Supplementary Data

Saleem et al. 2024. eDNA Metabarcoding-based Source Attribution of Fecal Indicator Bacteria Exceedances in Urban Freshwater Beaches, Sand and Rivers.

Supplementary dPCR method information

Sequences of dPCR primers and probes are shown below. Primer oligos (250 nM) were manufactured by Intregrated Technologies Incorporated. Probes (6000 pM) were manufactured by Life Technologies Inc. dPCR reactions were run in singleton format as previous analyses have shown high concordance between sample dPCR replicates (Edge et al. 2021). The mean number of qualified wells for dPCR assays was 17,466 (HF183/Gull4), 17,951 (HuMt/GooseMt) 17,808 (Dog3), 17,925 (Rum2Bac), and 17,627 (Pig2Bac).

dPCR Oligonucleotide	Sequence
Human HF183	
F primer HF183	ATCATGAGTTCACATGTCCG
R primer BacR287	CTTCCTCTCAGAACCCCTATCC
Probe BacP234MGB	FAM-CTAATGGAACGCATCCC-MGB
Gull4	
F primer qGull7F	CTTGCATCGACCTAAAGTTTTGAG
R primer qGull8R	GGTTCTCTGTATTATGCGGTATTAGCA
Probe qGull7Pb	VIC-ACACGTGGGTAACCTGCCCATCAGA-QSY
Dog3	
F primer DG3F	TTTTCAGCCCCGTTGTTTCG
R primer DG3R	TGAGCGGGCATGGTCATATT
Probe DG3	FAM-AGTCTACGCGGGCGTACT-MGB
Ruminant Rum2Bac	
F primer BacB2-590F	ACAGCCCGCGATTGATACTGGTAA
R primer Bac708Rm	CAATCGGAGTTCTTCGTGAT
Probe BacB2-626P	VIC-ATGAGGTGGATGGAATTCGTGGTGT-QSY
Pig Pig2Bac	
F primer Pig2Bac41F	GCATGAATTTAGCTTGCTAAATTTGAT
R primer Pig2Bac163Rm	ACCTCATACGGTATTAATCCGC
Probe Pig2Bac113MGB	FAM-TCCACGGGATAGCC-MGBNFQ
Human mitochondrial	
F primer HumMtF	CAGCAGCCATTCAAGCAATGC
R primer HumMtR	GGTGGAGACCTAATTGGGCTGATTAG
Probe HumMtPr	VIC - TATCGGCGATATCGGTTTCATCCTCG-QSY
Canada Goose mitochondrial	
F primer GooseMtF	CTAACATCCAAATCCCTCGACCCA
R primer GooseMtR	TCCTATTCAGCCTCCTAGTGCTCT
Probe GooseMtPr	FAM-TACTCACCGCCATAGCCCTAGCCT-QSY

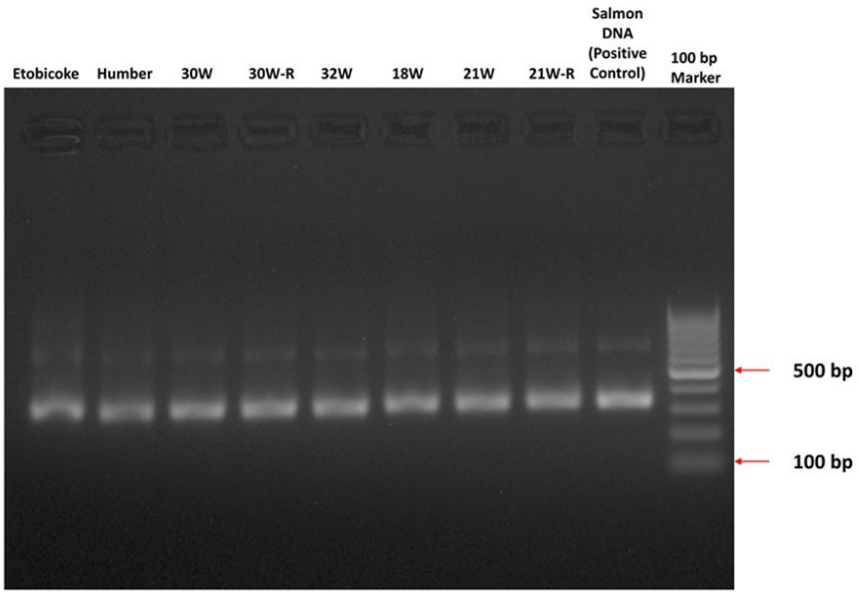
Supplementary Table 1. Description of the 48 samples processed for digital PCR and eDNA metabarcoding.

Sample Name	Sample Description	Sample Type	Sampling Location
MC-Ex-June10	Marie Curtis BAV Exceedance Day	Beach Water	Toronto
MC-Ex-June30	Marie Curtis BAV Exceedance Day	Beach Water	Toronto
MC-Ex-July7	Marie Curtis BAV Exceedance Day	Beach Water	Toronto
MC-Non-ex-June2	Marie Curtis BAV Non-Exceedance Day	Beach Water	Toronto
MC-Non-ex-July19	Marie Curtis BAV Non-Exceedance Day	Beach Water	Toronto
MC-Non-ex-August25	Marie Curtis BAV Non-Exceedance Day	Beach Water	Toronto
SS-Ex-June29	Sunnyside BAV Exceedance Day	Beach Water	Toronto
SS-Ex-June30	Sunnyside BAV Exceedance Day	Beach Water	Toronto
SS-Ex-August25	Sunnyside BAV Exceedance Day	Beach Water	Toronto
SS-Non-ex-June2	Sunnyside BAV Non-Exceedance Day	Beach Water	Toronto
SS-Non-ex-July7	Sunnyside BAV Non-Exceedance Day	Beach Water	Toronto
SS-Non-ex-August5	Sunnyside BAV Non-Exceedance Day	Beach Water	Toronto
Hum-Ex-June29	Humber River BAV Exceedance Day	Source Water	Toronto
Hum-Ex-June30	Humber River BAV Exceedance Day	Source Water	Toronto
Hum-Ex-August25	Humber River BAV Exceedance Day	Source Water	Toronto
Hum-Non-ex-June2	Humber River BAV Non-Exceedance Day	Source Water	Toronto
Hum-Non-ex-July7	Humber River BAV Non-Exceedance Day	Source Water	Toronto
Hum-Non-ex-August5	Humber River BAV Non-Exceedance Day	Source Water	Toronto
ETOB-Ex-June10	Etobicoke Creek BAV Exceedance Day	Source Water	Toronto
ETOB-Ex-June30	Etobicoke Creek BAV Exceedance Day	Source Water	Toronto
ETOB-Ex-July7	Etobicoke Creek BAV Exceedance Day	Source Water	Toronto
ETOB-Non-ex-June2	Etobicoke Creek BAV Non-Exceedance Day	Source Water	Toronto
ETOB-Non-ex-July28	Etobicoke Creek BAV Non-Exceedance Day	Source Water	Toronto
ETOB-Non-ex-August26	Etobicoke Creek BAV Non-Exceedance Day	Source Water	Toronto
LK-Ex-August4	Lakeside Beach BAV Exceedance Day	Beach Water	Niagara
LK-Ex-August9	Lakeside Beach BAV Exceedance Day	Beach Water	Niagara
Sun-Ex-August11	Sunset Beach BAV Exceedance Day	Beach Water	Niagara
LK-Non-ex-August10	Lakeside Beach BAV Non-Exceedance Day	Beach Water	Niagara
LK-Non-ex-August17	Lakeside Beach BAV Non-Exceedance Day	Beach Water	Niagara
Sun-Non-ex-August30	Sunset Beach BAV Non-Exceedance Day	Beach Water	Niagara
Sun-Ex-June7	Sunset Beach BAV Exceedance Day	Beach Water	Niagara
Sun-Ex-July21	Sunset Beach BAV Exceedance Day	Beach Water	Niagara
Sun-Ex-August9	Sunset Beach BAV Exceedance Day	Beach Water	Niagara
Sun-Non-Ex-June8	Sunset Beach BAV Non-Exceedance Day	Beach Water	Niagara

