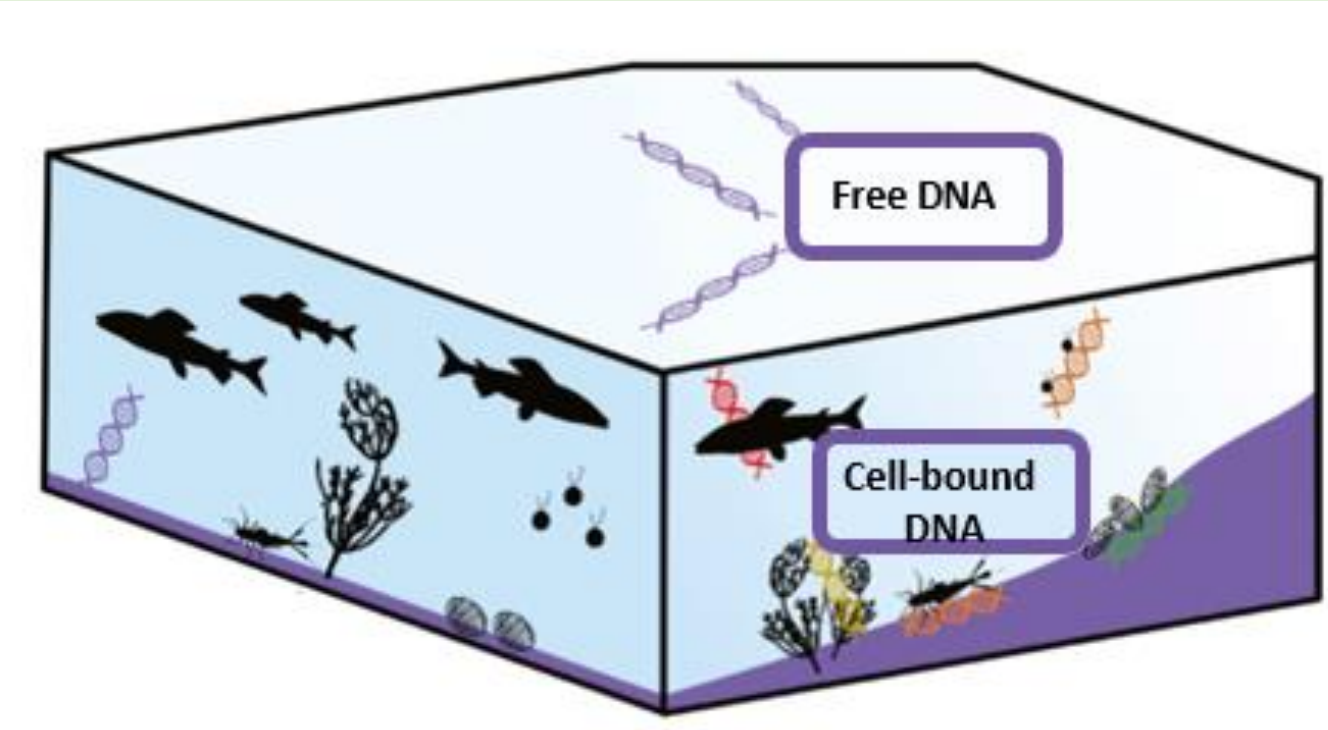


Background

Environmental monitoring programs are essential for effective estuarine management, but they are often time-consuming, expensive, and subject to technical and resource limitations. Traditional monitoring methods may also miss early detection of newly-arrived invasive species or confirm losses of rare native species that occur in low densities or at locations that are difficult to survey. Environmental DNA (eDNA) refers to the DNA in an environmental sample, which comes from whole microorganisms, fragments of tissue, cellular material, or waste products.



eDNA methods allow resource managers to detect species present in an aquatic system without the need to capture and identify individual organisms.

