Monitoring Fish Communities in Nova Scotia using Environmental DNA

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1 Executive Summary

Fisheries and Oceans Canada (DFO) is using environmental genomics to monitor the fish biodiversity of harbour and offshore marine ecosystems in and around Nova Scotia. Here, we report on the analysis of 96 water samples and four field blanks collected from harbours in August 2021, received as filters and preserved in Longmire's buffer, and six samples collected in October 2020 from offshore sites in the Scotian Shelf bioregion for the Atlantic Zone Monitoring Program, received as DNA extracts. All samples analyzed for this report were successfully extracted and sequenced.

Two DNA markers targeting fish (12S and 16S) were amplified and sequenced in all samples. Based on the available reference data, we identified 20 fish families, 24 genera, and 23 species across all harbour and offshore samples, including commercial pelagic fish species such as herring, mackerel, and tuna.

Fish taxa were detected at all 16 bottom harbour sites, 15 of the 16 surface harbour sites, and five of the six offshore sites and in 79.4% of all samples. The most frequently detected families were Belonidae (35.3% of samples) and Scrombidae (33.3% of samples). The most frequently detected fish species included commercial pelagic fish, Atlantic mackerel, *Scomber scombrus* (32.4% of samples) and Atlantic herring, *Clupea harengus* (15.7% of samples), as well as Atlantic saury, *Scomberesox saurus* (30.4% of samples) and cunner, *Tautogolabrus adspersus* (15.7% of samples). Twelve of the 21 species found in harbour samples were found in both surface and bottom samples. More species were identified in bottom samples than surface samples, with eight species identified in only bottom samples, but one species was only identified in surface samples.

This report presents biodiversity data generated using environmental genomics for fish communities in two different marine habitats to support monitoring programs in the Maritimes. Taxonomy tables with fish detections by site have been provided to facilitate further analysis. In addition to taxonomic data, unique DNA sequences can provide a high-resolution, taxonomy-free approach to measuring and tracking biodiversity.

2 Background

Environmental genomics is a novel approach to biodiversity characterization that does not require collection of whole biological specimens but instead relies on recovery and analysis of DNA from the physical environment in which they live (e.g., water, soil, sediment, etc.). This environmental DNA (eDNA) is released from organisms through various mechanisms including cell shedding and the excretion of various bodily fluids and feces [1–3]. DNA is isolated from samples, biodiversity informative regions of DNA are amplified through polymerase chain reaction (PCR), and then these regions are sequenced using high-throughput genomic sequencing platforms. The resulting DNA sequences are filtered through a variety of quality control and assurance steps and then compared to publicly available databases (e.g., GenBank) where the genomic information for known specimens has been deposited. When a match is found, a taxonomic identification can be assigned to a DNA sequence from an environmental sample[1–3].

There are several advantages to environmental genomics compared to the conventional approach to biodiversity characterization. It is non-invasive and does not require the capture of a whole biological specimen. Very small samples of water (~250 mL) or sediment (~5 g) are required for a biodiversity assessment because, unlike conventional surveys, organisms at all trophic levels from algae to large mammals can be detected from the same environmental sample. The technique is highly sensitive, which means rare or elusive species, including endangered and invasive species, can be detected through their DNA in addition to the more common species. It is cost effective with time savings during field sampling and sample analysis. Furthermore, high-throughput DNA sequencing technology allows for simultaneous analysis of a large set of samples.

Because environmental genomics is a novel approach to biodiversity characterization, it is not widely applied yet. However, in recent years, there has been a steady increase in its application to various environmental characterization efforts [4–6]. While the sensitivity of environmental genomics is one of the many appeals of this technology, it is also one of its potential limitations as contaminants of biological nature can also be detected within the environmental sample. To manage this, many precautions are taken during the acquisition, preservation, and processing of samples to limit the introduction of contaminants (e.g., collection of field blanks as a negative control). In addition, stringent quality control measures during data analysis can limit false positives.

Monitoring the biodiversity of marine ecosystems is important for assessing human impacts and informing management decisions. For this project, Fisheries and Oceans Canada (DFO) is using environmental genomics for biodiversity monitoring, particularly of fish communities, in the Maritimes region. This report includes the analysis of water samples collected in harbours as well as samples collected offshore from the Scotian Shelf bioregion for the Atlantic Zone Monitoring Program.