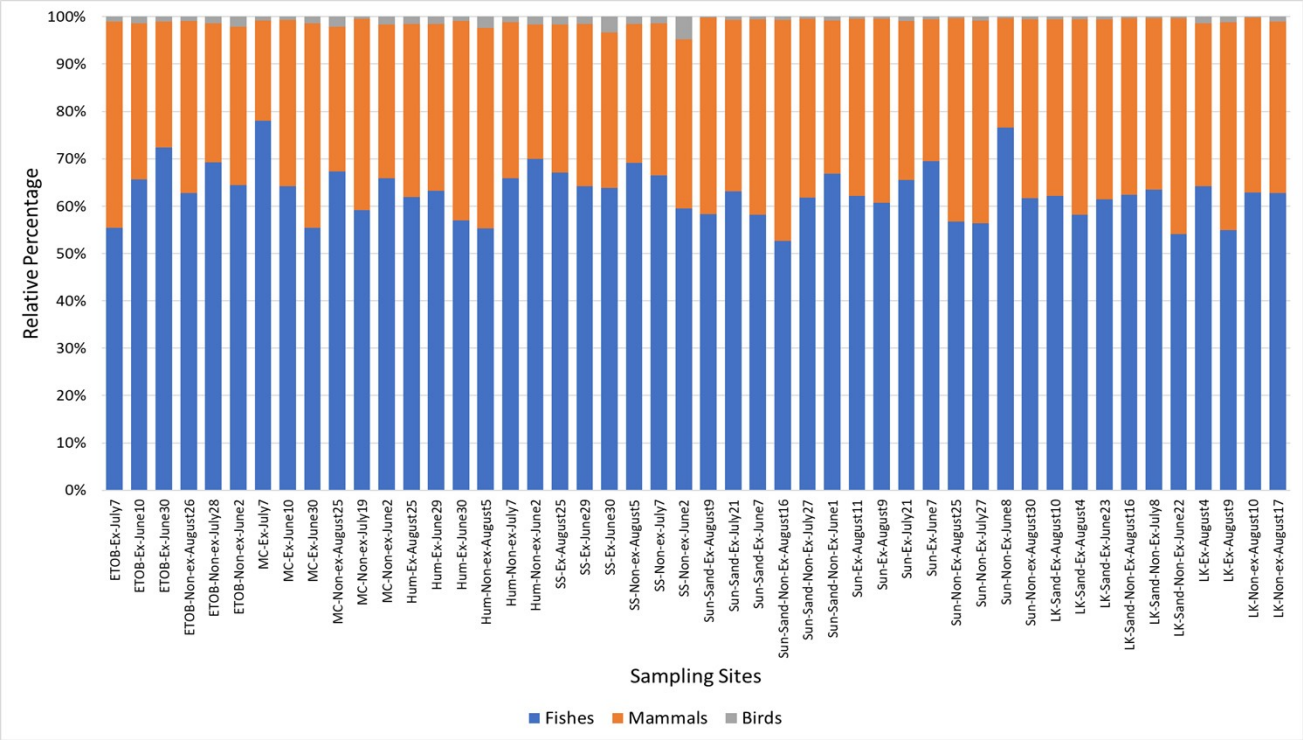
Sun-Non-Ex-July27	Sunset Beach BAV Non-Exceedance Day	Beach Water	Niagara
Sun-Non-Ex-August25	Sunset Beach BAV Non-Exceedance Day	Beach Water	Niagara
LK-Sand-Ex-June23	Lakeside Sand	Shoreline Sand-porewater	Niagara
LK-Sand-Ex-August4	Lakeside Sand	Shoreline Sand-porewater	Niagara
LK-Sand-Ex-August10	Lakeside Sand	Shoreline Sand-porewater	Niagara
LK-Sand-June22	Lakeside Sand	Shoreline Sand-porewater	Niagara
LK-Sand-July8	Lakeside Sand	Shoreline Sand-porewater	Niagara
LK-Sand-August17	Lakeside Sand	Shoreline Sand-porewater	Niagara
Sun-Sand-Ex-June7	Sunset Sand	Shoreline Sand-porewater	Niagara
Sun-Sand-Ex-July21	Sunset Sand	Shoreline Sand-porewater	Niagara
Sun-Sand-Ex-August9	Sunset Sand	Shoreline Sand-porewater	Niagara
Sun-Sand-Non-Ex-June1	Sunset Sand	Shoreline Sand-porewater	Niagara
Sun-Sand-Non-Ex-July27	Sunset Sand	Shoreline Sand-porewater	Niagara
Sun-Sand-Non-Ex-August16	Sunset Sand	Shoreline Sand-porewater	Niagara

Supplementary Table 2. Quality control analytics before and after each eDNA analysis pipeline filtration step (n = 48).

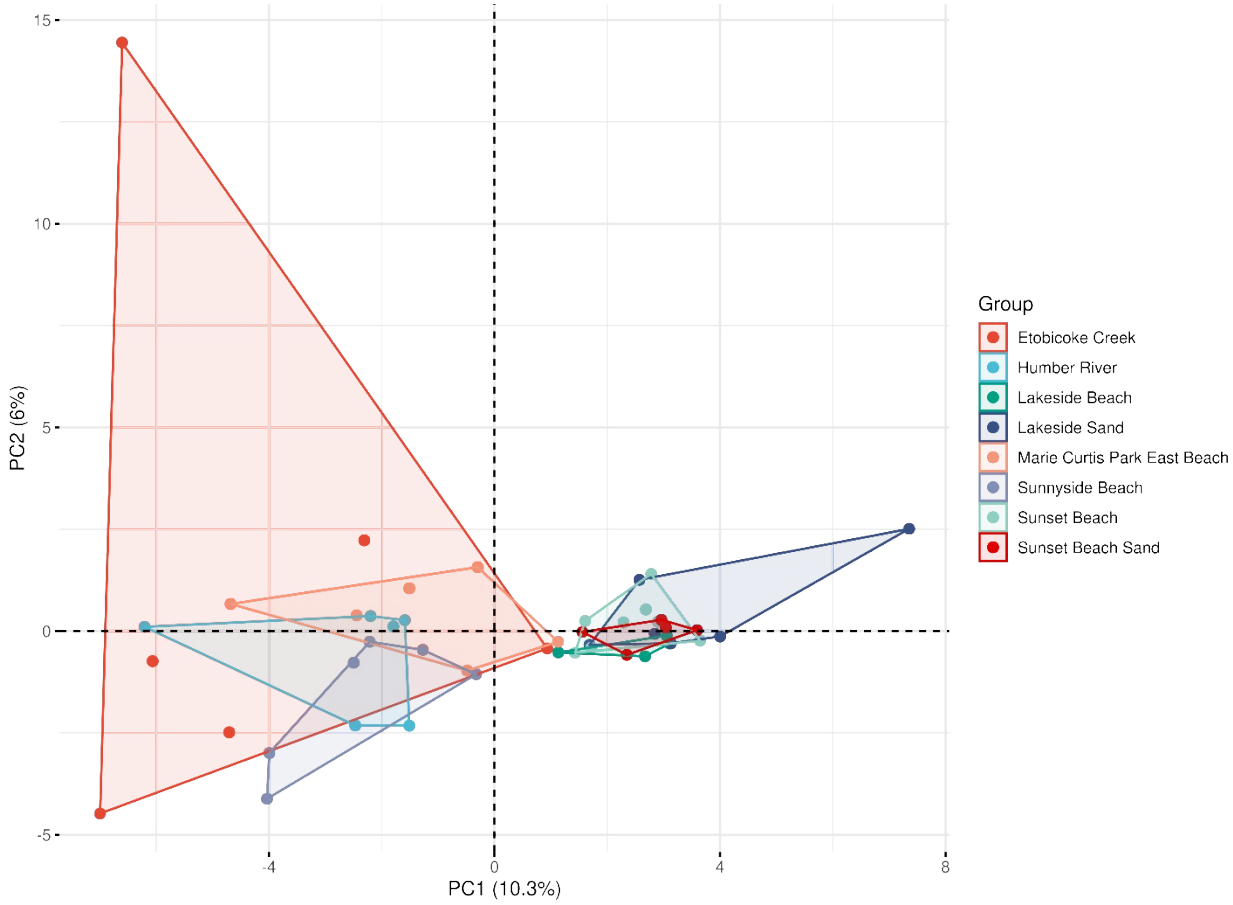
Data Quality Step	Process Summary	
Paired-end Merging	Before Merging	222805 ± 224
	After Merging	219605 ± 222
Trimming	Before Trimming	219605 ± 222
	After Trimming	219551 ± 224
Quality Filtering	Processed Sequences	219551 ± 224
	Passed Sequences	215993 ± 222
Dereplication	Processed Sequences	215993 ± 222
	Unique Sequences	40653 ± 732
Exact Sequence Variants (ESVs)	Before Filtration	369
	After Filtration	200



