Metabarcoding quick looks

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Metabarcoding detections

Through metabarcoding, we can make progress towards characterizing the community of nearshore fishes of Casco Bay without physically handling or even viewing them. A subset of the Summer 2023 GMRI CBASS eDNA filters were analyzed by the University of New Hampshire, a partner with metabarcoding equipment. Here's a quick look into what was detected.

Table 1: Native species metabarcoding detections

Genus	Species	Common name
Acipenser	brevirostrum	shortnose sturgeon
Alosa	aestivalis	blueback herring
Alosa	pseudoharengus	alewife
Alosa	sapidissima	american shad
Ameiurus	nebulosus	brown bullhead
Anguilla	rostrata	american eel
Apeltes	quadracus	fourspine stickleback
Brevoortia	patronus	atlantic menhaden
Catostomus	commersonii	white sucker
Clupea	harengus	atlantic herring
Couesius	plumbeus	lake chub
Fundulus	diaphanus	banded killifish
Fundulus	heteroclitus	mummichog
Gasterosteus	aculeatus	threespine stickleback
Glyptocephalus	cynoglossus	witch flounder
Lepomis	gibbosus	pumpkinseed
Lophius	americanus	american angler
Menidia	menidia	atlantic silverside
Microgadus	tomcod	atlantic tomcod
Micropterus	salmoides	largemouth bass
Micropterus	treculii	smallmouth bass
Morone	saxatilis	striped bass
Myoxocephalus	scorpius	shorthorn sculpin
Notemigonus	crysoleucas	golden shiner
Osmerus	mordax	rainbow smelt
Perca	flavescens	yellow perch
Pholis	gunnellus	rock gunnel
Pseudopleuronectes	americanus	winter flounder
Pungitius	pugnitius	ninespine stickleback
Rhinichthys	atratulus	blacknose dace
Semotilus	atromaculatus	creek chub
Semotilus	corporalis	fallfish
Tautogolabrus	adspersus	cunner

Though it offers an incredible remote sensing opportunity to detect cryptic species in marine environments, metabarcoding is not perfect. Sequences may be ascribed to closely-related, yet incorrect species. These data have several examples of this occurring. For many of these species, these are likely misattributions of native species genetic sequences (alewife, sand lance, sculpin, chain or redfin pickerel, various local minnows) to congeners native to other regions (allis shad, lesser sand-eel, great sculpin, grass pickerel, striped shiner, fathead minnow). In the future, we may "fix" these data by manually changing these suspected misattributions to the appropriate native species.

Other detected non-natives are a bit more of a mystery. Some may be true detections of range expansions or aquarium releases (ill-fated to die in the estuary in the winter). The unexplained remainder could be an error in the metabarcoding process.

Table 2: Non-native species metabarcoding detections

Genus	Species	Common name	Native Range
Alosa	alosa	allis shad	Europe
Ammodytes	tobianus	lesser sand-eel	Europe
Ancistrus	temminckii	armored suckermouth catfish	South America
Esox	niger	grass pickerel	Inland North America
Gambusia	affinis	western mosquitofish	Inland North America
Luxilus	cornutus	striped shiner	Inland North America
Myoxocephalus	polyacanthocephalus	great sculpin	North Pacific
Pimephales	promelas	fathead minnow	Inland North America
Salmo	trutta fario	river trout	Europe
Salvelinus	leucomaenis	whitespotted char	North Pacific

Seine-metabarcoding detection agreement

We can pair this subset of eDNA data to our seine catch data to identify species and locations which have good agreement between the detection methods.

Caught and identified via metabarcoding

Out of 968 species detections over 22 samples, relatively few species were successfully detected via both metabarcoding and the seine. These were alewife, atlantic silverside, atlantic tomcod, and mummichog. It should be noted that these species are among the 10 most commonly-caught species in the seine survey. With the exception of tomcod, they are all also pelagic species which occupy the water column. DNA shed by individuals of these species has a good chance of ending up in the surface layer that we sample.

Table 3: Detected via both metabarcoding and seine

Genus	Species	Common name	Date	Site	Count
Alosa	pseudoharengus	alewife	2023-07-19	Audubon	38
Alosa	pseudoharengus	alewife	2023-07-19	Mackworth Beach	2500
Alosa	pseudoharengus	alewife	2023-07-19	Presumpscot Mooring	2
Alosa	pseudoharengus	alewife	2023-07-19	Skitterygusset	13
Alosa	pseudoharengus	alewife	2023-07-26	Audubon	17
Alosa	pseudoharengus	alewife	2023-07-26	Skitterygusset	75
Alosa	pseudoharengus	alewife	2023-08-07	Back Cove	2
Fundulus	heteroclitus	mummichog	2023-07-19	Back Cove	1
Fundulus	heteroclitus	mummichog	2023-07-26	Skitterygusset	13
Fundulus	heteroclitus	mummichog	2023-08-07	Back Cove	1
Fundulus	heteroclitus	mummichog	2023-08-07	Mackworth Beach	16
Fundulus	heteroclitus	mummichog	2023-08-07	Mackworth North	1
Fundulus	heteroclitus	mummichog	2023-08-07	Mussel Cove	99
Menidia	menidia	atlantic silverside	2023-07-19	Audubon	3
Menidia	menidia	atlantic silverside	2023-07-19	Skitterygusset	1
Menidia	menidia	atlantic silverside	2023-07-26	Audubon	29
Menidia	menidia	atlantic silverside	2023-08-07	Back Cove	18
Menidia	menidia	atlantic silverside	2023-08-07	Mackworth Beach	159
Menidia	menidia	atlantic silverside	2023-08-07	Mackworth North	131
Menidia	menidia	atlantic silverside	2023-08-07	Mussel Cove	699
Microgadus	tomcod	atlantic tomcod	2023-07-11	SMCC	1
Microgadus	tomcod	atlantic tomcod	2023-08-07	Mackworth Beach	1

Caught, but not detected via metabarcoding

There are very few instances where species caught in seines are not also detected in metabarcoding results. Most of these – sand lance, sculpins, pipefish, eel – are demersal (bottom-dwelling) fishes. There is no guarantee that shed DNA from demersal or benthopelagic species would be mixed through the water column to be captured at the surface. This is likely a factor in their limited detections.