3 Methods

3.1 Sample Submission

Water samples were collected by DFO from 16 harbour sites in Halifax, Nova Scotia in August 2021. At each site, 1L samples were collected in triplicate from the bottom and the surface. Field negative controls were also collected during sampling. The samples and field blanks were filtered onto Sterviex filters by DFO, preserved in Longmire's buffer, and submitted to CEGA for laboratory analysis. Additionally, 1L environmental water samples were taken from six offshore sites around Nova Scotia for the Atlantic Zone Monitoring Program in October 2020. These samples were filtered, and DNA was extracted by DFO. DNA extracts were submitted to CEGA for laboratory analysis.

In total, CEGA received 113 filters (96 environmental samples and 17 field blanks) preserved in Longmire's buffer from Maritime harbours and six DNA extracts from the Atlantic Zone Monitoring Program. Thirteen field blanks were excluded from subsequent analysis at DFO's request. All samples were received frozen and were stored at -80°C until processing.

3.2 Sample Processing

DNA was extracted from filters for each harbour sample and four field blanks were processed as requested. The DNA was quantified for all harbour samples (**Table 1**), the four field blanks that were processed (**Table 2**), as well as all the offshore samples (**Table 3**). In total, 106 samples (102 environmental samples and 4 field blanks) were processed.

Two DNA markers for fish biodiversity were amplified from two gene regions: 12S using the MiFishU primer set[7] and 16S using the primer set from McInnes et al. (2017)[8]. Additional negative controls were added during extraction and PCR stages and all negative controls, including field blanks, were carried through subsequent stages. All samples, including field and lab negative controls, were sequenced on an Illumina NovaSeq 6000 instrument using a 2 x 150 cycle SP kit and a minimum target depth of 1,000,000 reads per sample per marker.

3.3 Data Analysis

Sequences were filtered to meet minimum quality and length thresholds. DADA2[9] was used to truncate, merge, denoise, and detect chimeric sequences for 12S sequences to create exact sequence variants (ESVs), each of which represent a unique sequence from the sample. For 16S sequences, these steps were conducted using VSEARCH's implementation of UNOISE3 and UCHIME3[10] to create ESVs because our empirical testing showed that VSEARCH produces better results for 16S than DADA2.

ESVs were then assigned taxonomy by comparing each sequence against NCBI's Nucleotide reference database (downloaded July 2022), using the megablast algorithm. The taxonomy presented here matches the naming conventions used in NCBI's taxonomy database and was assigned based on a sequence similarity score (the product of the % sequence identity and the % query coverage). Species was assigned at a score of 99%, genus was assigned at a minimum score of 98%, family was assigned at minimum score of 95%, order and class were assigned at a minimum score of 90%, and kingdom and phylum were assigned at minimum score of 85%. Taxonomic identifications were verified against the World Register of Marine Species (WoRMs), the Global Biodiversity Information Facility (GBIF), and the

Encyclopedia of Life (EOL) to ensure that spurious matches resulting from poor reference database coverage were not included in the list. For this report, only ESVs that were attributed to fish (class Actinopteri and class Chondrichthyes with score ≥ 90%) were retained.

Table 1. List of water eDNA samples collected from Halifax harbours in August 2021 that were received for analysis as filters with their DNA concentrations.

Sample Name	[DNA] (pg/µL)
485525	621
485526	1650
485532	383
485533	1995
485539	741
485540	1213
485543	707
485544	1049
485545	807
485546	1893
485547	523
485548	2082
485563	1792
485564	1882
485568	334
485569	2161
485573	822
485574	1821
485589	1135
485590	1644
485594	1928
485595	1063
485599	3961
485600	1714
485603	500
485604	317
485605	572
485606	1722
485607	325
485608	1922
485610	454
485611	1456
485612	902
485613	1716
485614	781
485615	1705