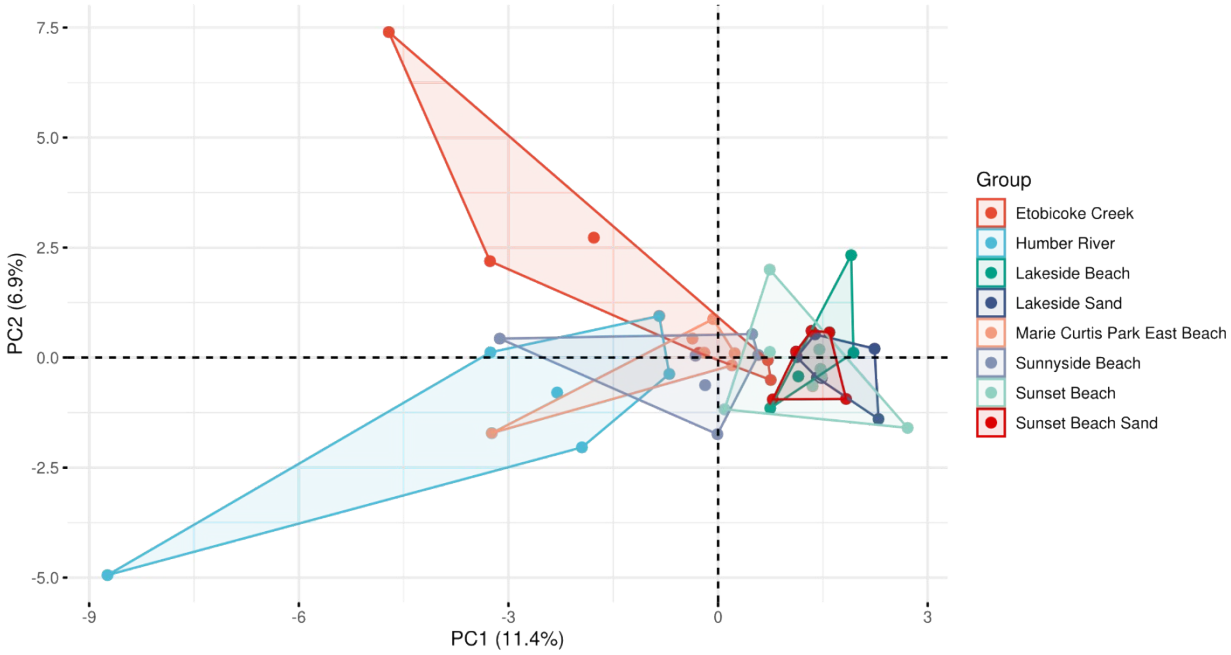
Supplementary Figure 1. Amplicon PCR for mitochondrial 16S rRNA gene.



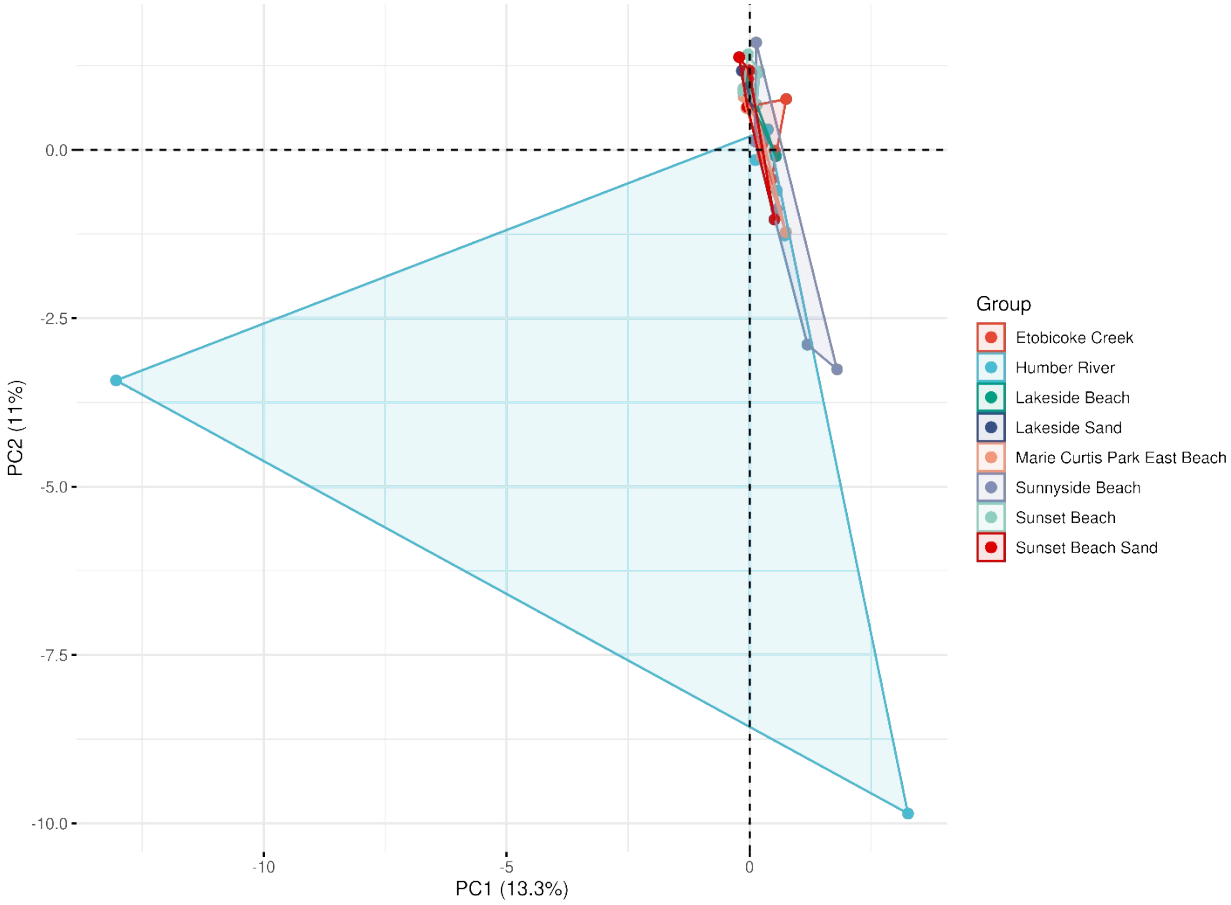
Supplementary Figure 2. Relative abundance of Exact Sequence Variants associated with Fishes, Mammals and Birds in all samples (n = 48).



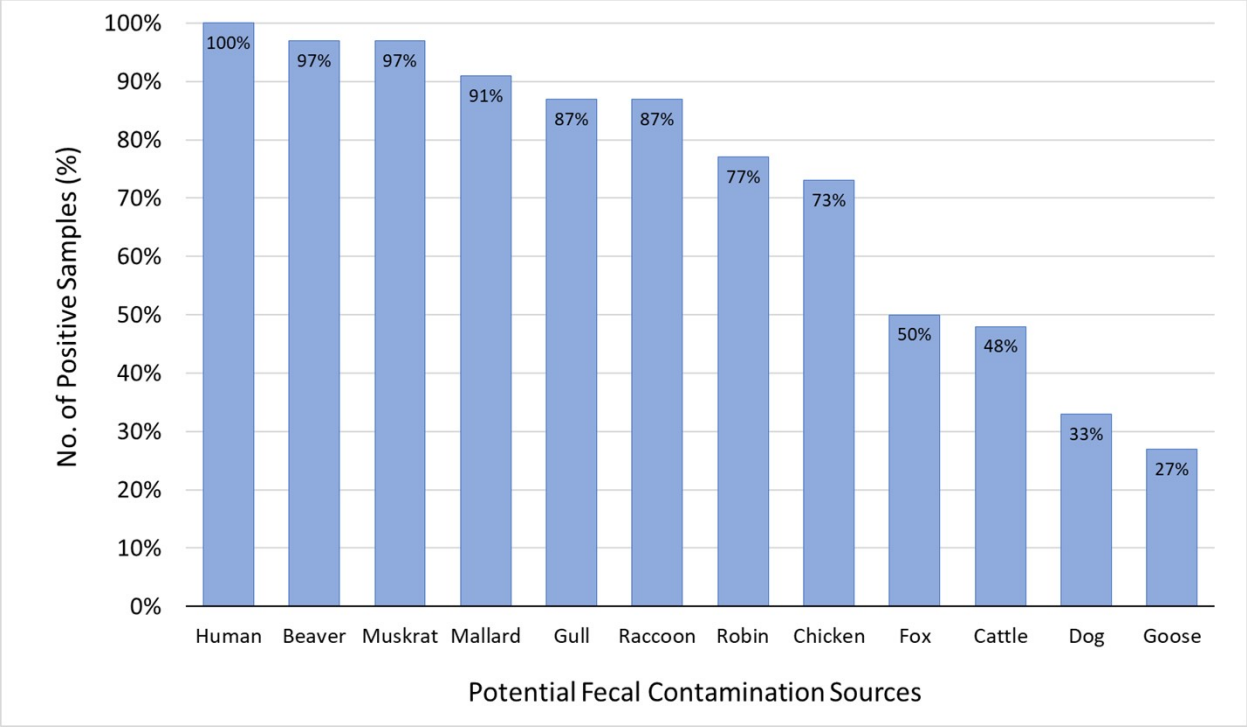
Supplementary Figure 3. Principle Component Analysis (Beta Diversity) of Fish species for Toronto and Niagara beaches, rivers and sand samples.



