Some Adventures with eDNA

Jesse H. Ausubel and Mark Y. Stoeckle

Program for the Human Environment
The Rockefeller University

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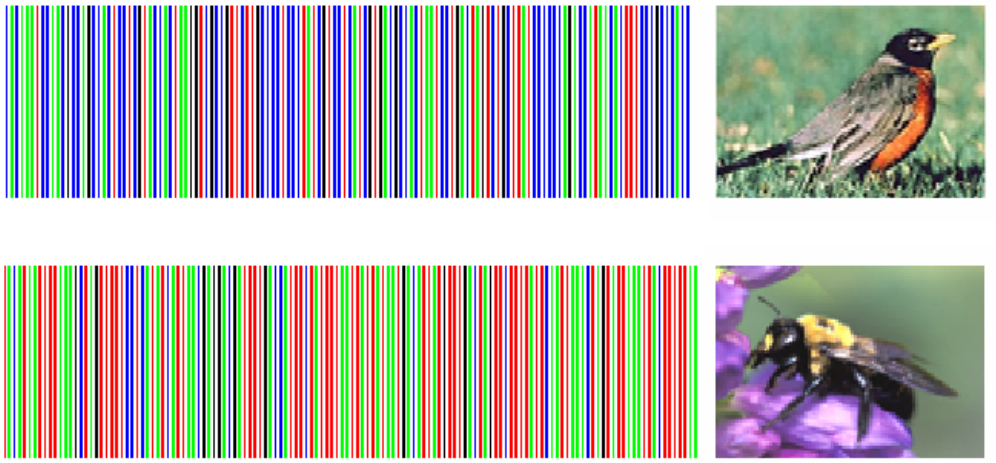
Monmouth University
eDNA: A Forensic Approach to Fish Detection

Alex Bettencourt woke up his brother to get this photo of his very big striped bass. — Alex Bettencourt

This story might have ended up as the Martha's Vineyard version of the Loch Ness monster, only in this fish story Nessie got eaten. But of course, Island fishermen don't wear kilts.

2003: Start of DNA barcoding movement

DNA barcoding: identifying species using short, standardized gene regions



Just as the UPC label identifies a product, a short DNA sequence, shown here schematically, can be an identifier for species, a "DNA barcode."