Sample Name	[DNA] (pg/µL)
485617	1867
485618	2984
485619	1277
485620	1609
485621	1251
485622	2455
485624	721
485625	793
485626	1254
485627	1745
485628	454
485629	1375
485631	781
485632	3257
485633	905
485634	3719
485635	845
485636	4361
485638	758
485639	5165
485640	741
485641	4376
485642	509
485643	3666
485645	1098
485646	2665
485647	1133
485648	2817
485649	1375
485650	3687
485652	1176
485653	3852
485654	1358
485655	4887
485656	1699
485657	4691

Sample Name	[DNA] (pg/µL)
485659	615
485660	911
485661	454
485662	1517
485663	598
485664	756
485666	388
485667	1896
485668	1006
485669	1801
485670	331
485671	2353
485673	919
485674	2762
485675	948
485676	2548
485677	603
485678	4198
485680	1285
485681	2504
485682	635
485683	2820
485684	1511
485685	2750

Table 2. List of field blanks collected in August 2021 that were received as filters with their DNA concentrations. Only field blanks that were processed are listed here. BQL indicates that the DNA in that sample was below quantification limit.

Sample Name	[DNA] (pg/µL)
485609	22
485658	BQL
485686	BQL
485549	BQL

Table 3. List of water eDNA samples collected from offshore Nova Scotia in October 2020 that were received for analysis as DNA extracts with their DNA concentrations. BQL indicates that the DNA in that sample was below quantification limit.

Sample Name	[DNA] (pg/µL)
DFOGUL21_41102	51
DFOGUL21_41103	91
DFOGUL21_41104	265
DFOGUL21_41105	BQL
DFOGUL21_41106	1138
DFOGUL21_41107	279

4 Results

DNA was successfully extracted and sequenced from all samples analyzed for this report. Across all 96 harbour samples and 6 offshore samples, an average of 887,008 reads (range: 1 – 3,027,804 reads) per sample per marker were generated. After filtering sample data for target taxa and screening out possible contamination, a total of 49,142,452 target sequence reads were generated, resulting in an average of 431,072 reads (range: 1 – 2,293,819 reads) per sample per marker with an average of 346 target ESVs (range: 1 – 1873 ESVs) per sample for 12S and 9 target ESVs (range: 1 – 64 ESVs) per sample for 16S.

We identified 20 families, 24 genera, and 23 species of fish from all harbour and offshore samples, including several commercially important pelagic fish species such as Atlantic herring, *Clupea harengus*, Atlantic mackerel, *Scomber scombrus*, and tuna, *Thunnus obesus* and *Thunnus thynnus* (see **Table A1** for a complete list of fish taxa identified).

Fish taxa were detected in 79.4% of all samples and at all 16 bottom harbour sites, 15 of the 16 surface harbour sites, and five of the six offshore sites (see **Supplementary Table 1** for a taxonomy table of detections by site and **Supplementary Table 2** for a table of ESV detections by site with taxonomic information). The most frequently detected family was Belonidae (35.3% of samples), followed by Scombridae (33.3% of samples; **Figure 1**). The most frequently detected fish species were Atlantic mackerel, *Scomber scombrus* (32.4% of samples), followed by Atlantic saury, *Scomberesox saurus* (30.4% of samples), Atlantic herring, *Clupea harengus* (15.7% of samples) and cunner, *Tautogolabrus adspersus* (15.7% of samples; **Figure 2**).

Of the 21 fish species identified in the harbour samples, more species were identified in the bottom samples (20 species) than in the surface samples (13 species; **Figure 3**). While the bottom and surface samples shared 12 species, unique fish species were identified in both bottom (8 species) and surface (1 species) samples.

Sequence data and taxonomy tables listing fish taxa detected across all sites will be provided (see **Table B1** for a list of all files to be shared). In the taxonomy tables, please note that rows with taxa that do not have higher resolution taxonomy assigned (i.e., no species or genus level identification) represent sequences that could not be assigned any higher resolution taxonomy. These rows represent unique observations from rows that were assigned higher resolution taxonomy. For example, in **Table 4** below, the observations in row 3 do not include the observations in row 1 and 2. Row 1 and 2 represent sequences that were identified to each species respectively and Row 3 only includes sequences that were identified as *Sebastes* but could not be assigned a species name.