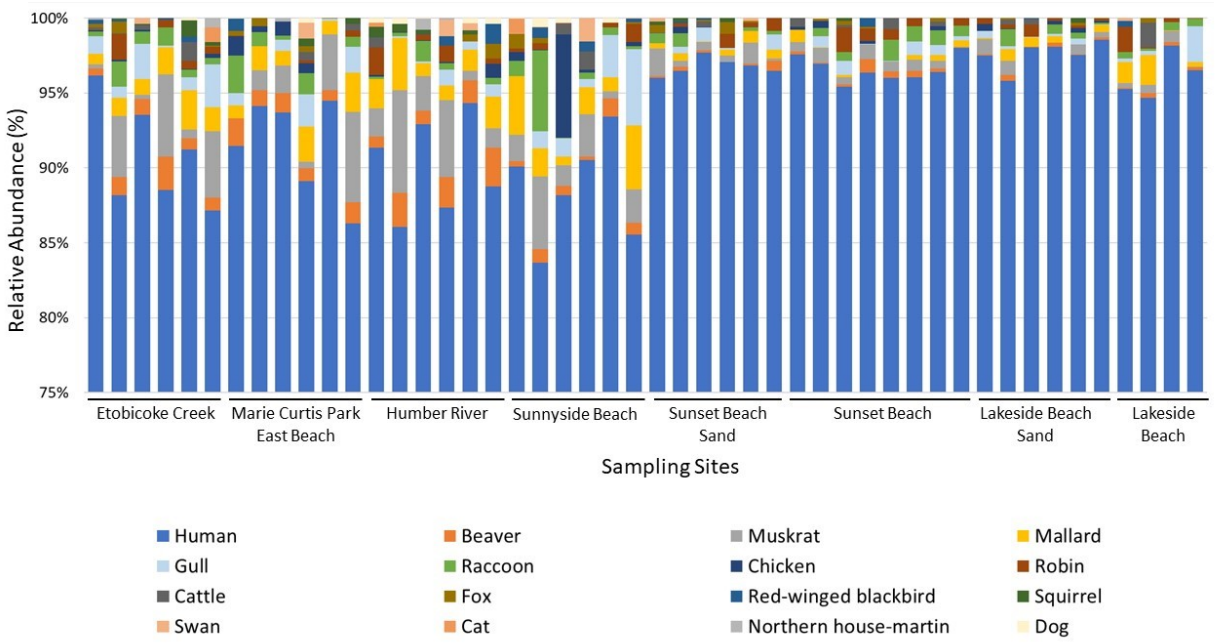
Supplementary Figure 4. Principle Component Analysis (Beta Diversity) of Mammal species for Toronto and Niagara beaches, rivers and sand samples.



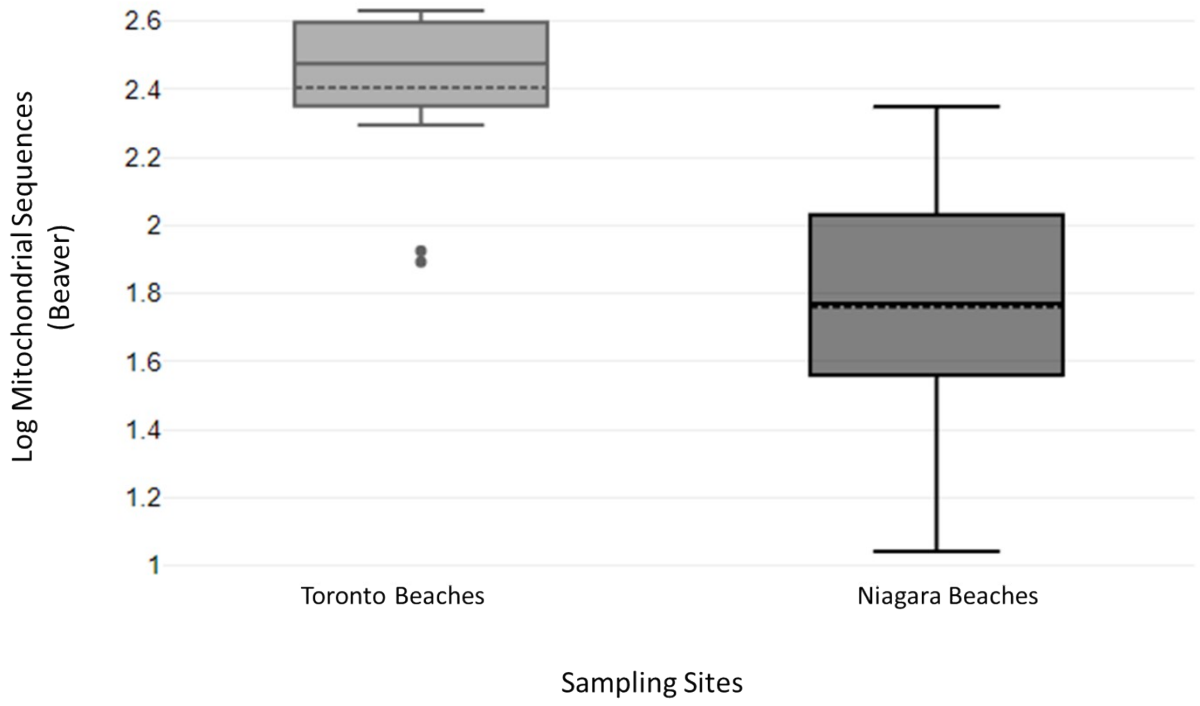
Supplementary Figure 5. Principle Component Analysis (Beta Diversity) of Bird species for Toronto and Niagara beaches, rivers and sand samples.



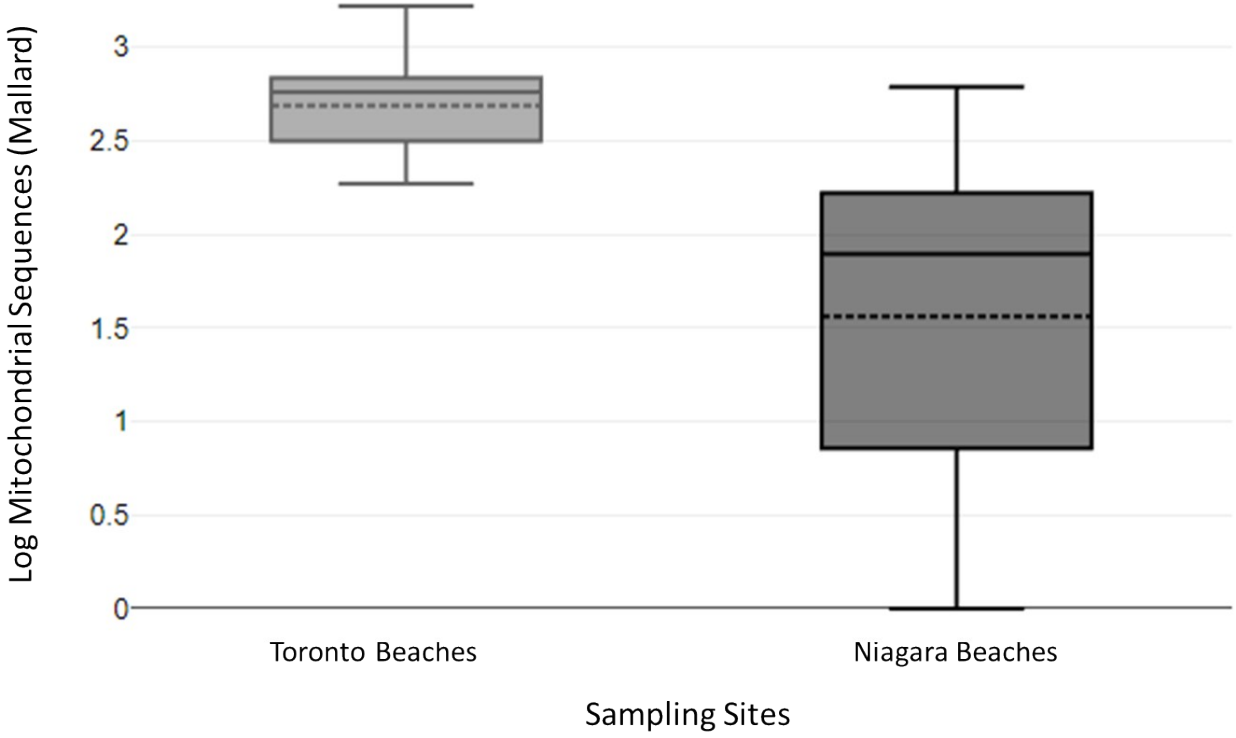
Supplementary Figure 6. Detection percentage (Number of positive samples) of common fecal contamination sources in the beach, river, and sand samples (n = 48).