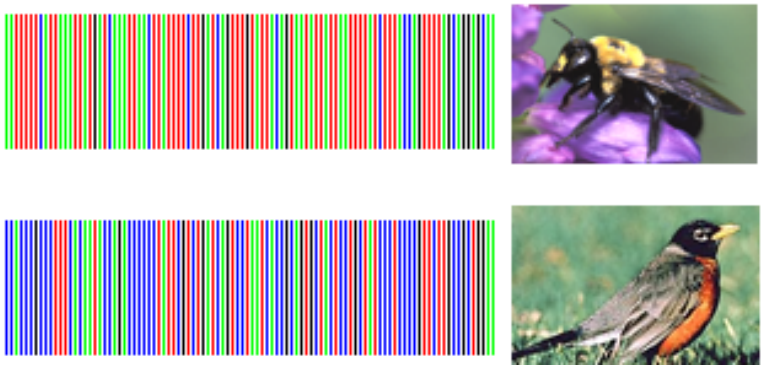
Anyone, anything, anywhere

1. Fragments

3. Look-alikes

2. Life stages

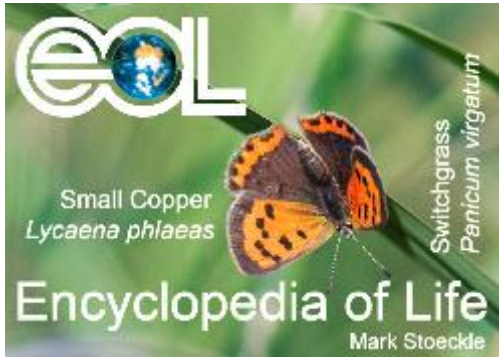
4. High accuracy



Anyone, anything, anywhere

5. Empowers more experts

6. Speeds discovery



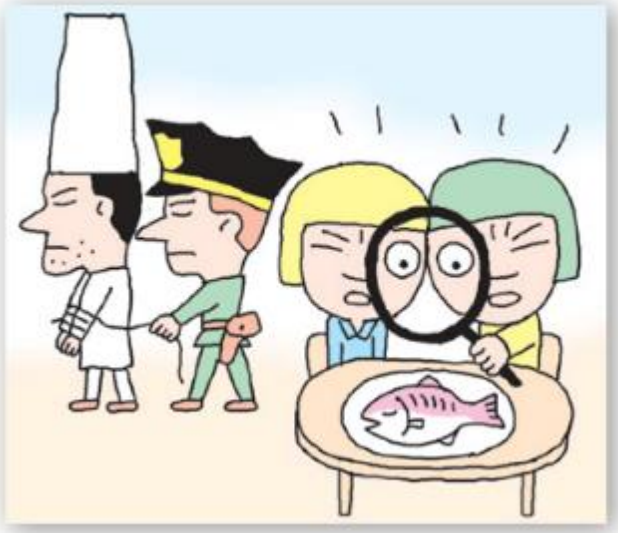
7. Simplifies, miniaturizes

2008: "Sushi-gate!"

THE NEW YORK TIMES, FRIDAY, AUGUST 22, 2008

A Fish Story With a DNA Hook

By JOHN SCHWARTZ



Korean Daily News

CBS Early Show

One-quarter of samples mislabeled, all as more expensive or more desirable fish

Mislabeling in 6 of 10 grocery stores/fish markets, and 2 of 3 restaurants

Sold as:
White (Albacore) Tuna
$8.50/lb wholesale

Photo FishBase M Bariche

DNA ID:
Mozambique Tilapia
$1.70/lb wholesale

Photo FishBase B Gratwicke

Could we collect DNA without collecting specimens or tissue?

Collecting fish

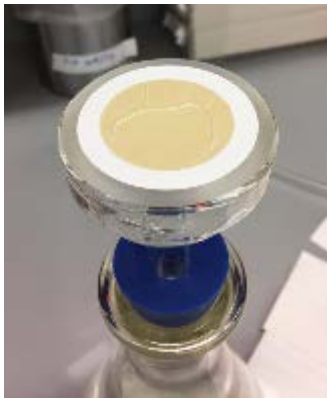
Collecting water

All samples analyzed to date are near-shore, surface

Fishing for DNA

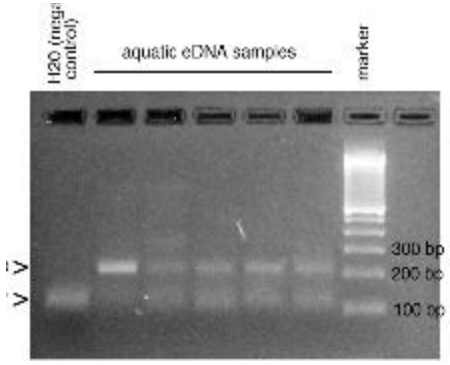
1. Collect water samples

2. Filter to concentrate



3. Extract DNA

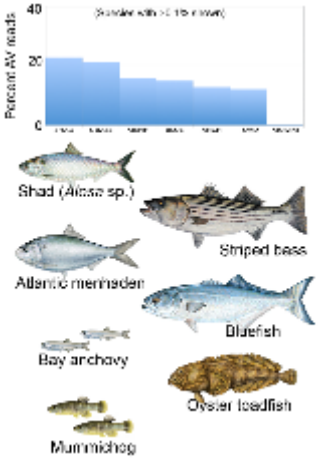
4. Amplify target (mtDNA 12S 110 bp)



