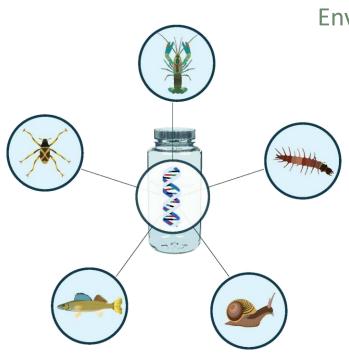


A little introduction to me...





A little introduction to eDNA

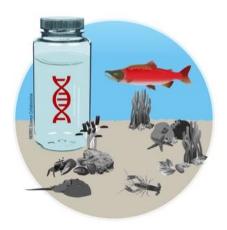


Environmental DNA (eDNA)

- All living things have and shed DNA
- eDNA is DNA released from an organism into the environment
- eDNA can come from:
 - Hair, scales, skin
 - Waste products
 - Reproductive cells
 - Entire organism (diatoms)

Often only used for qPCR or amplicon analysis

eDNA Methods



Single-species PCR (qPCR)

- + Simple, cheap, fast
- Only identifies one species
- Needs follow-up confirmation

Invasive or rare species



Metabarcoding (amplicon)

- + Identifies multiple species
- More complex, harder to interpret

Community assemblages, IBI, trophic structures

eDNA Process

Sample

 Collect samples (water, sediment, ect)

Extract DNA

- Standard kits
- QIAGENPower Soil Blood and Tissues
- Biofilm, etc
- Lab specific methods

DNA Analysis

- PCR: Amplify DNA with species specific probes
- Metabarcode: Amplify and sequence DNA

Interpretation

- Analysis and interpretation (bioinformatics)
- That's me!

eDNA Reality Check... It's Not Magic

eDNA can:

- Provide information on species presence
- Help target field sampling programs
- Reduce sampling effort
- Provide non-destructive, noninvasive sampling method

eDNA cannot:

- Confirm absolute absence
- Determine species abundance
- Determine life stage or condition
- Identify species without known DNA sequences

Finding fish (and other critters) with eDNA in NH

Results from 2018 Sampling season:

- Concurrent Seining/eDNA by Great Bay NERR
- Anadromous* fish returns in the Oyster and Lamprey Rivers
- Concurrent Larval Fish tow/eDNA by Wells NERR



2018 Great Bay Watershed Sampling Sites



Benchmark eDNA with Traditional Biological Monitoring Methods

New Hampshire - seining, coastal streams

Oregon - seining, crab trapping

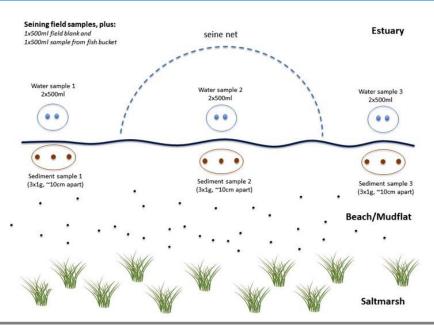
Maine - plankton tows, crab trapping

How does eDNA compare?





Seining sample collection

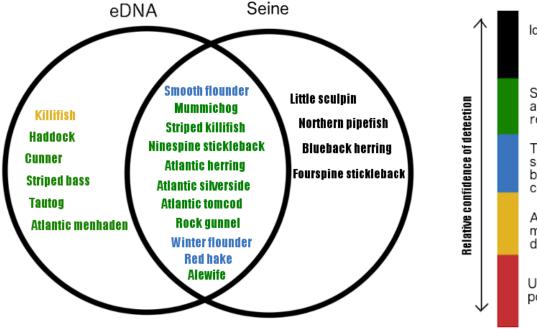


1-L water samples at 3 locations, 3 composite sediment samples at tideline



Seining – New Hampshire

5 sites, 2 sampling events



Identified via net count

Sure - Backed up by a strong hit to a reference sequence

Theorized - Missing species level reference, but reason to believe certain species

Abstracted - Due to missing species level database reference

Unlikely to be correct; possible contaminant

Anadromous fish counts



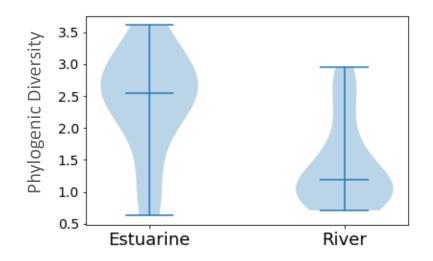
Triplicate 1-liter water samples collected above dam and at base of fish ladder, 2-3 times/week May-June (Oyster and Lamprey Rivers, NH)

26 fish species

Also American beaver, Common Muskrat, Common Tern, Cow, Eastern Gray Squirrel, Eastern Newt, Pig, Mallard, Human & invertebrates

eDNA & Anadromous Fish

Can we measure the difference in fish assemblage above and below a stream barrier? Yes, different species and higher diversity in estuarine samples





47 species, including non-fish, detected with 12S primer (MyFish)

eDNA & Anadromous Fish

How does our survey of two rivers (Oyster & Lamprey) compare to the NH Resident Freshwater species list from Fish and Game?