Overarching Project Goals

Researchers from several National Estuarine Research Reserves (NERRs) and the University of New Hampshire (UNH) are working collaboratively to:

- Design and implement a **pilot eDNA monitoring program** at several NERR sites.
- Identify estuarine target **species of concern**, with a focus on invasive invertebrates and migratory fish.
- Develop **eDNA sample collection and analysis protocols**, with training materials and recommendations for the appropriate use of eDNA in estuarine monitoring.

eDNA Methods

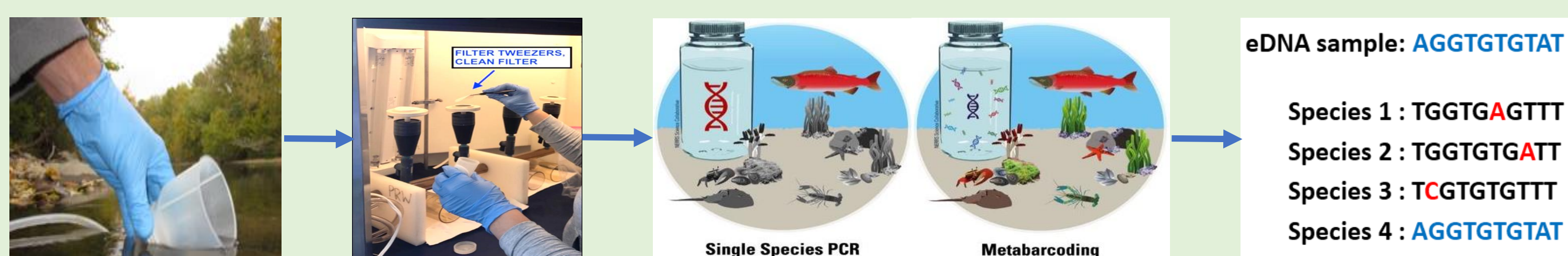
Step 1: Collect water and/or sediment samples with sterilized equipment

Step 2: Filter sample to capture eDNA

Step 3: Extract DNA

Step 4: Amplify and sequence DNA using PCR (detects only a single species) or Metabarcoding (detects multiple species in a sample)

Step 5: Assign taxonomy to the sequenced DNA to determine species present in the sample (Bioinformatics)



Lessons Learned

Advantages:

- Lower cost than traditional methods
- Reduced sampling effort
- Results within days or weeks
- Detect cryptic species
- Repeatability
- Non-destructive, non-invasive
- Target multiple phyla in a single sequence run

Challenges:

- Determines presence, not abundance
- Cannot confirm absolute absence
- Cannot determine life stage or condition
- Samples easily contaminated
- Other DNA sources (i.e. bait fish, runoff)
- Methods (collection, processing, interpretation) will affect results

Team Members

- University of New Hampshire
- Great Bay NERR (New Hampshire)
- Wells NERR (Maine)
- South Slough NERR (Oregon)
- He'eia NERR (Hawaii)
- Apalachicola NERR (Florida)
- Hudson NERR (New York)



