**Flatfish distributions across the northeast U.S. continental shelf: comparing bottom trawl and eDNA metabarcoding results for Fall, 2019**

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**Abstract**

Environmental DNA holds promise for monitoring biodiversity in dynamic ecosystems and may have broad applications for resource assessments. Here, we evaluate the sufficiency of a common eDNA mitochondrial marker (i.e., the Riaz 12S primers) to match all 47 pleuronectiform species (8 families) documented from North Carolina to the Gulf of Maine. For this marker, 51% of the 47 species lack reference sequences; only 21 full and 2 partial sequences were available in GenBank (<https://www.ncbi.nlm.nih.gov>) as of September, 2022. We also compared flatfish taxa observed by a fishery-independent bottom trawl survey to flatfishes observed using the Riaz 12S primers across the same sampling range (35.4 – 43.0oN) of the US east coast during Fall, 2019. The bottom trawl survey identified 12 flatfish taxa (3 identified as sp.) using morphological traits. The eDNA metabarcoding survey using filtered water samples identified 11 taxa (2 identified to family). Five common species were identified in both surveys. Much less water was strained by the eDNA method to reveal the same biodiversity: 301 20-minute trawl tows vs. a total of 346 liters of filtered seawater for eDNA metabarcoding. Additional reference sequences in GenBank are needed to improve estimates of flatfish diversity. Regarding fishery applications, 5 flatfishes with formal stock assessments are represented by sequences in GenBank, as well as an additional 2 sequences that can only be identified to family (Pleuronectidae). An initiative by NOAA Fisheries’ National Systematics Laboratory, Smithsonian, will add reference sequences from vouchered specimens to GenBank. Use of multiple markers will also improve taxonomic identifications.

250-260 words now.

**Introduction**

Delineating species distributions is basic information for defining stock structure of fishery resources and also forms the baseline when examining changes in faunal distributions related to broad-scale environmental changes, such as global warming.

Understanding the spatial and temporal distribution of biodiversity is important for siting windmills and assessing the effects of wind energy development

This region (northeast US) is perhaps a best case example, as it is well studied and moderately diverse compared to other regions (Tom has more perspective on writing this).

The point from Stoeckle about fewer samples (eDNA v trawl)

Multi-tiered

Already cleared eDNA ‘works’

But how well does it work for

1. biodiversity vs.
2. resource assessments
3. Geographic shits (biogeograph, climate ecology, for example, crossing faunal breaks [extralimital taxa])

Other tiers?

**Field Methods**

Two independent surveys, Fall 2019

1. Bottom trawl survey (Ship, dates, map of stations), morphological identification of species. cite Politis Tech Rep
2. eDNA (Ship, dates, map of stations), metabarcoding for species identification, cite a ECOMON survey

Define overlapping region of both surveys for all comparisons

**Reference collections**

What can we expect to match?

Flatfishes (Order Pleuronectiformes) in the northeast region (Cape Hatteras to Gulf of Maine):

6 bothids

1 scophthalmid

9 paralichthyids

11 cyclopsettids

7 pleuronectids

1 poecilopsettid

3 achirids

10 cynoglossids

47 flatfish species in 8 families for the region

Table 1 legend. Species diversity of flatfishes (Order Pleuronectiformes) in coastal and marine waters from Cape Hatteras to the Gulf of Maine. This table uses (Page et al., 2013) as a primary source of nomenclature, with modifications by Munroe (1998) and Campbell et al. (2019).. Regional documentation is referenced in footnotes to the table.

Rich has move Table 1 to a google sheets file at <https://docs.google.com/spreadsheets/d/1JkbpHpTaZvx5MsL_6DlBJeuo6v1lmc0XkaK1Im8HhdQ/edit#gid=0>

Image for slide: AFS book of names, WORMS, etc., a Qcode for a bar code

Work with Yuan on, YL uses genbank ( MitoFish? http://mitofish.aori.u-tokyo.ac.jp/)

**Results**

Strength of reference collections

Pie chart of: 1) those that are solid, those that have questions (% match) (or maybe are AOK to the genus or family level) and those missing

Do for all flatfish species and for fishery species

YL: Talking about rabbit holes... I put the Winter Flounder/American Plaice sequence and the Winter Flounder/Yellowtail Flounder sequences together to compare. There are 4 versions of sequences in total. If I include the primer sites, then there are even more than 4 versions! This reminds me that sequencing errors can also play a role in creating various versions of sequences that match 98-100% to reference sequences.

Possibilities: sequencing error (wrong species), or different haplotypes

Geographic (hortizontal) distribution

Paired maps ( left, BTS; right, eDNA)

Run through by family/species that have a minimum of data

For eDNA any level (surface to bottom at a station is a positive)

Vertical distribution

For eDNA, a positive hit for any bottle at a depth is a hit

Or Pie charts ??