Table 4. Example taxonomic data to illustrate how taxonomy results are presented in the data files.

	Order	**Family**	**Genus**	**Species**
1	Perciformes	Sebastidae	*Sebastes*	*Sebastes fasciatus*
2	Perciformes	Sebastidae	*Sebastes*	*Sebastes mentella*
3	Perciformes	Sebastidae	*Sebastes*	

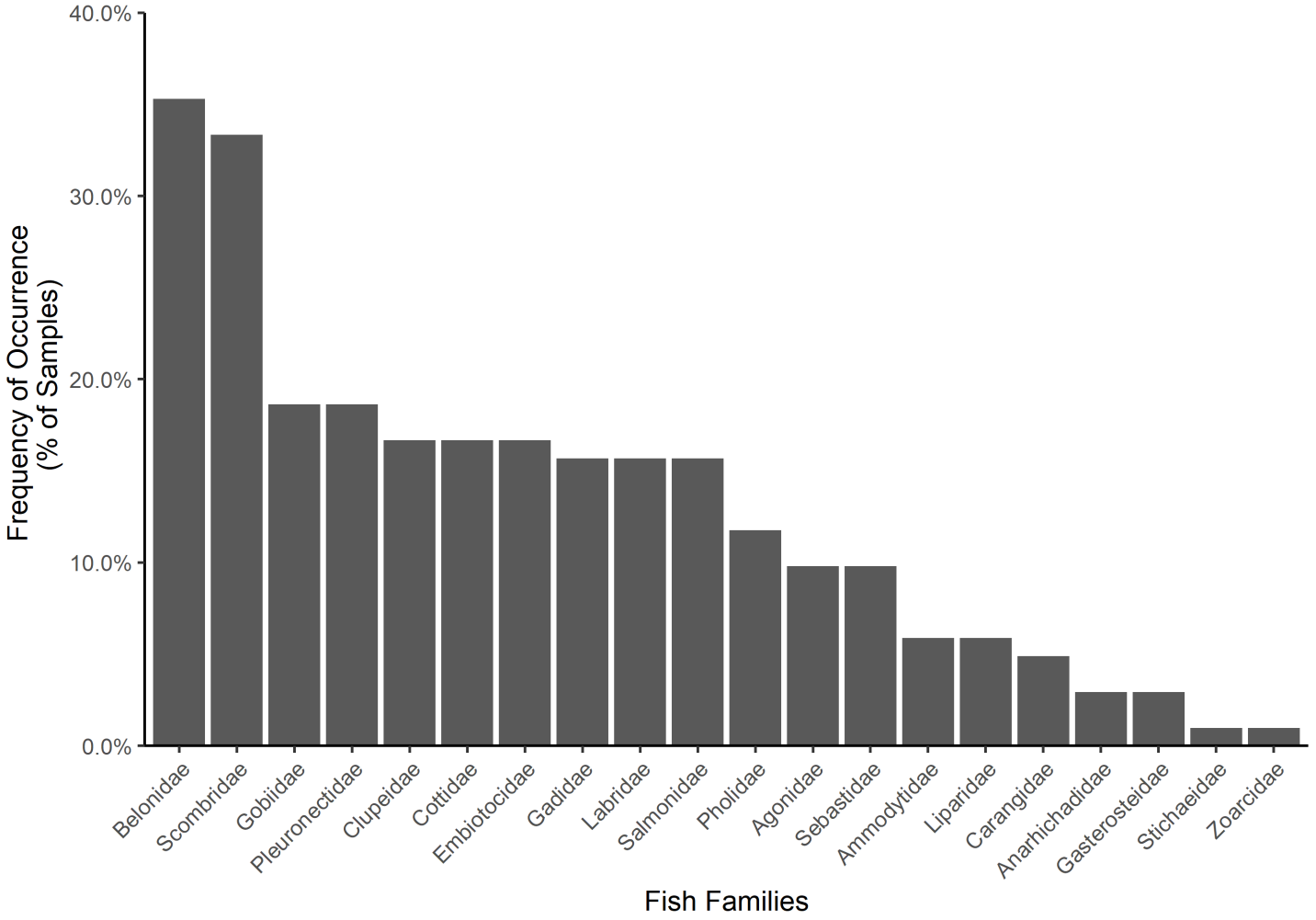

Figure 1. Frequency of occurrence of fish families (% of samples) across all harbour and offshore samples collected in Nova Scotia in 2020 and 2021 (n = 102). The most frequently detected families were Belonidae (35.3% of samples) and Scombidae (33.3% of samples).