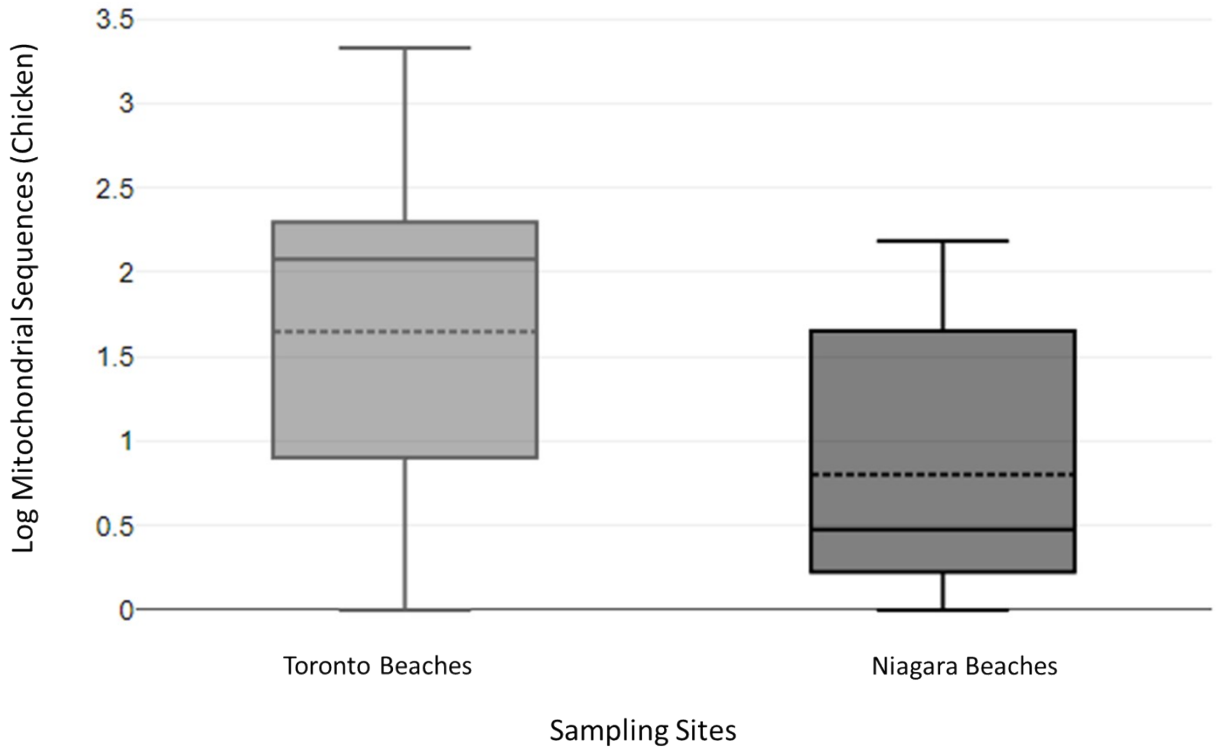
Supplementary Figure 7. Relative abundance of potential mammalian and bird fecal contamination sources for beaches, rivers and sand samples.



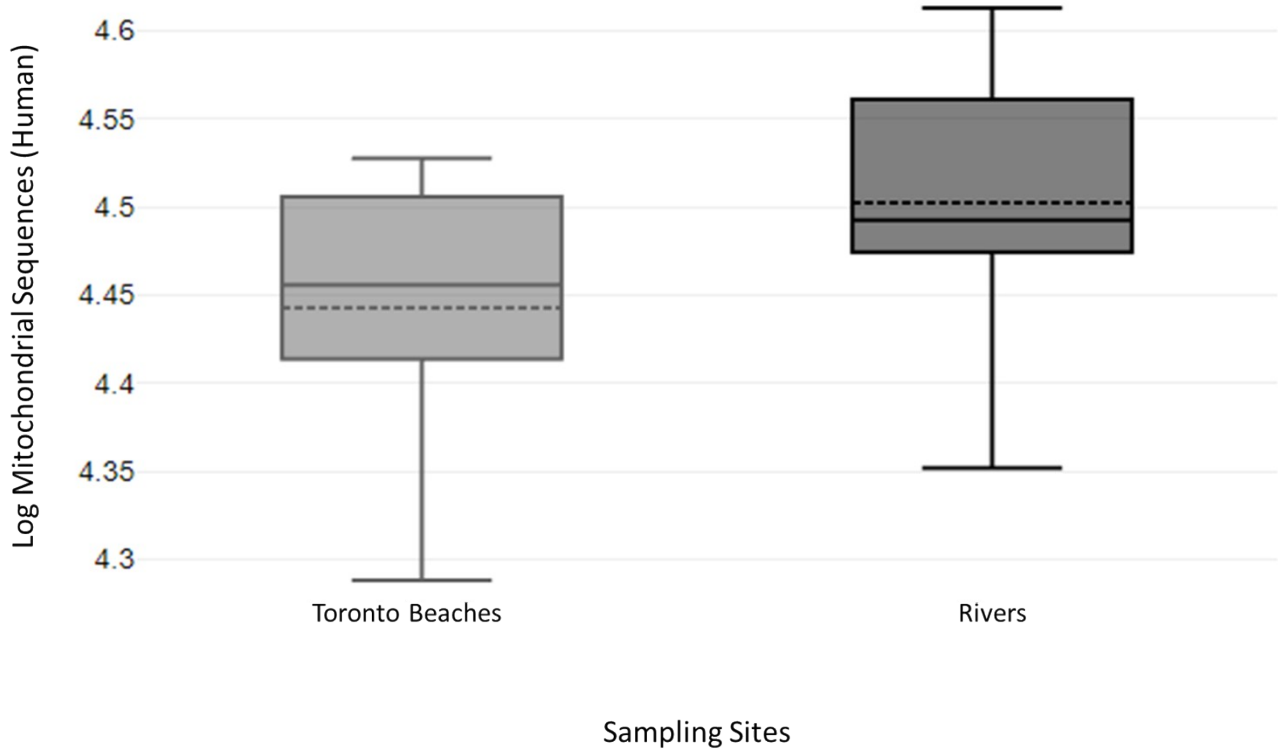
Supplementary Figure 8. Comparative abundance of Beaver mitochondrial sequences between Toronto and Niagara beaches.



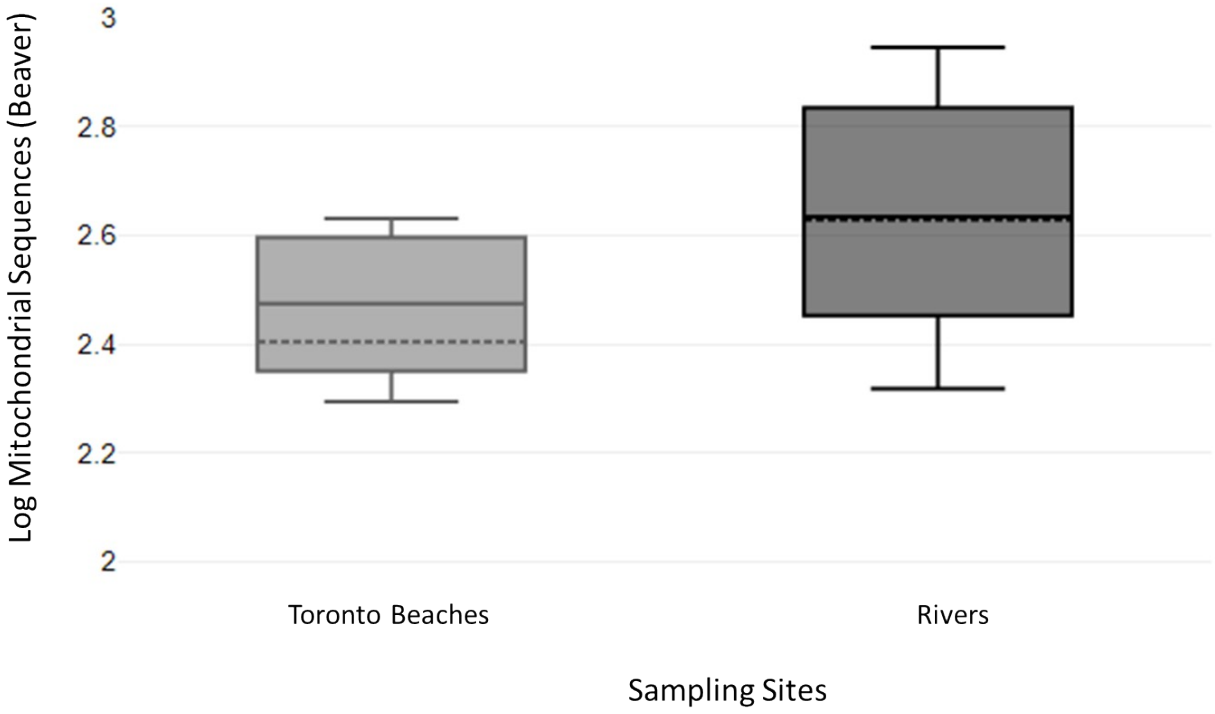
Supplementary Figure 9. Comparative abundance of Mallard mitochondrial sequences between Toronto and Niagara beaches.