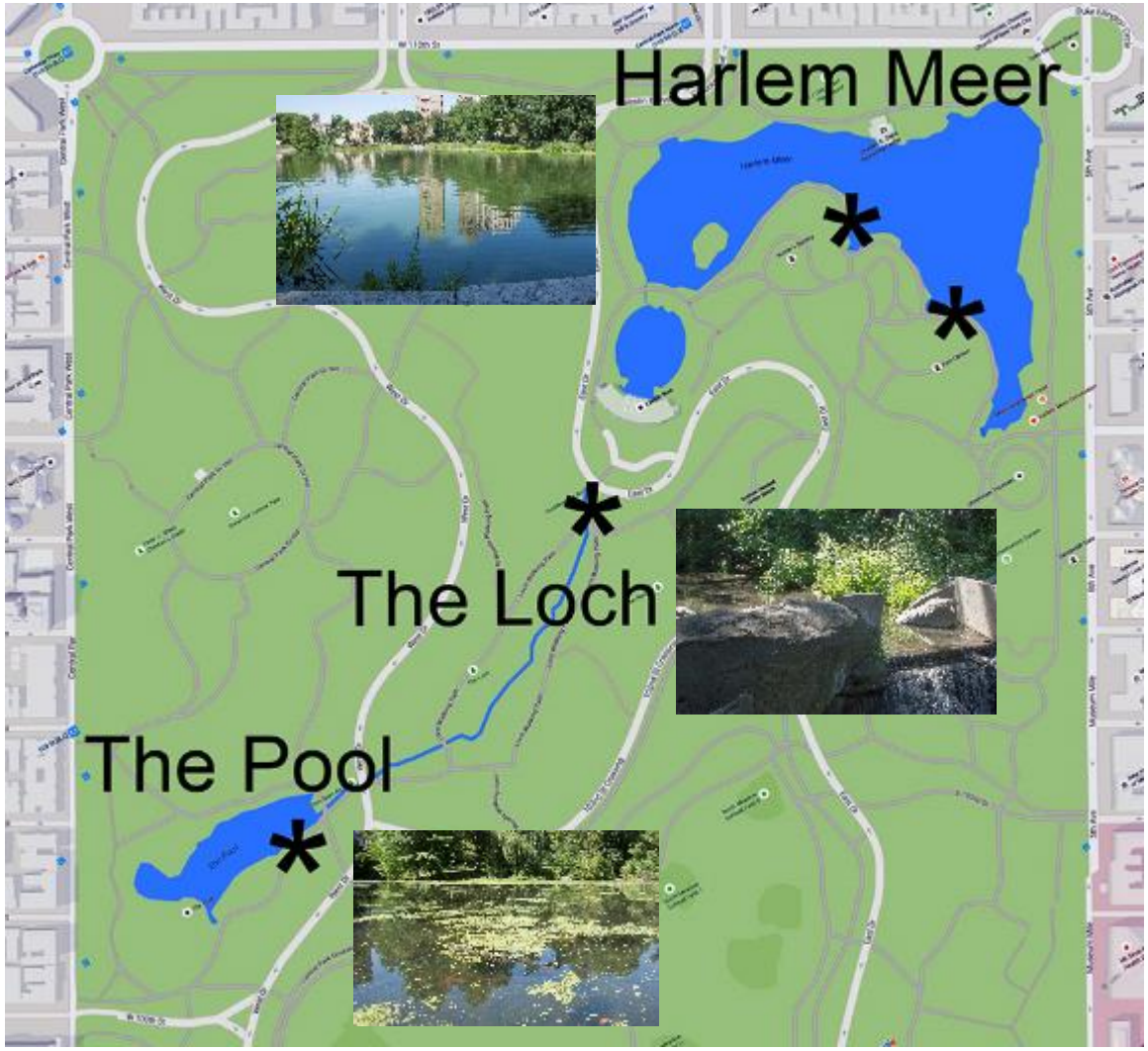
5. Sequence

Illumina MiSeq

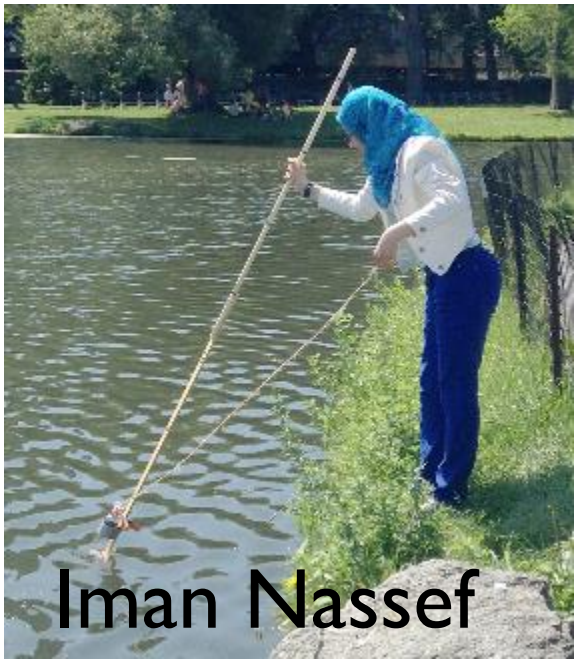
6. Match to library



City Fish: NYC Central Park eDNA survey



*sampling sites summer 2015