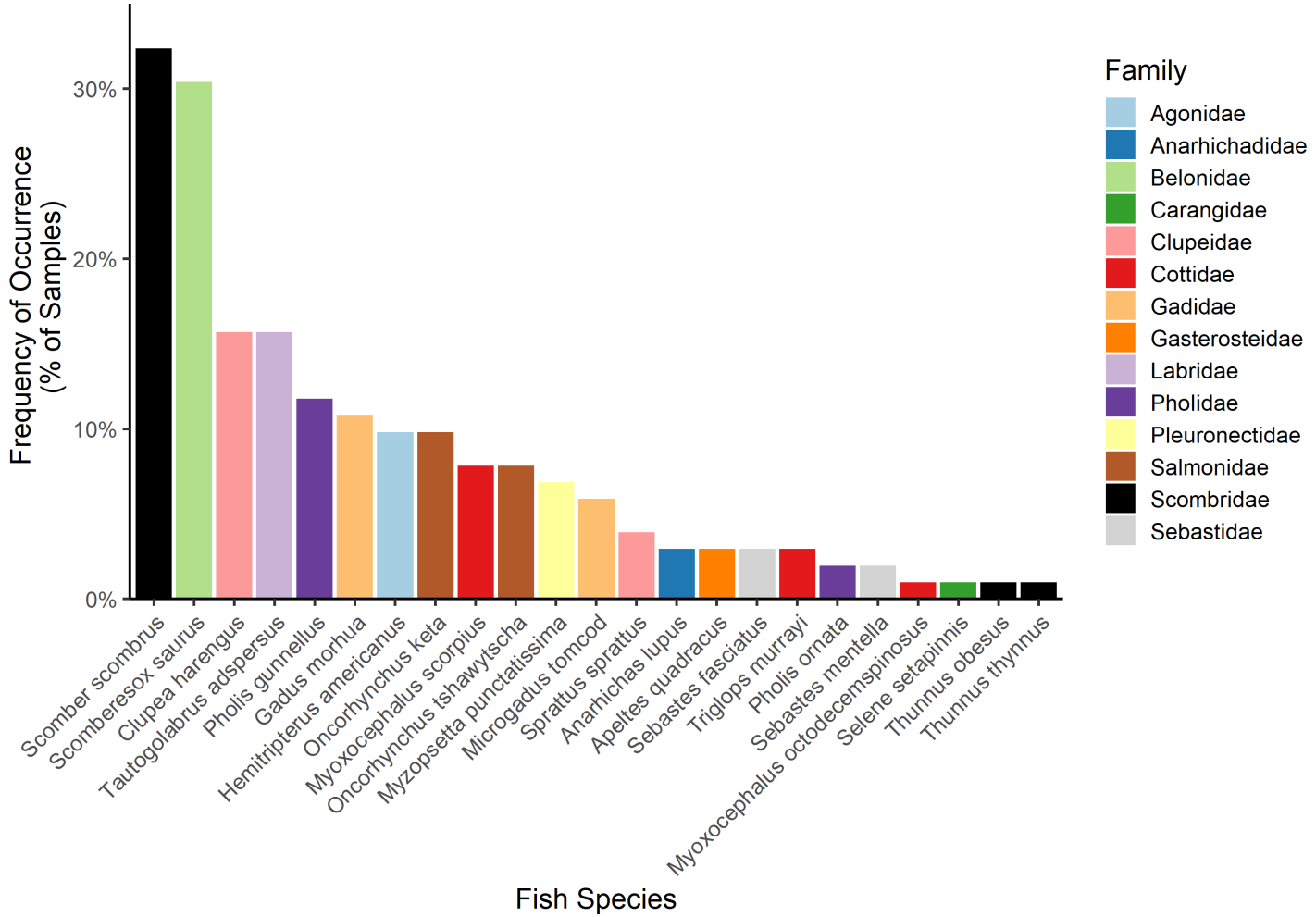

Figure 2. Frequency of occurrence of fish species (% of samples) across all harbour and offshore samples collected in Nova Scotia in 2020 and 2021 (n = 102). The most frequently detected species were *Scomber scombrus* (32.4% of samples), *Scomberesox saurus* (30.4% of samples), *Clupea harengus* (15.7% of samples) and *Tautogolabrus adspersus* (15.7% of samples).