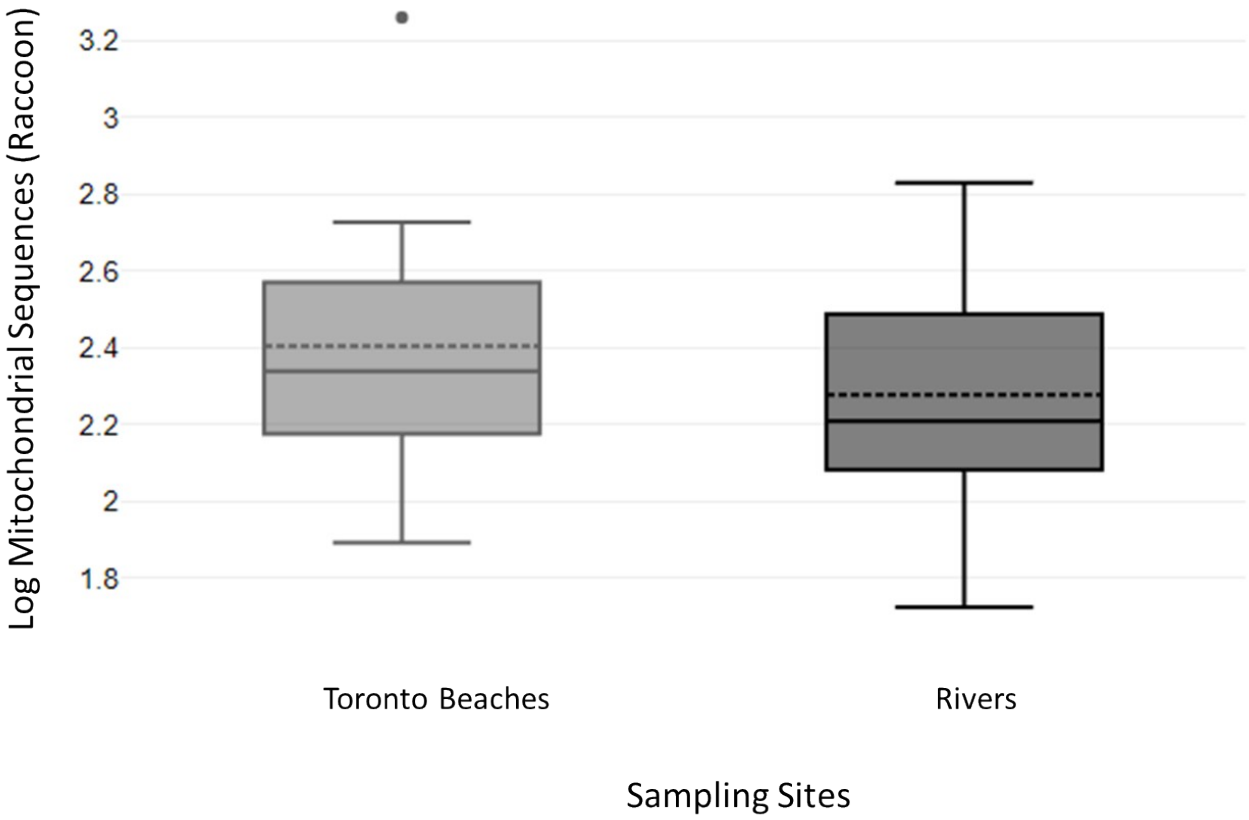
Supplementary Figure 10. Comparative abundance of Chicken mitochondrial sequences between Toronto and Niagara beaches.



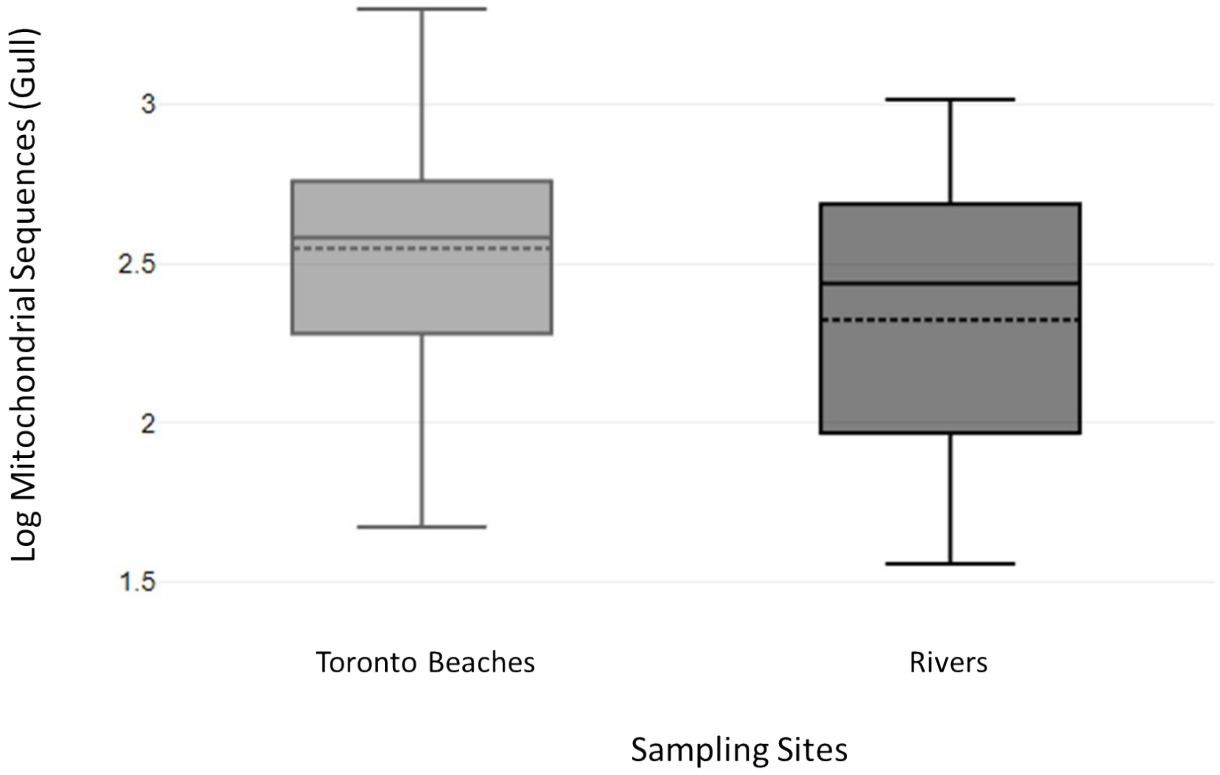
Supplementary Figure 11. Comparative abundance of Human mitochondrial sequences between Toronto beaches and river samples.



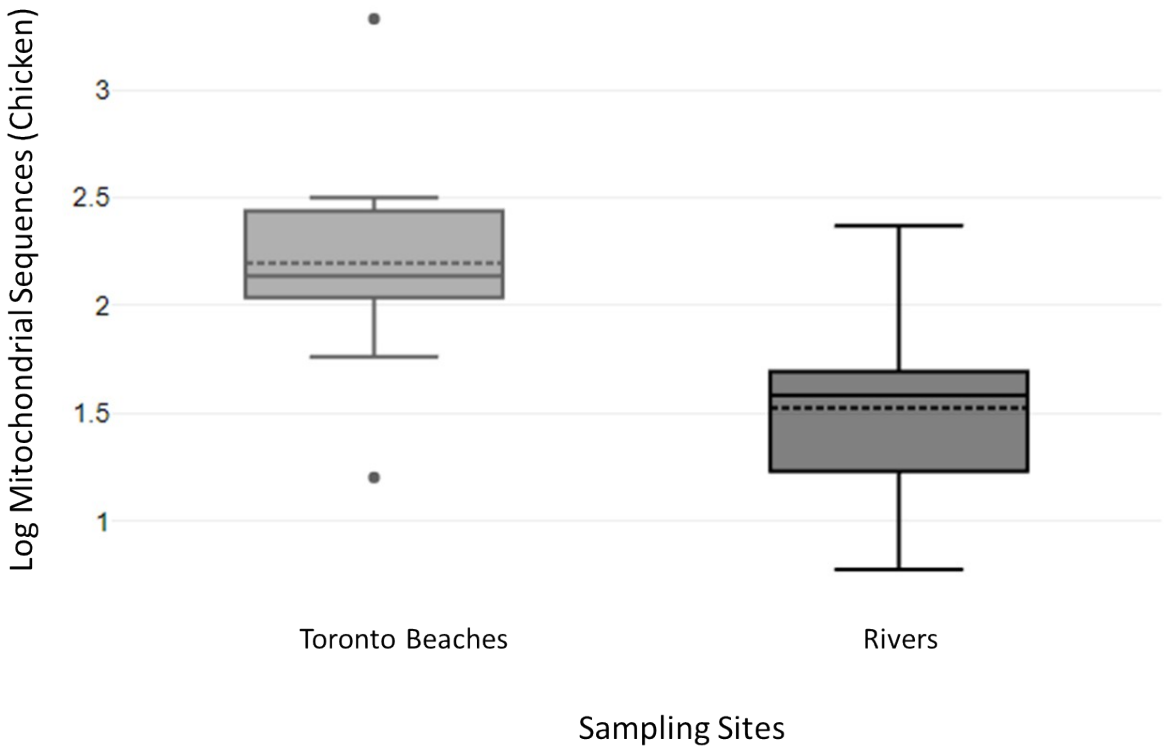
Supplementary Figure 12. Comparative abundance of Beaver mitochondrial sequences between Toronto beaches and river samples.