In the case of sand lance *Ammodytes americanus*, we detected a sequence from its non-native congener the lesser sand-eel *Ammodytes tobianus* at the same sites on the same days. This is a huge clue that we can reassign detections of *A. tobianus* to *A. americanus*.

There are some similar congener matches for sculpins. We did not always identify to the species level, though our two most commonly-caught sculpin species of the last several years have been grubbies and shorthorn sculpin. There is one instance in which we caught a grubby scuplin *Myoxocephalus aenaeus* at the same place and time as we detected its congener the great sculpin *Myoxocephalus polyacanthocephalus*.

The pelagic (water column) fishes not detected via eDNA but caught in the seine included "killifish"– a catchall term which here may have referred to mummichog *Fundulus heteroclitus*, which was detected via metabarcoding at the same time and place.

Table 4: Detected via seine, but not metabarcoding

Common name	Date	Site	Count
american eel	2023-07-26	Presumpscot Mooring	1
grubby sculpin	2023-07-24	Cushing Island	1
grubby sculpin	2023-08-07	Mackworth North	3
killifish	2023-08-07	Mussel Cove	1
ninespine stickleback	2023-07-24	SMCC	2
pipefish	2023-07-11	SMCC	13
pipefish	2023-07-24	Brothers	3
pipefish	2023-07-24	SMCC	2
pipefish	2023-08-07	Mackworth North	3
sand lance	2023-07-11	Cushing Island	3
sand lance	2023-07-14	Alewife Cove	3
sand lance	2023-07-26	Alewife Cove	12
sculpin	2023-07-11	Cushing Island	3
sculpin	2023-07-11	Great Diamond Island	1
sculpin	2023-07-11	SMCC	3
sculpin	2023-07-24	Brothers	1
sculpin	2023-07-24	SMCC	1
sculpin	2023-08-07	Mussel Cove	2
white mullet	2023-08-07	Mackworth Beach	161

Detected via metabarcoding, but not caught

We know that DNA persists in aquatic environments for variable durations depending on currents, sunlight, temperature, and other factors. It is not surprising that there are many species that we detected via metabarcoding, but did not physically sample. DNA may remain in the areas around the seine sites, or be carried there, without the fish being there.

Species that are detected via metabarcoding, but not caught in our seines likely include many larger-bodied fishes that would inhabit deeper areas just outside the reach of the net. These include shortnose sturgeon, witch flounder, American angler (monkfish), and striped bass. This group could also contain many demersal fishes which aggregate within and around complex habitat (boulders, etc) not well sampled by the net. These would include cunner, rock gunnel, and sculpins.

Still others species in this group include fishes that form large, dense schools. These include many species we commonly catch, include alewife, Atlantic herring, Atlantic menhaden, and Atlantic silverside. These fishes could produce large amounts of shed DNA in the areas near our seine site without actually being in the towpath.

Table 5: Detected via metabarcoding, but not caught

Genus	Species	Common name	Edna detections
Acipenser	brevirostrum	shortnose sturgeon	4
Alosa	aestivalis	blueback herring	5
Alosa	alosa	allis shad	13
Alosa	pseudoharengus	alewife	4
Alosa	sapidissima	american shad	1
Ameiurus	nebulosus	brown bullhead	3
Ammodytes	tobianus	lesser sand-eel	2
Ancistrus	temminckii	armored suckermouth catfish	1
Anguilla	rostrata	american eel	12
Apeltes	quadracus	fourspine stickleback	4
Brevoortia	patronus	atlantic menhaden	12
Catostomus	commersonii	white sucker	10
Clupea	harengus	atlantic herring	5
Couesius	plumbeus	lake chub	3
Esox	niger	grass pickerel	2
Fundulus	diaphanus	banded killifish	1
Fundulus	heteroclitus	mummichog	7
Gambusia	affinis	western mosquitofish	1
Gasterosteus	aculeatus	threespine stickleback	3
Glyptocephalus	cynoglossus	witch flounder	1
Lepomis	gibbosus	pumpkinseed	9
Lophius	americanus	american angler	1
Luxilus	cornutus	striped shiner	4
Menidia	menidia	atlantic silverside	4
Microgadus	tomcod	atlantic tomcod	1
Micropterus	salmoides	largemouth bass	2
Micropterus	treculii	smallmouth bass	7
Morone	saxatilis	striped bass	11
Myoxocephalus	polyacanthocephalus	great sculpin	5
Myoxocephalus	scorpius	shorthorn sculpin	1
Notemigonus	crysoleucas	golden shiner	4
Osmerus	mordax	rainbow smelt	1
Perca	flavescens	yellow perch	6
Pholis	gunnellus	rock gunnel	5
Pimephales	promelas	fathead minnow	1
Pseudopleuronectes	americanus	winter flounder	2
Pungitius	pugnitius	ninespine stickleback	5
Rhinichthys	atratulus	blacknose dace	5
Salmo	trutta fario	river trout	2
Salvelinus	leucomaenis	whitespotted char	1
Semotilus	atromaculatus	creek chub	4
Semotilus	corporalis	fallfish	3
Tautogolabrus	adspersus	cunner	2