Discussion

Refer to efforts at Smithsonian on gene skimming of voucher material (update?)

Conclusions

1. Reference libraries (I suspect that these will have low coverage for all species but reasonable coverage for fishery species).

1. Geographic distribution (Venn diagrams of trawl, eDNA and both for the season)

**eDNA species match notes**

No ambiguity (n = 5)

**Cyclopsettidae:** *Syacium papillosum* (Dusky flounder)

* Description: A genus with 3 species, southern to NC

**Cyclopsettidae:** *Citharichthys arctifrons* (Gulf stream flounder)

* Description: A small common on cont shelf; other species are inshore, bays and estuaries

**Cyclopsettidae:** *Etropus microstomus* (Smallmouth flounder)

* Description:

**Bothidae:** *Bothus robinsi* (Twospot flounder)

* Description:

**Scophthalmidae:** *Scophthalmus aquosus* (Window pane)

* Description: Continental shelf and inshore

Resolved ambiguity (n = 4)

**Paralichthyidae:** *Paralichthys oblongus* (Fourspot Flounder)

* This is ascribed to wrong genus in the initial match (American fourspot flounder, Hippoglossina oblonga)

**Paralichthyidae:** *Paralichthys dentatus* (Summer flounder)

* Matches also *Paralichthys adspersus*, an eastern Pacific species

**Pleuronectidae:** *Hippoglossus hippoglossus* (Atlantic Halibut)

* Atlantic halibut based on locality (other two matches are Pacific species: Pacific halibut *Hippoglossus stenolepis* and shotted halibut *Eopsetta grigorjewi*)

**Pleuronectidae:** Witch flounder, *Glyptocephalus cynoglossus*

* Matches alsoRex sole, *Glyptocephalus zachirus,* a Pacific species

Unresolved ambiguity (n = 2)

**Pleuronectidae spp1:** American plaice or winter flounder Hippoglossoides platessoides

* This should be ascribed to the common family (Pleuronectidae spp.)

**Pleuronectidae spp2:** Winter and Yellowtail Flounder Pleuronectidae spp. The winter flounder assignment may be wrong, since that may be an error in GenBank considering the morphological similarities between winter flounder and yellowtail flounder.

* This sequence also matches 100% with Limanda limanda (Common Dab; an Eastern Atlantic species), Pleuronectes platessa (European Plaice; an Eastern Atlantic fish), Platichthys flesus (European Flounder; an Eastern Atlantic species. Its introduction to the Great Lakes, Canada, and US has not been established.), Limanda sakhalinensis (Sakhalin Sole; a western Pacific species) and x number others (listing genera!).

Old abstract

Analysis of environmental DNA holds promise for monitoring biodiversity in a changing ecosystem and may have broad applications for resource assessments. In a case study of flatfish, we check for the sufficiency of a common eDNA mitochondrial marker (i.e., the Riaz 12S primer set) to match all 47 known species (8 families) of the Pleuronectiformes documented in the western Atlantic Ocean, from North Carolina to the Gulf of Maine. Referencing nucleotides posted to GenBank ([https://www.ncbi.nlm.nih.gov](https://www.ncbi.nlm.nih.gov/)), there are full sequences for 21 flatfish species (plus 2 partial sequences) relative to the Riaz 12S marker, meaning that 51% of the 47 species do not have reference sequences, as of September, 2022. In a real test of the Riaz 12S marker, we compare the flatfish taxa actually observed by two surveys across this region in the fall of 2019: 1) a bottom trawl survey that identified fish with traditional morphological traits, and 2) an oceanographic survey that identified species from amplicon sequence variants (ASVs) detected from filtered water samples using the 12S marker. The bottom trawl caught 12 different flatfish taxa in X tows (2 were identified as sp.). The eDNA survey ‘caught’ 11 flatfish taxa in X water samples (2 were identified to the family level), and X taxa were observed by both survey methods. In terms of identifying flatfish diversity, more work is needed to shore up the reference material in GenBank. In terms of fishery applications, 5 flatfishes with formal assessments are represented by sequences in GenBank (*Scophthalmus aquosus*, *Paralichthys dentatus*, *P. lethostigma*, *Glyptocephalus cynoglossus*, *Hippoglossus hippoglossus*), and an additional 2 sequences that can only be identified to the family level (Pleuronectidae 1: either *Hippoglossoides platessoides* or *Pseudopleuronectes americanus*; Pleuronectidae 2: *Pseudopleuronectes americanus* or *Myzopsetta ferruginea*). There is an initiative by NOAA Fisheries at the Smithsonian to improve reference materials, as well as potential for multiple markers to improve taxonomic identifications.

Too many words