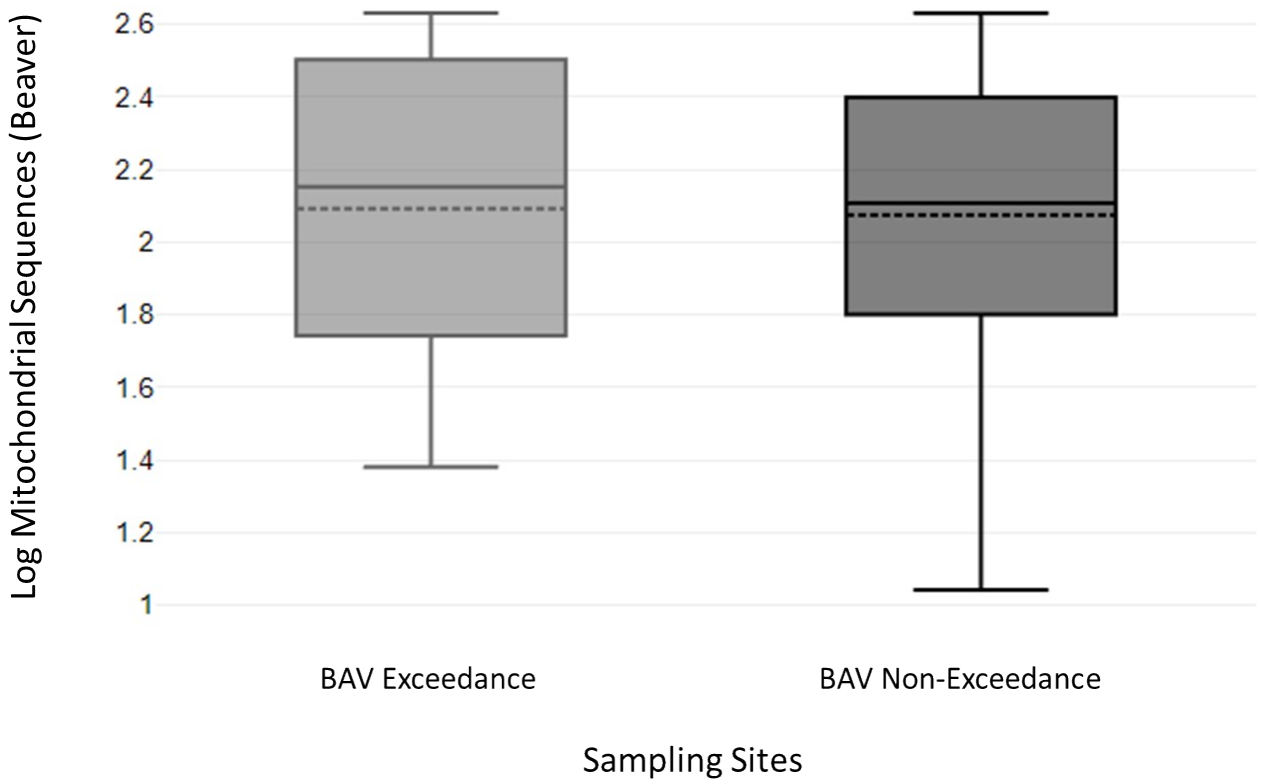
Supplementary Figure 13. Comparative abundance of Raccoon mitochondrial sequences between Toronto beaches and river samples.



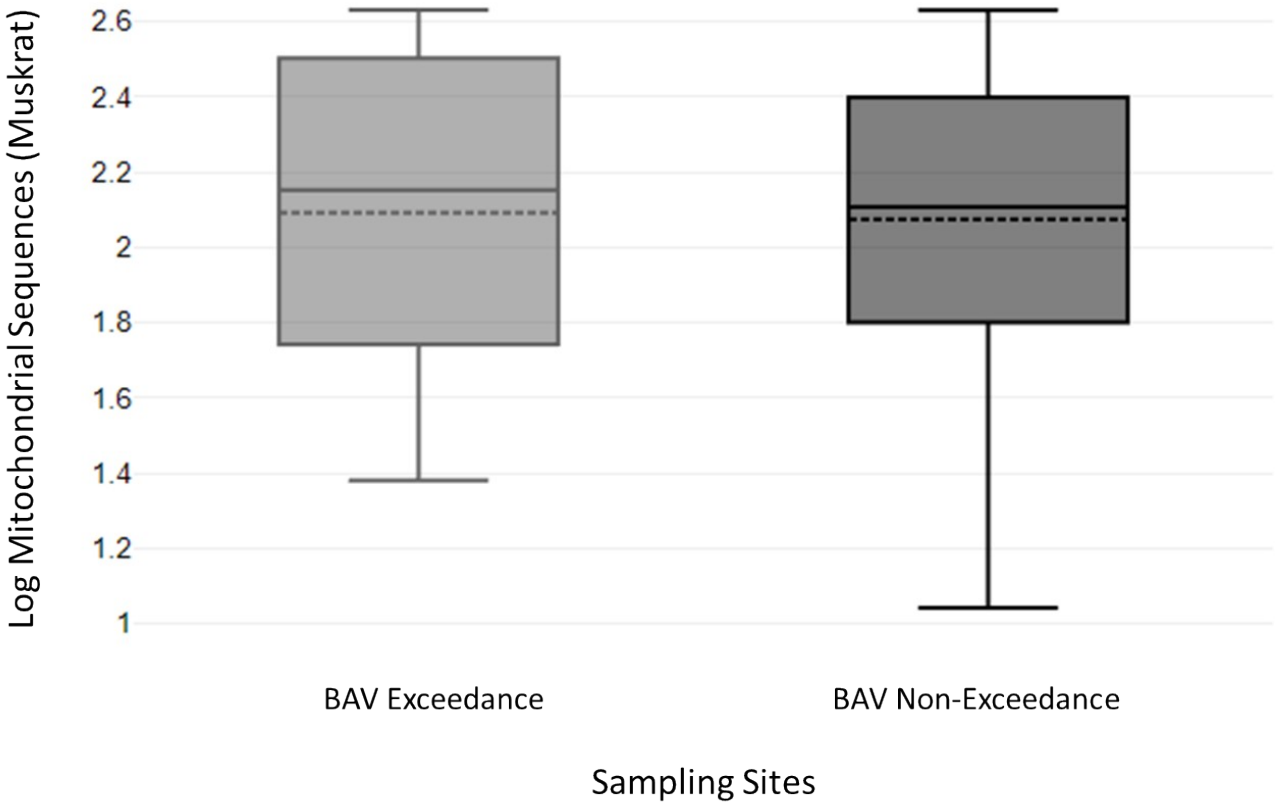
Supplementary Figure 14. Comparative abundance of Gull mitochondrial sequences between Toronto beaches and river samples.



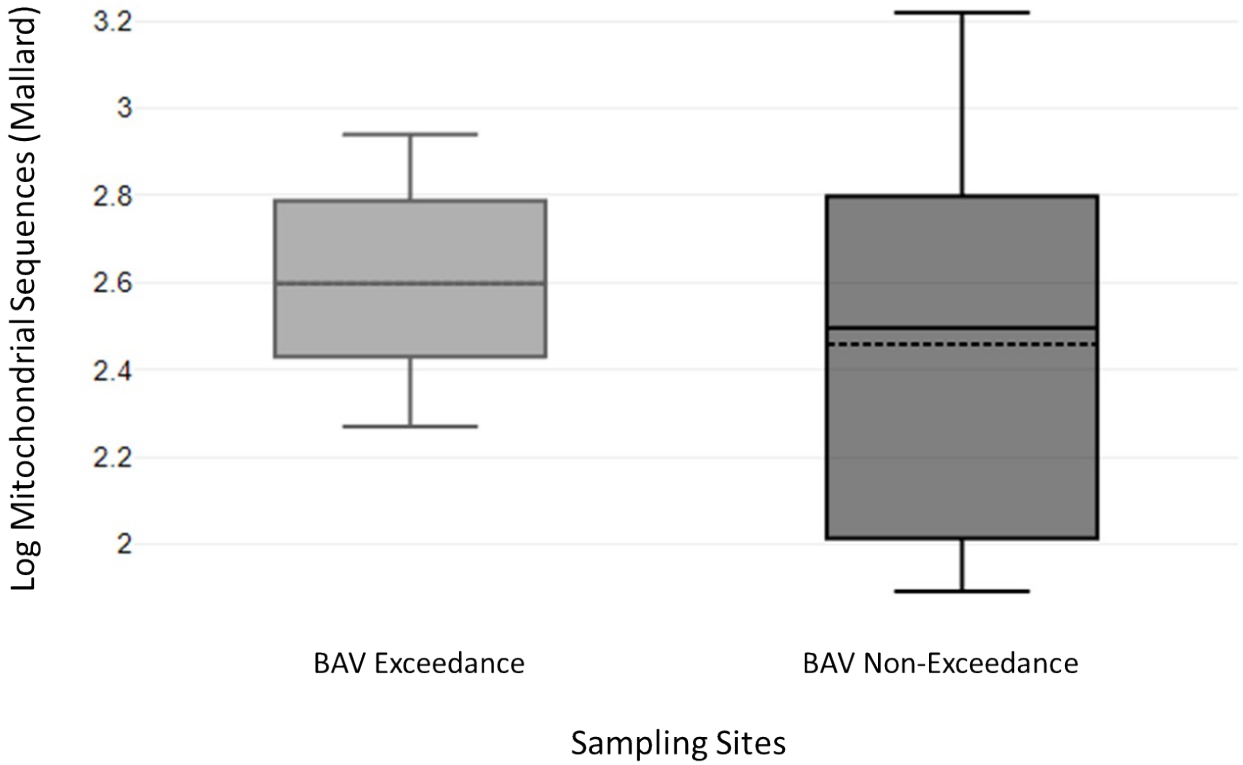
Supplementary Figure 15. Comparative abundance of Chicken mitochondrial sequences between Toronto beaches and river samples.