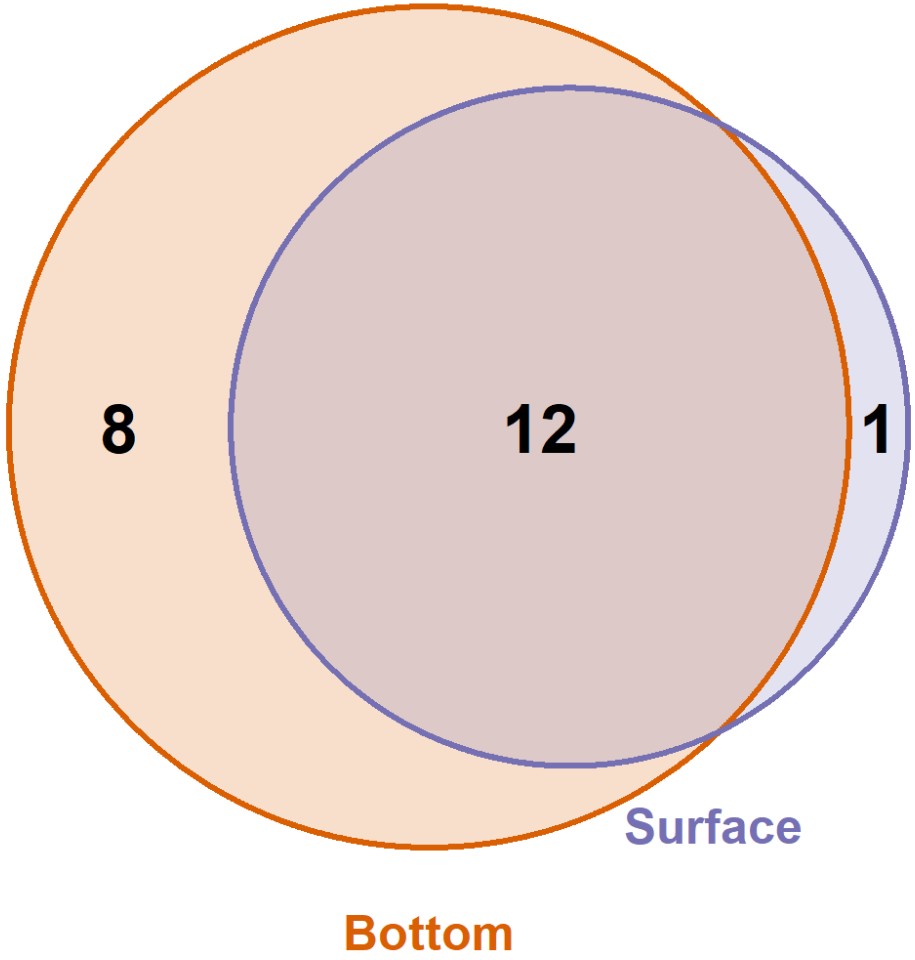

Figure 3. Venn diagram of the number of fish species detected in bottom (n = 48; 20 species total) and surface (n = 48; 13 species total) water samples collected in Halifax harbours in 2021. Twelve species were identified in both bottom and surface samples.

5 Conclusions

Using an environmental genomics approach with two DNA markers (12S and 16S), we identified 20 fish families, 24 genera, and 23 species based on available reference data across 102 harbour and offshore water eDNA samples. This included important commercial pelagic fish species such as Atlantic herring, *Clupea harengus*, Atlantic mackerel, *Scomber scombrus*, and tuna, *Thunnus obesus* and *Thunnus thynnus*.