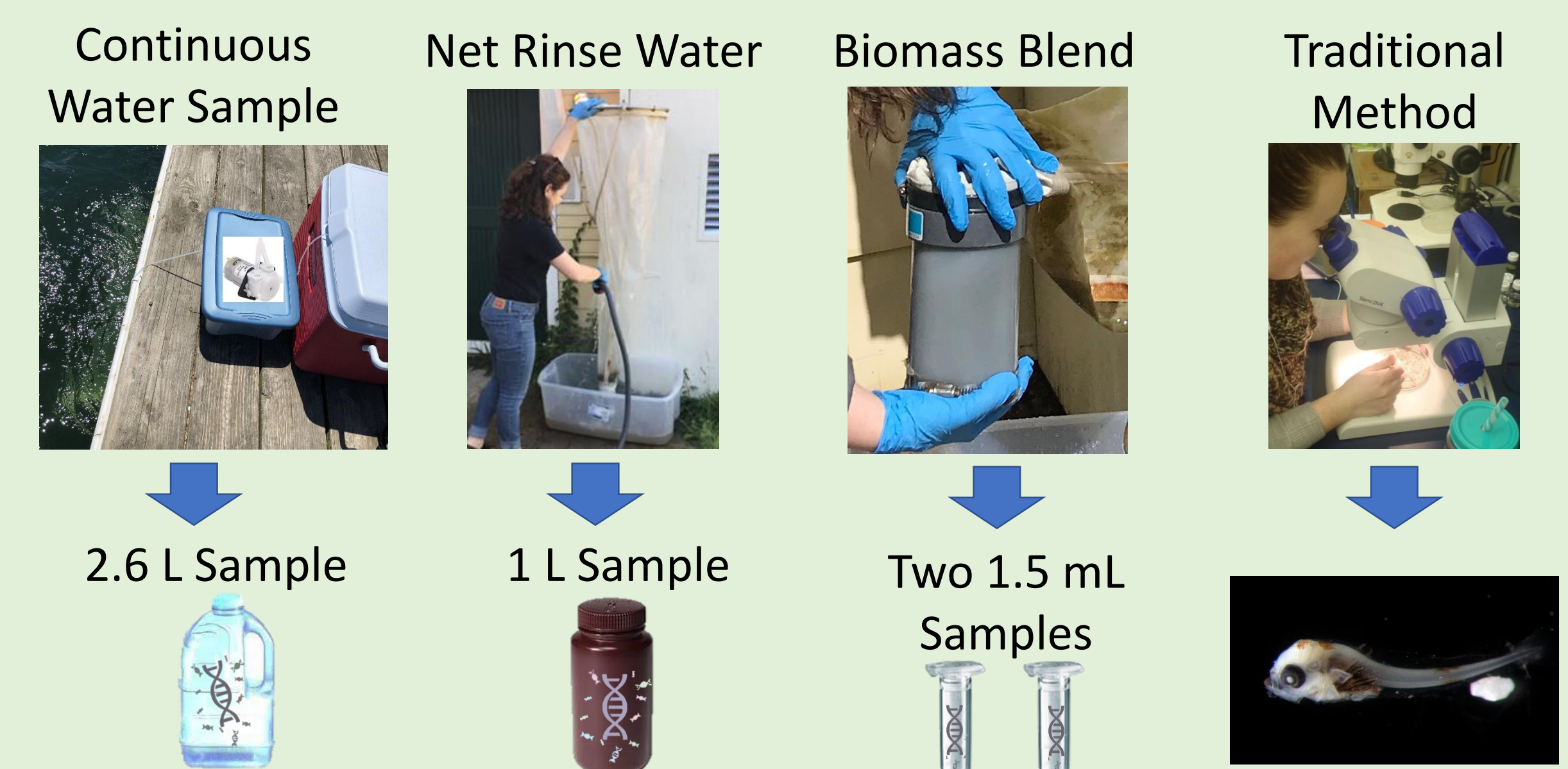
Case Study: Fish Biodiversity in the Gulf of Maine

Goal: Compare fish species detected in Wells Harbor using traditional methods of capture (larval fish tow) and several different eDNA methods of collecting water samples.



Methods:

1. Sterilized all sampling gear using 10% bleach solution.
2. Deployed two plankton nets (monthly) from Wells Harbor dock for 1 hour.
3. Continuously pumped 2.6 L seawater into a sterile collection jug throughout the tow (**continuous water sample**).
4. Hosed down the "eDNA net" and collected 1 L of the **net rinse water**.
5. Blended the contents of the cod end of the "eDNA net" in a food processor. Collected two 1.5 mL samples (**biomass blend**).
6. Identified the fish species present in the "traditional net" under a dissecting microscope (**traditional method**).
7. Filtered all water samples at Wells NERR. DNA extraction, metabarcoding, and bioinformatics conducted at UNH to determine fish species present.



Preliminary Results:

Species detected in July 2019 samples:

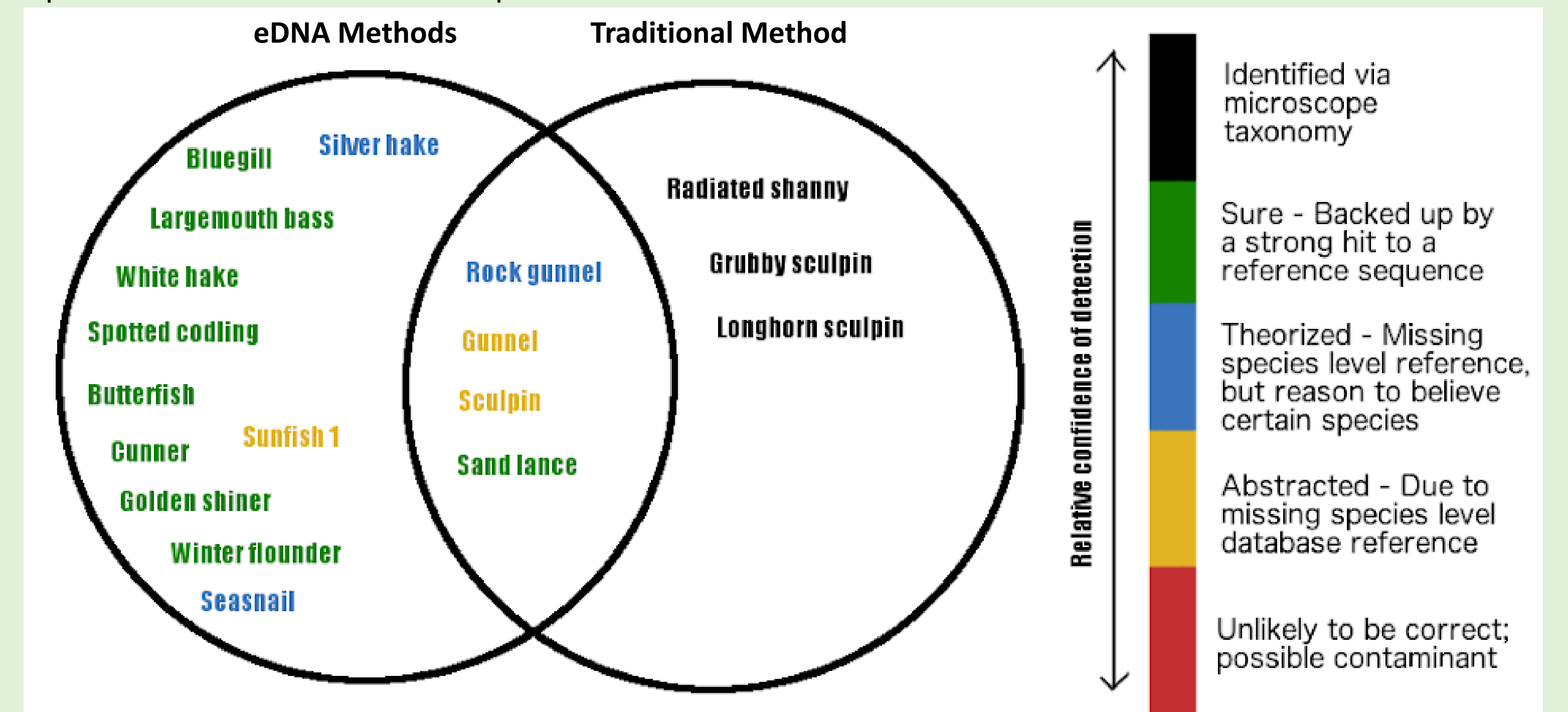
Method	Cunner	Menhaden	Silver Hake	Rock Gunnel	Northern Pipefish	Windowpane Flounder	Winter Flounder	Nine-Spine Stickleback
Net Rinse								
Continuous		X		X				
Biomass	X		X		X	X	X	
Traditional	5						1	1

Species detected in August 2019 samples:

Method	Cunner	Menhaden	Silver Hake	Red Hake	Northern Pipefish	Windowpane Flounder	Atlantic Halibut	Striped Bass	Atlantic Silverside	Grubby Sculpin	Fourbeard Rockling	Pacific Sand lance	Sea Raven	Atlantic Mackerel
Net Rinse	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Continuous		X												
Biomass	X		X	X										
Traditional	No fish caught													

(MIFISH Primer: Sato et al. 2015)

Species detected in all 2018 samples combined:



Acknowledgements:

We thank the following individuals for their support and help in the field and lab: Claire Gottsegen, Michelle Furbeck, Jake Aman, Katrina Zarrella Smith, Kayla Rexroth, Kathryn Kissam, Lee Pollock, and Sylvia Pollock. This work is sponsored by the National Estuarine Research Reserve System Science Collaborative, which supports collaborative research that addresses coastal management problems important to the National Reserve System. The Science Collaborative is funded by the National Oceanic and Atmospheric Administration (NOAA) and managed by the University of Michigan Graham Sustainability Institute (NAI4NOS4190145).