A dozen species in ¼ cup of water

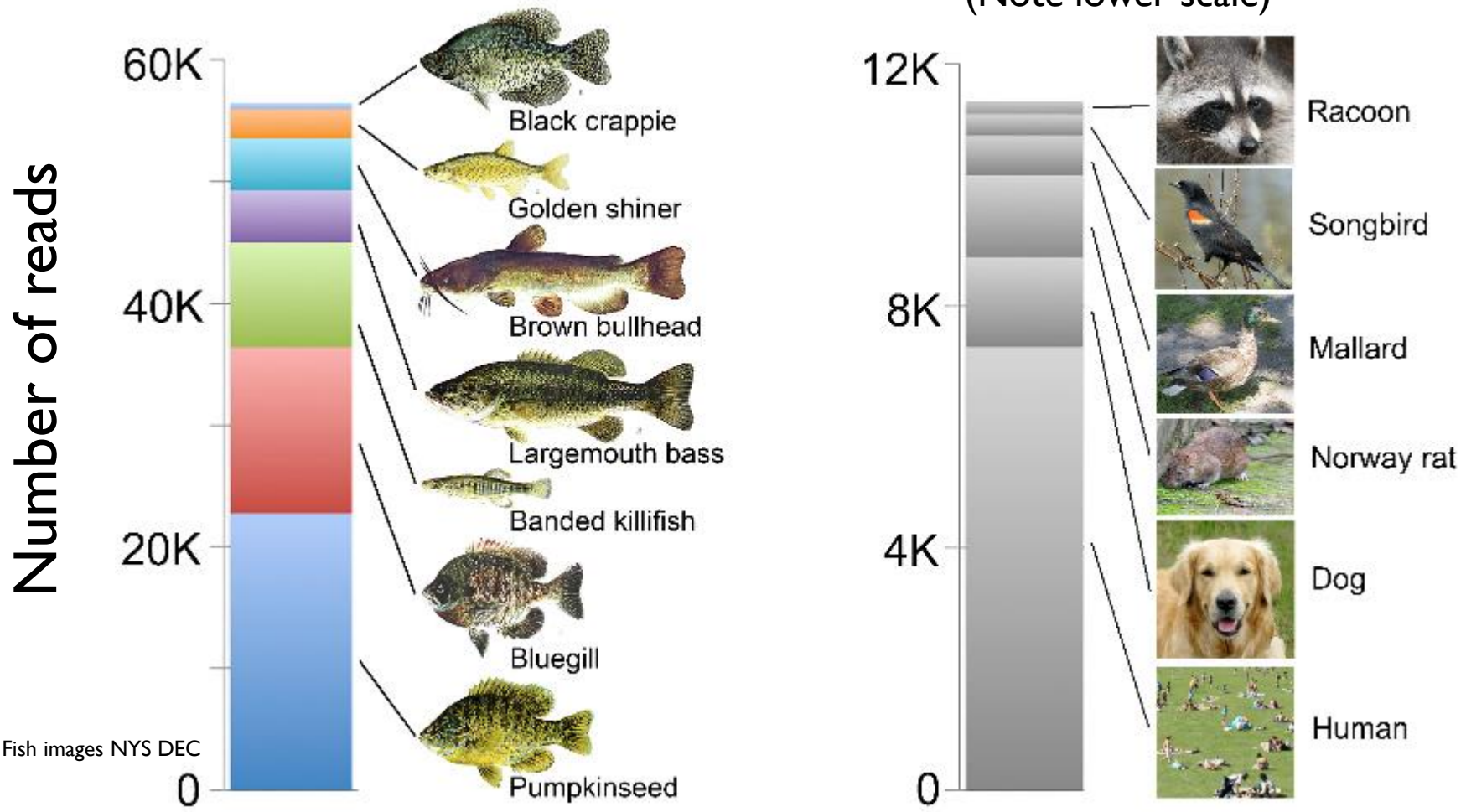
- Analyze 6.33 ng DNA (roughly 60 mL H_2O); 7 x 10^4 reads
- Detected 7 species of fish, also mammals, birds

