

# Environmental DNA Analysis of Water eDNA Samples from Musquash Estuary Marine Protected Area

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Centre for Environmental Genomics Applications



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 Centre for Environmental Genomics Applications	Environmental DNA Analysis of Water eDNA Samples from Musquash Estuary Marine Protected Area	
	RPT057-HE01	2023-03-15

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 Centre for Environmental Genomics Applications	Environmental DNA Analysis of Water eDNA Samples from Musquash Estuary Marine Protected Area	
	RPT057-HE01	2023-03-15

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 Centre for Environmental Genomics Applications	Environmental DNA Analysis of Water eDNA Samples from Musquash Estuary Marine Protected Area	
	RPT057-HE01	2023-03-15


## TABLE OF CONTENTS

1	Status Update.....	1
2	References .....	5

 Centre for Environmental Genomics Applications	Environmental DNA Analysis of Water eDNA Samples from Musquash Estuary Marine Protected Area	
	RPT057-HE01	2023-03-15

## LIST OF TABLES

<b>Table 1</b> List of water eDNA samples collected from Musquash Estuary Marine Protected Area in September 2022 that were submitted to CEGA for analysis with their DNA concentrations after extraction. ....	2
<b>Table 2</b> List of lab and field blanks samples collected as part of the Musquash Estuary Marine Protected Area sampling in August 2022 that were submitted to CEGA for analysis with their DNA concentrations after extraction. BQL indicates the DNA concentration was below the quantifiable limit (<0.1 ng/μL).....	4

 Centre for Environmental Genomics Applications	Environmental DNA Analysis of Water eDNA Samples from Musquash Estuary Marine Protected Area	
	RPT057-HE01	2023-03-15


## 1 Status Update

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.)<sup>1-4</sup>. This environmental DNA (eDNA) is released from organisms through various mechanisms including cell shedding and the excretion of various bodily fluids and feces. 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<sup>1-4</sup>. 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. Additionally, organisms at all trophic levels from algae to large mammals can be detected from the same environmental sample.

For this project, Fisheries and Oceans Canada (DFO) is using environmental genomics to characterize marine biodiversity in the Musquash Estuary Marine Protected Area. Eighty-one sea water samples and two field blanks (negative controls) were collected from the area between September 12-13, 2022. Water samples were filtered by DFO, and two lab blanks were generated. Filters were preserved in Longmire's buffer and frozen but thawed during transport to CEGA. To avoid further freeze-thaw cycles, the filters were stored at 4°C until processing.

DNA was extracted from all filters and the DNA in each sample was quantified for sample (**Table 1**) and field blanks (**Table 2**). The mean DNA concentration of samples was 2.07 ng/μL (range: 0.37 – 5.64 ng/μL). The DNA concentrations in the samples are within the typical range of DNA concentration for eDNA water samples. All field and lab blanks had DNA concentrations below the quantification limit (<0.1 ng/μL).


In summary, DNA was successfully extracted from 81 water samples and four blanks collected from Musquash Estuary Marine Protected Area in 2022. Based on the DNA concentrations presented here, we expect to amplify and sequence DNA from all samples. At this stage, we recommend proceeding with PCR amplification and sequencing for all samples. Following our standard practice, all blanks will be carried through the DNA metabarcoding workflow for screening.

 Centre for Environmental Genomics Applications	Environmental DNA Analysis of Water eDNA Samples from Musquash Estuary Marine Protected Area	
	RPT057-HE01	2023-03-15


**Table 1** List of water eDNA samples collected from Musquash Estuary Marine Protected Area in September 2022 that were submitted to CEGA for analysis with their DNA concentrations after extraction.

Sample Name	DNA Concentration (ng/μL)	Sample Name	DNA Concentration (ng/μL)
M06S2	2.54	M28S3	0.42
M04S3	2.33	M28S2	2.39
M04S2	3.26	M28S1	1.42
M04S1	2.20	M26S2	2.34
M02S1	5.64	M26S1	2.54
M01S3	3.59	M24S3	1.59
M01S2	3.44	M24S2	1.37
M01S1	3.73	M32B2	2.10
M11S2	3.84	M32B1	1.53
M11S1	2.89	M32S3	2.04
M08B1	1.18	M32S2	1.64
M08S3	2.36	M30B3	1.88
M08S2	4.06	M30B2	2.84
M07S2	3.06	M30B1	1.94
M07S1	3.20	M30S2	0.87
M06S3	3.02	2B03S3	1.60
M19S2	2.94	2B03S2	1.32
M19S1	2.45	2B03S1	1.19
M16S3	1.51	2B02S2	1.85
M16S2	2.63	2B02S1	2.16
M15S3	2.72	2B01S2	1.30
M15S2	3.54	2B01S1	2.61
M15S1	2.04	M32B3	1.85
M11S3	3.90	301B2	0.90
M24S1	1.33	301B1	1.23
M22S3	2.38	301S3	2.16
M22S2	3.84	301S2	1.93
M22S1	3.56	301S1	1.91
M20B3	2.00	2B03B3	1.25
M20B2	2.53	2B03B2	1.44
M20B1	0.47	2B03B1	1.03
M19S3	2.46	2A01S1	0.86
M30S1	1.59	303S3	0.87




 Centre for Environmental Genomics Applications	Environmental DNA Analysis of Water eDNA Samples from Musquash Estuary Marine Protected Area	
	RPT057-HE01	2023-03-15

Sample Name	DNA Concentration (ng/μL)
303S2	0.52
303S1	1.12
302S3	1.73
302S2	0.37
302S1	1.76
301B3	2.65
2A12B2	1.71
2A12B1	2.17
2A12S3	1.22
2A12S2	1.63
2A12S1	1.62
2A10S3	0.59
2A01S3	1.21
2A01S2	1.61
2A12B3	1.43

 Centre for Environmental Genomics Applications	Environmental DNA Analysis of Water eDNA Samples from Musquash Estuary Marine Protected Area	
	RPT057-HE01	2023-03-15

**Table 2** List of lab and field blanks samples collected as part of the Musquash Estuary Marine Protected Area sampling in August 2022 that were submitted to CEGA for analysis with their DNA concentrations after extraction. BQL indicates the DNA concentration was below the quantifiable limit (<0.1 ng/μL).

Sample Name	DNA Concentration (ng/μL)
LB02	BQL
LB01	BQL
B0913	BQL
B0912	BQL

 Centre for Environmental Genomics Applications	Environmental DNA Analysis of Water eDNA Samples from Musquash Estuary Marine Protected Area	
	RPT057-HE01	2023-03-15

## 2 References

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4. Compson, Z. G., McClenaghan, B., Singer, G. A. C., Fahner, N. A. & Hajibabaei, M. Metabarcoding From Microbes to Mammals: Comprehensive Bioassessment on a Global Scale. *Front. Ecol. Evol.* **8**, 581835 (2020).