Fish were detected at all 16 bottom harbour sites, 15 of the 16 surface harbour sites, and five of the six offshore sites and detected in 79.4% of all samples. The most frequently detected families were Belonidae and Scrombidae and the most frequently detected fish species included commercial fish, Atlantic mackerel and Atlantic herring, as well as Atlantic saury and cunner. Of the 21 species detected in harbour samples, 12 species were detected in both surface and bottom samples. More species were identified in bottom samples than surface samples, with eight species identified in only the bottom samples.

This report presents fish biodiversity data from two marine habitats to support monitoring programs in the Maritimes. Future eDNA samples from the study areas can be processed and analyzed using the same laboratory and bioinformatics protocols and compared to the results in this report. Raw sequence data and taxonomy tables listing fish taxa detected across all sites have been provided to facilitate further analysis.

5.1 Notes on the Interpretation of eDNA Results

Taxonomic annotation of environmental DNA is heavily reliant on the quality and completeness of reference databases, which varies between genetic markers [11]. The ESVs with unassigned taxonomies represent organisms whose DNA may not have been sequenced yet by the general scientific community and would therefore not be represented in the publicly available reference databases. Unidentified ESVs are useful in a general analysis of biodiversity and can be informative for comparative biodiversity analyses. As databases continue to be populated and bioinformatics methods are advanced, these unidentified ESVs may be able to be assigned taxonomy in future. Additionally, errors in public reference databases exist and can potentially introduce false positives. Despite our best efforts to minimize the impact of erroneous reference sequences, there is a possibility for false assignments from these errors.

While this report includes a comprehensive list of species detected using environmental genomics, it is possible that some species present in the environment were not detected. False negatives may be due to species being absent from the reference database (as discussed above), or due to a lack of genetic resolution (i.e., high genetic similarity) between sister taxa which can lead to ambiguous or false database assignments. Alternatively, inefficiencies during the PCR stage of sample processing due to the variability in genetic markers between taxonomic groups can bias the recovery of DNA sequences in environmental genomic analyses [11]. A multi-marker approach is used to minimize the potential impact of these limitations by increasing the likelihood of DNA recovery, sequence resolution, and taxonomic annotation for the taxa of interest relative to any one DNA marker.

6 References

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7 Appendix A

Table A1. Complete taxonomic list of fish (class Actinopteri and class Chondrichthyes) detected in all harbour and offshore samples collected in Nova Scotia in 2020 and 2021 (n = 102). The number of detections in each sample location (harbour surface, harbour bottom, or offshore) is listed for each taxon. See *Results* for guidance on interpreting the taxonomy presented in this table.

Order	Family	Genus	Species	Harbour Surface (n = 48)	Harbour Bottom (n = 48)	Offshore (n = 6)
Beloniformes	Belonidae	Scomberesox	*Scomberesox saurus*	18	11	2
Beloniformes	Belonidae			6	2	
Beloniformes				7	3	
Carangiformes	Carangidae	Selene	*Selene setapinnis*		1	
Carangiformes	Carangidae	Selene		1	2	2
Carangiformes	Carangidae				1	
Carangiformes					1	
Clupeiformes	Clupeidae	Alosa			1	
Clupeiformes	Clupeidae	Clupea	*Clupea harengus*	2	13	1
Clupeiformes	Clupeidae	Clupea			5	1
Clupeiformes	Clupeidae	Sprattus	*Sprattus sprattus*		4	
Clupeiformes	Clupeidae				6	
Clupeiformes				1	10	
Cypriniformes				1		1
Gadiformes	Gadidae	Gadus	*Gadus morhua*	2	8	1
Gadiformes	Gadidae	Microgadus	*Microgadus tomcod*	3	3	
Gadiformes	Gadidae	Microgadus		1		
Gadiformes	Gadidae			2	2	
Gadiformes				2	1	
Gobiiformes	Gobiidae			8	9	2
Gobiiformes					2	1