The Loch

Fish

Mammals, birds

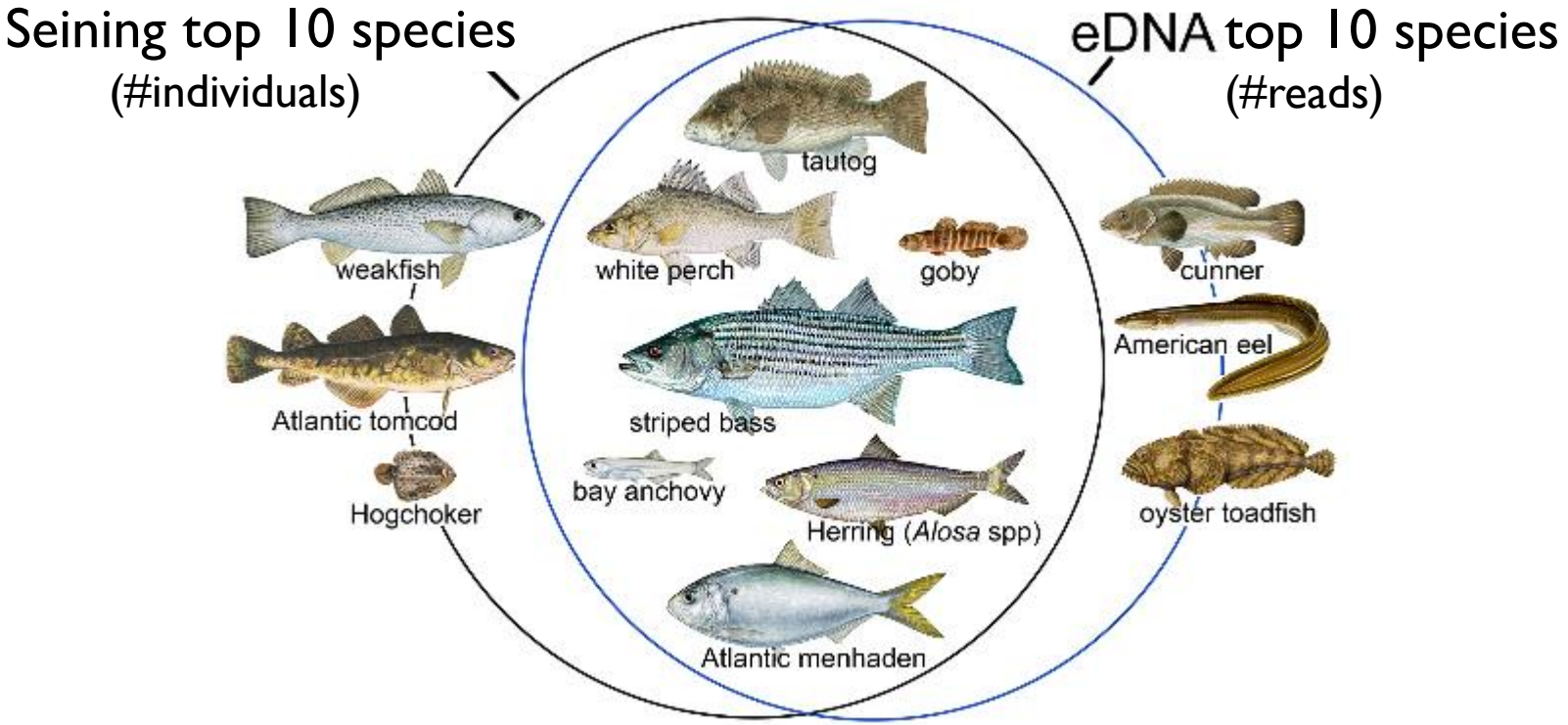
(Note lower scale)



