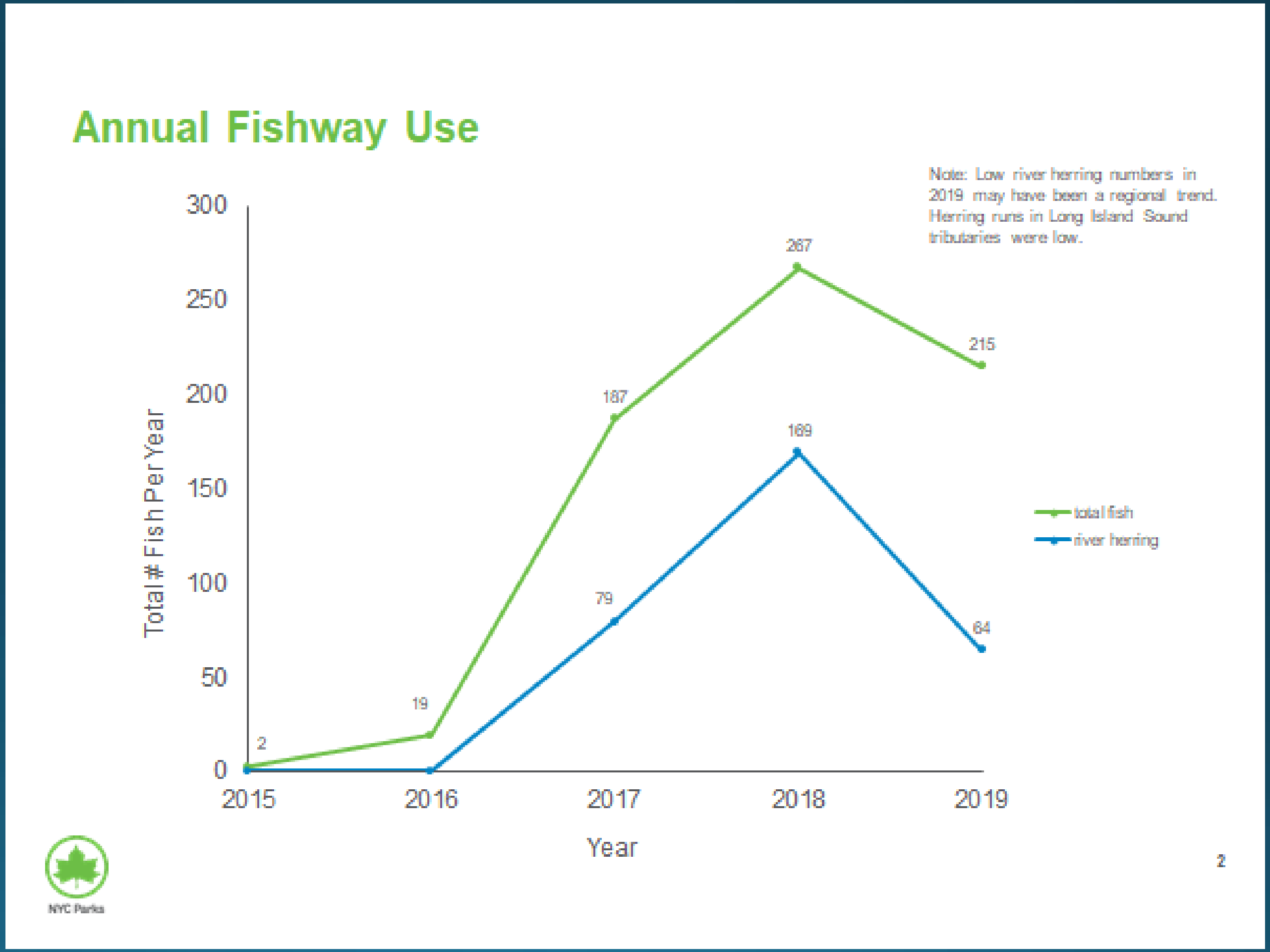
2007 - 400

2017 - 400

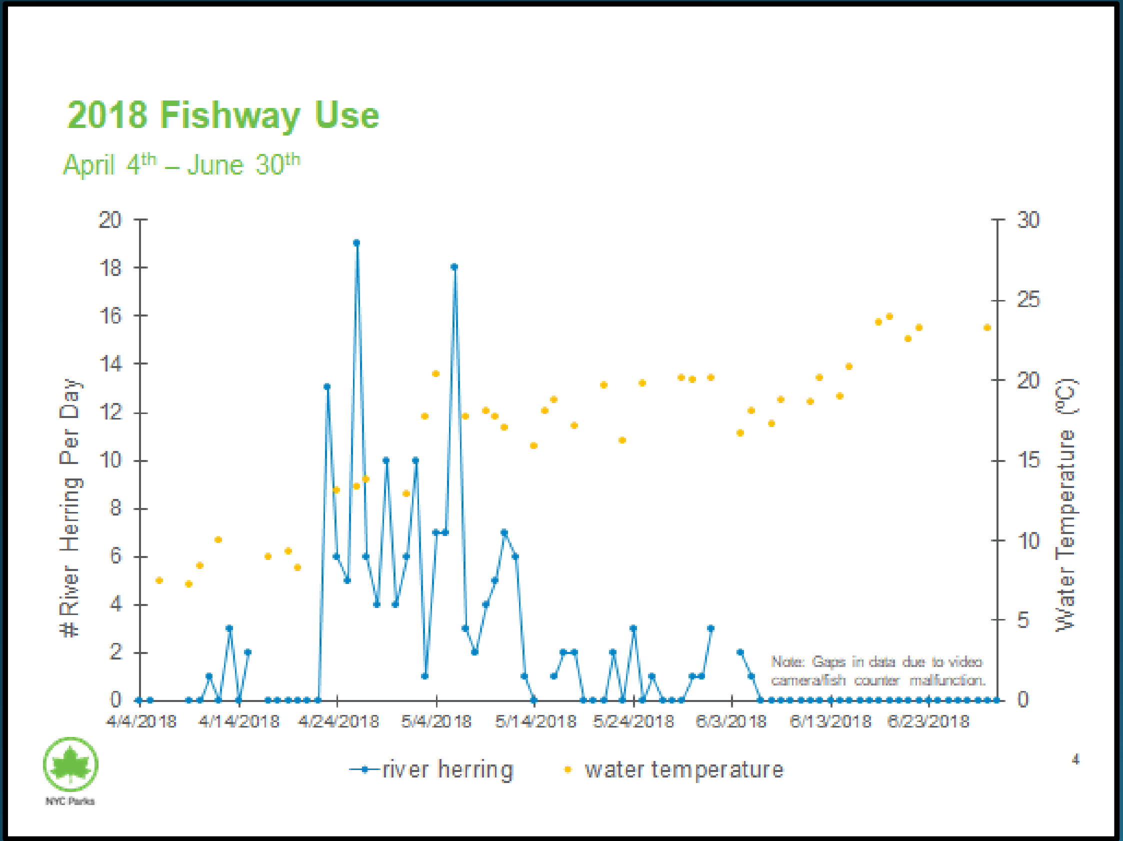
2018 - 400

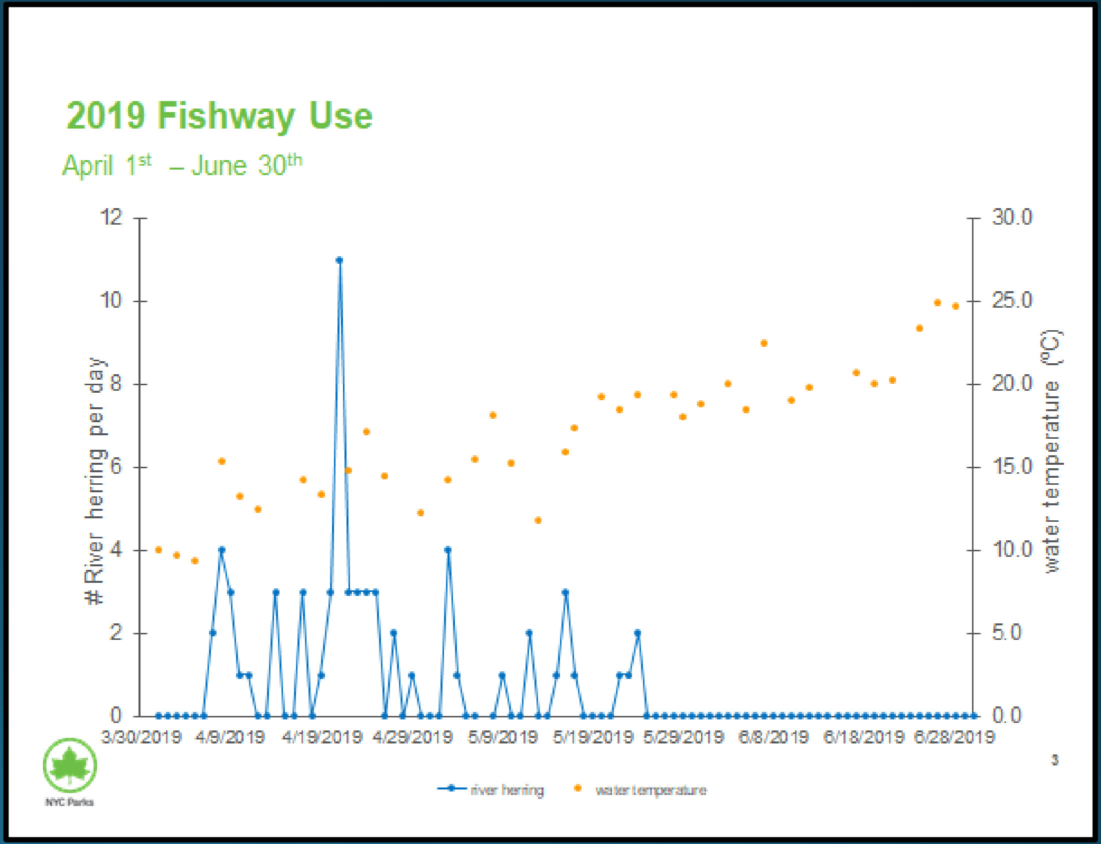
2019 - 400

2020 - zero



Seasonality of Adult Spawners

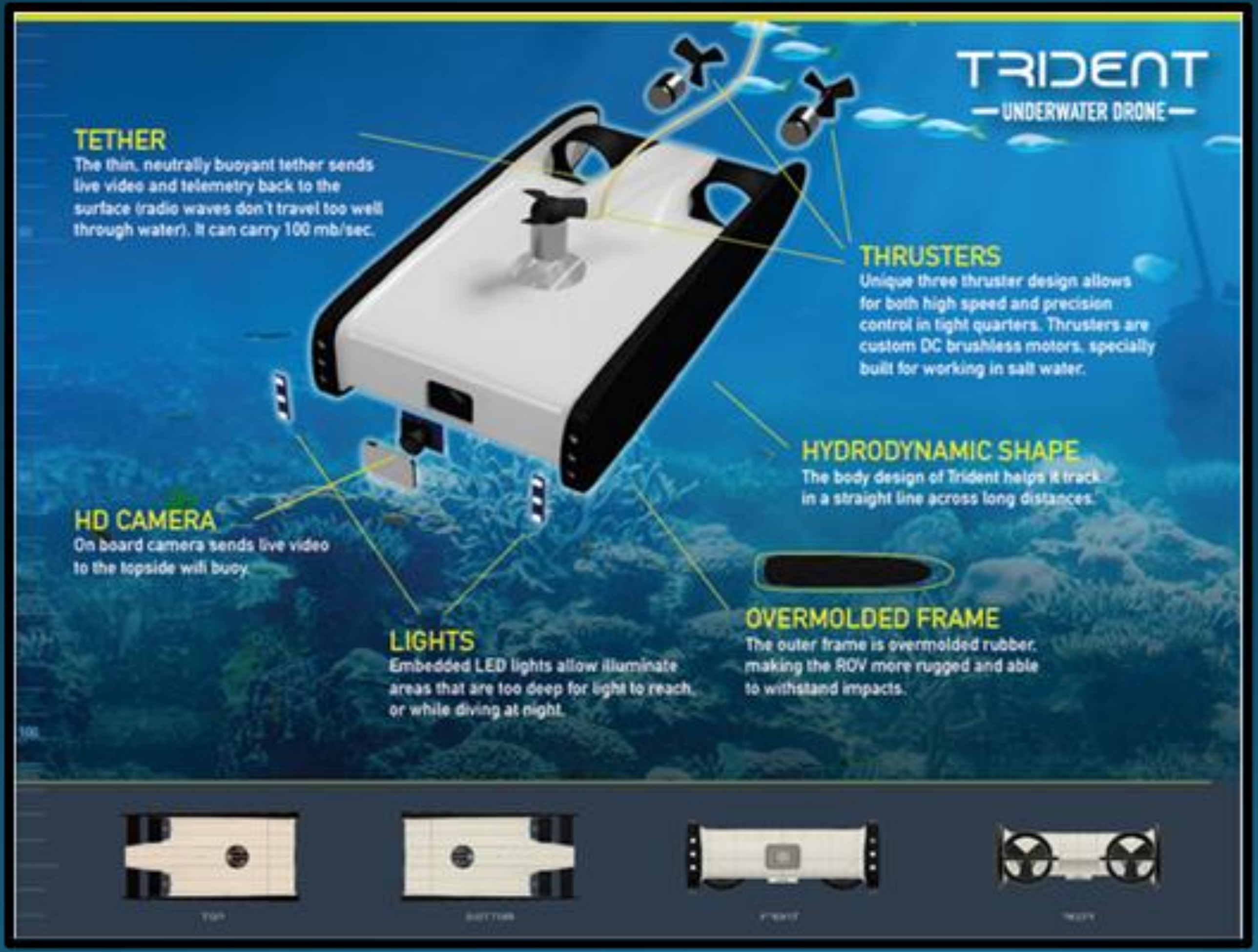




Study Plan --The Phenology of Migration in Bronx Lake

- Late winter – negative control, no alewife DNA expected
- April – before stocking – exact count of migrants through ladder (range to date)
- April – day of stocking (*n*=400)
  - Water in truck
  - 400 stockers briefly contained by block nets in river
  - 400 stockers + migrants within river, likely congregated at head of pool
- April & May – continued migration via ladder
- May & June (July & August??) – young-of-the year, documented with ROV
- October – adult & YOY alewife absent, documented with ROV, test for lingering signal. If detected, continue later.

ROV (Remotely Operated Vehicle) or “Underwater Drone”



Some Conclusions