Order	Family	Genus	Species	Harbour Surface (n = 48)	Harbour Bottom (n = 48)	Offshore (n = 6)
Labriformes	Labridae	Tautogolabrus	*Tautogolabrus adspersus*	5	10	1
Labriformes	Labridae	Tautogolabrus			3	
Labriformes				4	4	
Perciformes	Agonidae	Hemitripterus	*Hemitripterus americanus*	4	6	
Perciformes	Agonidae	Hemitripterus		1		
Perciformes	Agonidae			1		
Perciformes	Anarhichadidae	Anarhichas	*Anarhichas lupus*		3	
Perciformes	Cottidae	Myoxocephalus	*Myoxocephalus octodecemspinosus*		1	
Perciformes	Cottidae	Myoxocephalus	*Myoxocephalus scorpius*		6	2
Perciformes	Cottidae	Myoxocephalus		1	2	
Perciformes	Cottidae	Triglops	*Triglops murrayi*	2	1	
Perciformes	Cottidae			3	7	1
Perciformes	Gasterosteidae	Apeltes	*Apeltes quadracus*		3	
Perciformes	Gasterosteidae				1	
Perciformes	Liparidae	Liparis		1	2	
Perciformes	Liparidae			2	2	
Perciformes	Pholidae	Pholis	*Pholis gunnellus*	6	6	
Perciformes	Pholidae	Pholis	*Pholis ornata*	2		
Perciformes	Pholidae	Pholis		2		
Perciformes	Pholidae			3		
Perciformes	Sebastidae	Sebastes	*Sebastes fasciatus*		3	
Perciformes	Sebastidae	Sebastes	*Sebastes mentella*		2	
Perciformes	Sebastidae	Sebastes		4	5	
Perciformes	Sebastidae				2	
Perciformes	Stichaeidae			1		

Order	Family	Genus	Species	Harbour Surface (n = 48)	Harbour Bottom (n = 48)	Offshore (n = 6)
Perciformes	Zoarcidae	Gymnelus			1	
Perciformes	Zoarcidae				1	
Perciformes				4	12	1
Pleuronectiformes	Pleuronectidae	Myzopsetta	*Myzopsetta punctatissima*	3	2	2
Pleuronectiformes	Pleuronectidae	Platichthys			1	
Pleuronectiformes	Pleuronectidae	Pseudopleuronectes			1	
Pleuronectiformes	Pleuronectidae			5	8	3
Pleuronectiformes				5	5	2
Salmoniformes	Salmonidae	Oncorhynchus	*Oncorhynchus keta*	3	7	
Salmoniformes	Salmonidae	Oncorhynchus	*Oncorhynchus tshawytscha*	3	5	
Salmoniformes	Salmonidae				1	
Salmoniformes						1
Scombriformes	Scombridae	Scomber	*Scomber scombrus*	16	14	3
Scombriformes	Scombridae	Scomber		4	5	
Scombriformes	Scombridae	Thunnus	*Thunnus obesus*			1
Scombriformes	Scombridae	Thunnus	*Thunnus thynnus*			1
Scombriformes	Scombridae	Thunnus				1
Scombriformes	Scombridae			5	5	1
Scombriformes				6	4	1
Siluriformes						1
Stomiiformes				2	1	1
Unclassified	Embiotocidae			8	8	1
Uranoscopiformes	Ammodytidae	Ammodytes		1	4	1
Uranoscopiformes	Ammodytidae				1	1
Uranoscopiformes					1	

8 Appendix B

Table B1. List of supplementary files to be shared with the report.

Name	Filename	Description
Supplementary Table 1	RPT038-OP02 Supplementary Table 1 R1.xlsx	Complete taxonomic list of fish (class Actinopteri and class Chondrichthyes) identified from harbour and offshore samples in Nova Scotia and the sites they were detected in (presence/absence). See Results section in the report for guidance on interpreting the table.
Supplementary Table 2	RPT038-OP02 Supplementary Table 2 R1.xlsx	Complete list of fish ESVs (class Actinopteri and class Chondrichthyes) identified from harbour and offshore samples in Nova Scotia with the DNA marker, the taxonomic assignment, and the sites they were detected in (presence/absence).
Sequence Data	Multiple files	Compressed FASTQ files (one file per sample per marker per forward and reverse read) where Illumina adapters, indexes, and primer sequences have been trimmed. No other manipulation has been performed.