Fish images NYS DEC

eDNA vs traditional methods

- 7 of top 10 species same by seining, eDNA



- Seining data from 2013 Long River Survey, Hudson River Monitoring Program (thanks to Keith Dunton for sharing survey reports)
- Long River Survey Hudson only, NYC to Albany

Potential for coastal marine eDNA:

- Assessing coastal habitat restoration

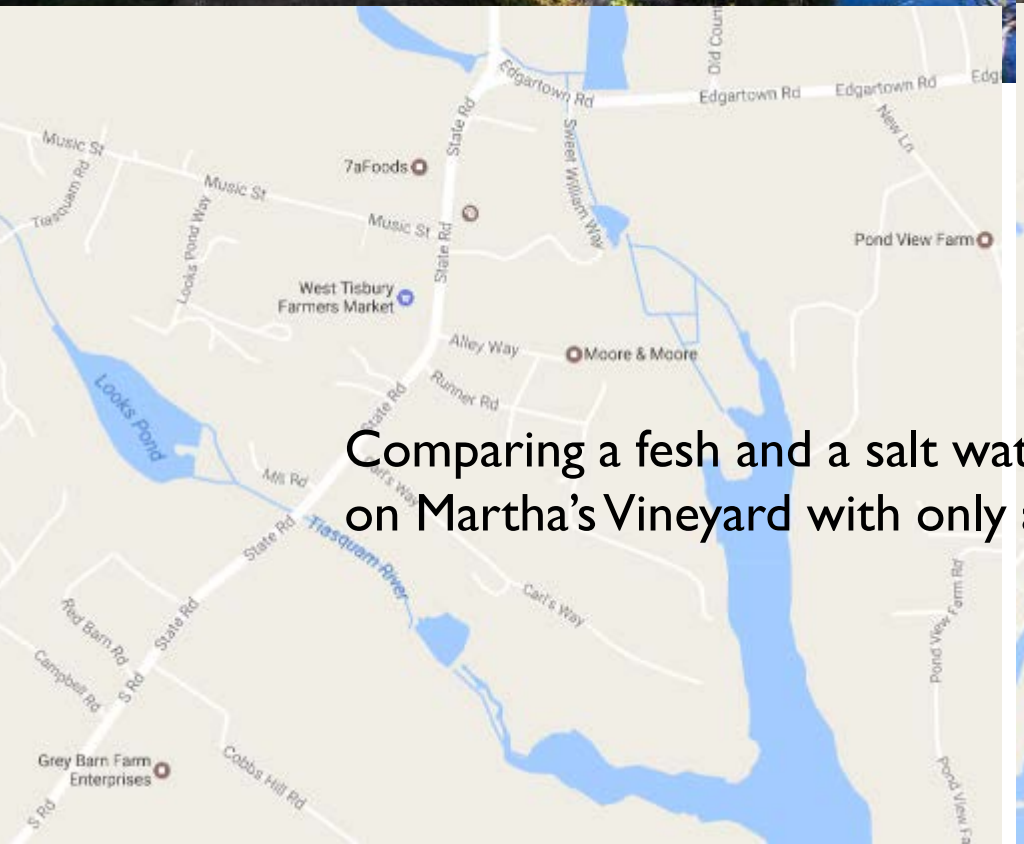
Wreck Pond restoration project

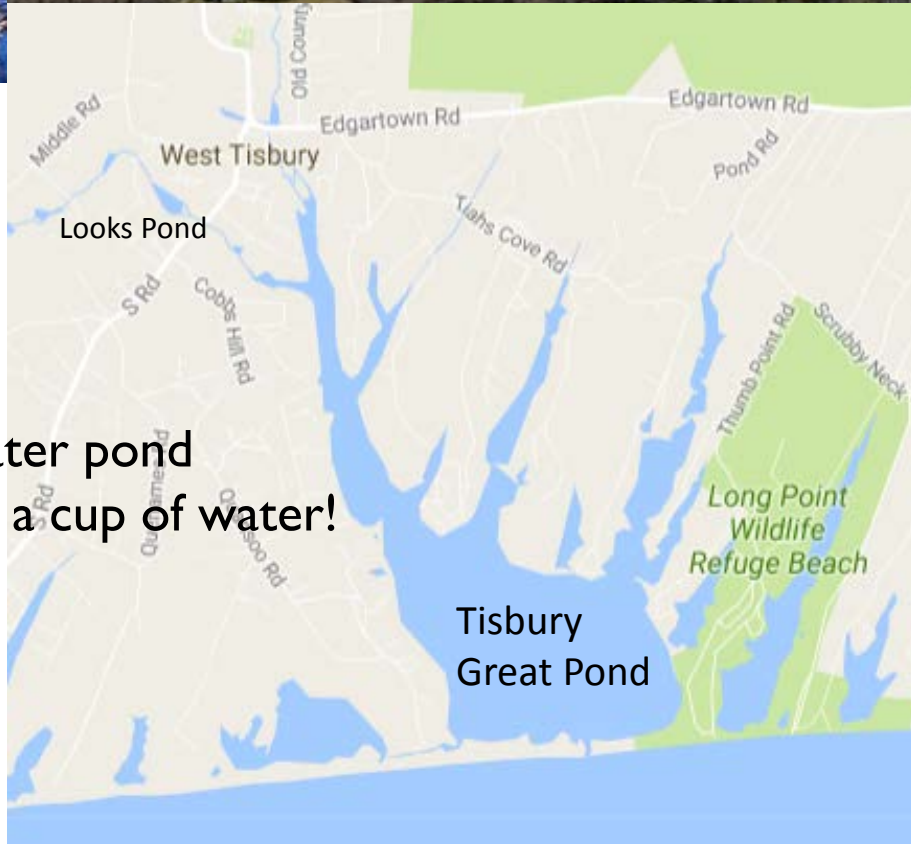
Seining at Wreck Pond

- Detecting endangered, invasive species

Atlantic sturgeon

Lionfish: "potential invasive, highly damaging"





Comparing a fesh and a salt water pond
on Martha's Vineyard with only a cup of water!








Vertebrate eDNA found in Look Pond (left, fresh) and Tisbury Great Pond (right, salt)

		mv_look_pond	mv_tisbury_great_pond
		8/27/16	8/27/16
		11:22 AM	5:28 PM
		aq157Aoct2016	aq158Aoct2016
White crappie probable	Pomoxis sp	37567	0
American eel	Anguilla rostrata	36667	0
Grass or American pickerel	Esox sp	25959	0
Golden shiner	Notemigonus crysoleuc	14831	0
Brown bullhead	Ameiurus nebulosus	7675	0
Banded killifish	Fundulus diaphanus	147	0
Mummichog	Fundulus heteroclitus	0	21723
Atlantic menhaden	Brevoortia tyrannus	0	12327
Inland silverside	Menidia beryllina	0	4151
Atlantic silverside	Menidia menidia	0	3711
Striped sea robin	Prionotus evolans	0	1437
Sheepshead minnow	Cyprinodon variegatus	0	526
Bay anchovy	Anchoa mitchilli	0	523
Human	Homo sapiens	6004	22613
Frog	Rana sp	14825	0
Canada goose	Branta canadensis	8341	0
Big brown bat	Eptesicus fuscus	596	0
Raccoon	Procyon lotor	257	0