Undetected	Detected	d	Limited		No ID	
Alewife	C	reek chu	b	Redf	in pickerel	
American brook la	mprey C	reek chu	bsucker	Rock	bass	
American eel	Ea	astern sil	very minno	wRour	nd whitefish	
American shad	Fa	allfish		Sea 1	amprey	
Atlantic salmon		Fathead minnow		Shor	Shortnose sturgeon	
Atlantic sturgeon	Fi	nescale	dace	Slim	y sculpin	
Banded killifish	G	olden sh	iner	Smal	llmouth bass	
Banded sunfish	La	ake chub	1	Spot	tail shiner	
Black crappie	La	ake trout		Swai	mp darter	
Blacknose dace	La	ake whit	efish	Tess	ellated darter	
Blueback herring	La	andlocke	d salmon	Wall	eye	
Bluegill	La	argemou	th bass	Whit	te perch	
Bridle shiner	Lo	ongnose	dace	Whit	te sucker	
Brook trout	L	ongnose	sucker	Yello	w bullhead	
Brown bullhead		Margined madtom		Yello	ow perch	
Brown trout	N	orthern I	Pike	Four	spine stickleback	
Burbot (freshwater	cusk) N	orthern r	edbelly dac	Men	haden	
Chain pickerel		umpkins		Perc	h 1	
Channel catfish	R	ainbow s	melt	Perc	h 2	
Common carp		ainbow t		Shad		
Common shiner	R	edbreast	sunfish	Myst	tery Fish -H. flammea	

We detected 26 of the 57 species on the NH Fish and Game "Resident Freshwater Species List" (listed to the right). Green indicates the species we identified that are on their list.



All Fish detections – Oyster & Lamprey Rivers

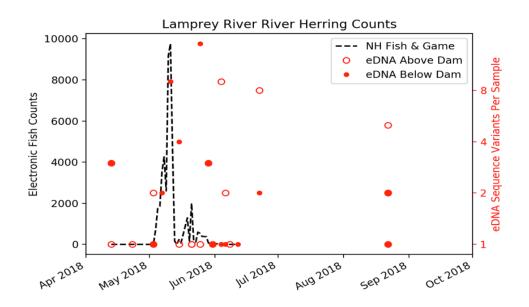
Detected 26 species
- most of the fish
that would be
expected to be in
that section of the
rivers.

Did NOT correlate well with fish counts

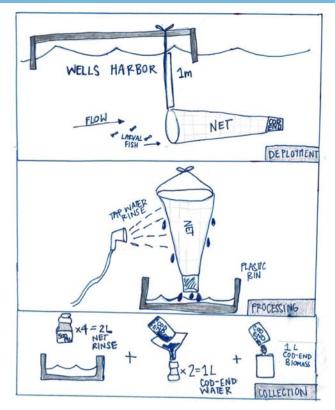


Anadromous fish counts

DNA increases when fish return, but doesn't correlate with abundance

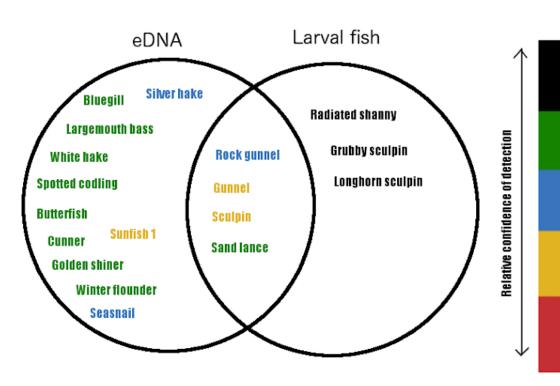


Larval Trawl (Wells)





Larval trawl results(Wells)



Identified via microscope taxonomy

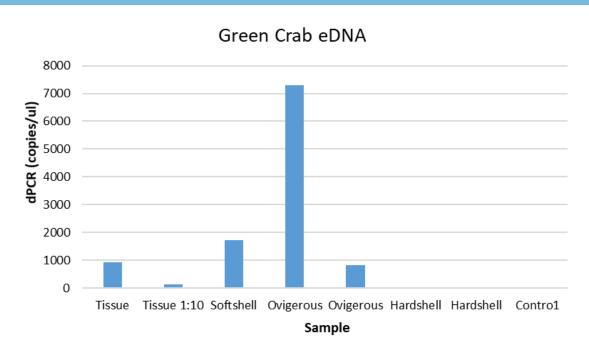
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Crab Experiment – Maine





Green crab DNA only detected from softshell and gravid crabs in tank experiment

Lessons so far:

 Methods matter! How samples are collected, processed, interpreted will effect results.

Contamination (primarily lab) is challenging.

 This is very interdisciplinary – biology, water quality, computer science, resource managers, communication.



Next steps for this project (summer 2019)

Still deciding, but possibly:

- Developing recommendations for fish surveys in estuaries
 how many samples, volume, analysis
- More larval fish surveys comparison between microscope, DNA from tow sample and DNA from water samples.
- Sampling algae in streams
- Invasive species (zebra mussels) in lakes



Questions?

HOME

ABOUT US

ABOUT eDNA



PROTOCOLS

DATA & RESULTS

CONTACT US



The only thing you have to remember from this talk

If you are developing any form of taxonomy based criteria you should be considering molecular methods

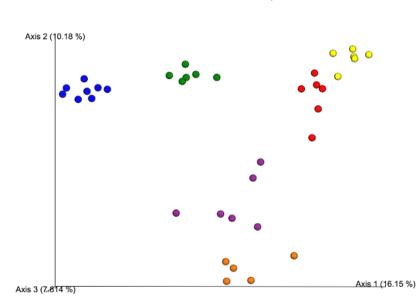
Really

Take home slide from Northeast Aquatic Biologists Conference, Feb 2019

Biodiversity

18S analysis of estuarine sediment samples Several hundred eukaryote species in each sample

UniFrac (ordination) Analysis of South Slough Sediment Samples





Samples collected in Dec 2017. The different colors represent each site. Samples that are close together have similar biological communities. Samples that are separated are statistically different.