- --eDNA is an exciting BUT STILL RAW tool with many potential applications to fish research for estimation of abundance, biodiversity, and phenology.

- --We likely need a decade of experimentation and play to suss out sensitivities.

Some Conclusions

- --Near term, calibration through manipulations and testing is more important than a rush to routine application.
- --Calibrations should take advantage of:
  - *Natural experiments*, e.g., Bronx River alewife migration cycle
  - *Manipulated experiments*, e.g., caging fish in rivers; tank studies with dead vs. live specimens; test temperature, salinity effects; length-weight-abundance relationships; decay and dispersion; etc.

Some Conclusions

- --eDNA looks to be reliable for common species. Detecting rare species may require considerable sampling effort. *Scaling effort to size of system appears very important.*
- --eDNA utility is strongly linked to questions being asked. *We need good questions!*
- --eDNA seems especially well suited to forensic concerns, e.g., presence of an invasive species (such as Asian carps in Great Lakes).
- --Pluses are clear, but some minuses in comparison with standard approaches.
  - e.g., occasional resolution only to higher taxonomic levels; lack of "carcass" verification; translation of qPCR to intuitive quantitative terms such as counts; uncertainties as to positives and negatives and need for subjective decisions.

The Challenge in a Big River -- Cumulative Catch Curves & Recognition of System Complexity in Sampling Design

Type of Detection Error

Source of Detection Error	False Positive (Type I)	False Negative (Type II)
Method	**Problem:** Detect species when no target species eDNA is present in the sample **Sources:** (1) Incorrect detection of non-target species (i.e., insufficient assay sensitivity) or (2) DNA contamination **Solution:** Improve assay specificity and exercise care when collecting, handling, and processing samples. **Include negative controls in experimental design.**	**Problem:** Fail to detect species when target species eDNA is present in the sample **Sources:** (1) Insufficient assay sensitivity or (2) method failure during sample processing **Solution:** Improve assay specificity and exercise care when collecting, handling, and processing samples. **Include positive controls in experimental design.**
Process	**Problem:** Detect target-species eDNA when species is absent from the ecosystem **Sources:** (1) Persistence of eDNA in the environment or (2) transport of eDNA from distant sources (e.g., barge traffic, boaters, avian deposition) **Solution:** Improve knowledge of the "ecology" of eDNA in the environment	**Problem:** Fail to detect species when present in the ecosystem because viable target-species eDNA absent in sample **Sources:** (1) Failure to collect eDNA in sample or (2) eDNA degraded in sample **Solution:** Improve sample collection, handling, and processing methods.

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