Comparison of vertebrate eDNA in six aquatic locales on Martha's Vineyard Island

Left to right columns:
Freshwater pond
Saltwater pond
Freshwater but with salt species too
Stream
Freshwater pond
Freshwater pond

	Filter #	157	158	280	283	284,285	281,282
	Collection date	########	########	########	########	########	########
		Look Pond	Tisbury Great Pond	Upper Lagoon	Mill Brook below Mill Pond	Mill Pond	Priesters Pond
Freshwater fish	grass or chain pickerel	20135	0	465	0	84663	0
	American eel	34904	0	25746	35479	1129	0
	black crappie	30549	0	0	0	0	0
	golden shiner	15814	0	4940	689	213	821
	brown bullhead	6832	0	0	0	1523	2692
	yellow perch	0	0	0	42	174	2938
	darter species	0	0	0	1415	1061	240
	banded killifish	179	0	0	818	328	759
	bluegill	191	0	3252	0	335	654
Saltwater fish	mummichog	0	21723	0	0	0	0
	four-spine stickleback	0	0	20790	0	0	0
	menhaden	0	12327	1595	0	0	0
	inland silverside	0	4151	0	0	0	0
	Atlantic silverside	0	3711	0	0	0	0
	menhaden or herring	0	0	3528	0	0	0
	striped sea robin	0	1437	0	0	0	0
	white perch	0	0	977	0	0	0
	striped bass	0	0	949	0	0	0
	sheepshead minnow	0	526	0	0	0	0
	bay anchovy	0	523	0	0	0	0
Other vertebrates	Canada goose	7497	0	6900	2196	1749	48479
	bullfrog or green frog	10856	0	0	5701	20229	22463
	goose or swan species	0	0	31240	0	0	0
	brown rat	0	0	14132	0	1198	888
	duck species	0	0	11496	334	348	340
	black crowned night heron	0	0	4662	0	0	0
	white-tailed deer	0	0	705	2878	69	813
	northern short-tailed shrew	0	0	0	3405	0	0
	gray squirrel	0	0	0	2458	0	0
	cormorant	0	0	1189	0	0	0
	racoon	171	0	0	156	116	634
	muskrat	0	0	0	524	403	138
	black-capped chickadee	0	0	0	886	0	0
	dog	0	0	0	415	0	0
	big brown bat	398	0	0	0	0	0
	snapping turtle	117	0	0	0	0	0
	river otter	0	0	0	0	111	0
	cedar waxwing	0	0	0	0	56	0

My first thought was that the striper was a fresh arrival, and had followed the herring into the pond. Alex suggested it was one of a number of schoolies he had heard about that people caught on the saltwater side and released on the freshwater side. Striped bass spawn in freshwater and have been introduced into a number of lakes and reservoirs across the country, where they grow in excess of 40 pounds, so he is probably correct.

"I think it's been swimming around in there for about 13, 14 years, and I just happened to catch it."

surprise

Alex Bettencourt thought he heard something in the water behind him, so the fisherman decided to try a cast.

By **Nelson Sigelman** - June 1, 2016 0

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eDNA: can sample adaptively, spontaneously, e.g., after storms, heat waves, pollution episodes

East River

Hudson River

January 2016

July 2016

People, Partners, Sponsors

MURU Marine Science and Policy Initiative

A collaboration between:

MONMOUTH UNIVERSITY
Urban Coast Institute
Marine Science & Policy Initiative

Keith Dunton
Jason Adolf and Co.

The Rockefeller University
Program for the Human Environment

Jesse Ausubel
Mark Stoeckle

WCS

Howard Rosenbaum and Co.
Wildlife Conservation Society

THE ROCKEFELLER UNIVERSITY
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Summer Science Research Program (SSRP)
Jeanne Garbarino

LABORATORY OF GENETICALLY ENCODED SMALL MOLECULES
Zachary Charlop-Powers

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Thomas Noji and Co.