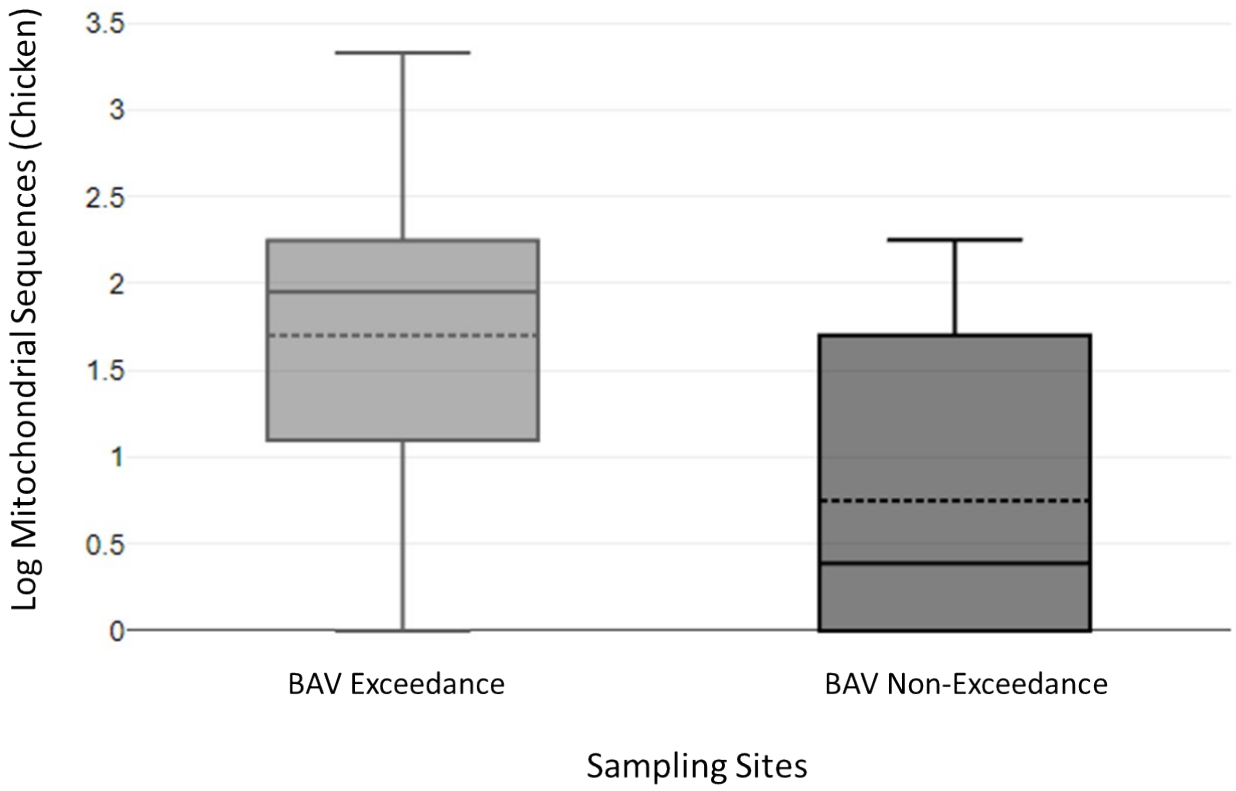
Supplementary Figure 16. Comparative abundance of Beaver mitochondrial sequences between Beach Action Value Exceedance and Non-exceedance Beach water samples.



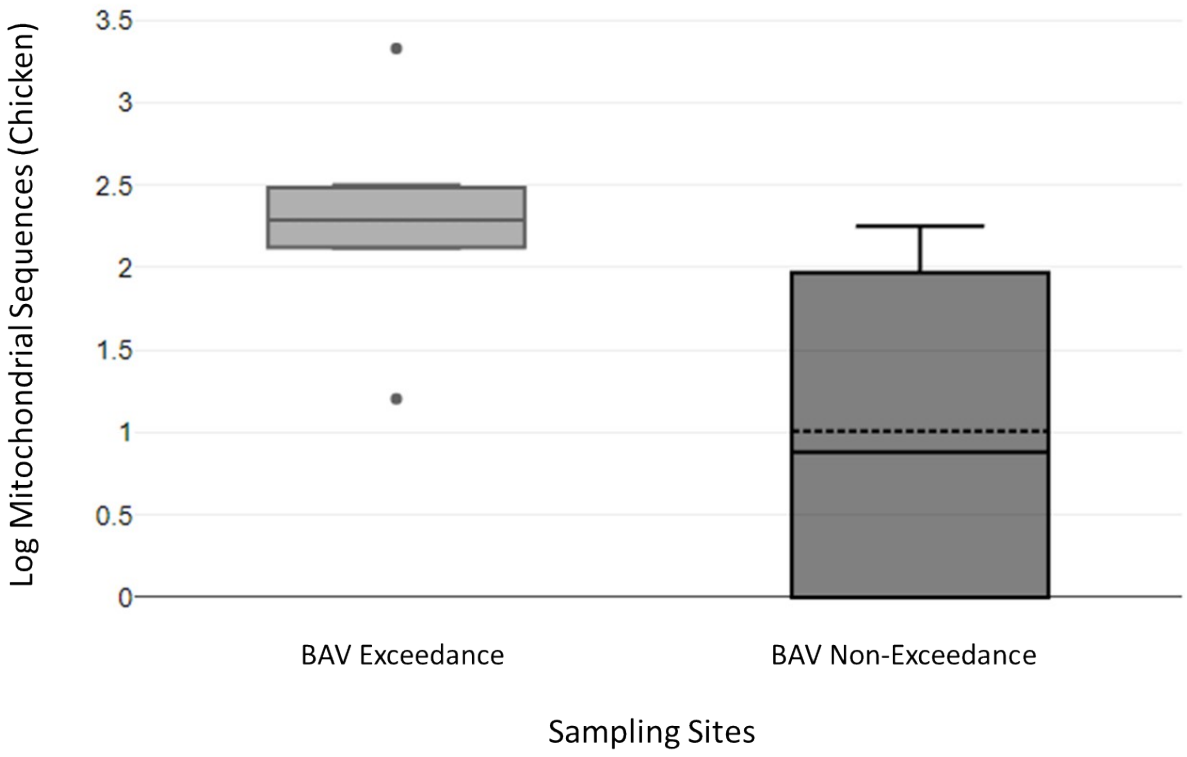
Supplementary Figure 17. Comparative abundance of Muskrat mitochondrial sequences between Beach Action Value Exceedance and Non-exceedance Beach water samples.



Supplementary Figure 18. Comparative abundance of Mallard mitochondrial sequences between Beach Action Value Exceedance and Non-exceedance Beach water samples.



Supplementary Figure 19. Comparative abundance of Chicken mitochondrial sequences between Beach Action Value Exceedance and Non-exceedance Beach water samples.



Supplementary Figure 20. Comparative abundance of Chicken mitochondrial sequences between Beach Action Value Exceedance and Non-exceedance shoreline water samples from Toronto beaches.

Supplementary Table 5. Comparison of the frequency of occurrence of food animal eDNA sequences (chicken, cow, pig) with human HF183 and mt dPCR markers and human eDNA sequences.

		HF183 % +ve	**HuMt % +ve**	**Human eDNA % +ve**
Toronto beaches n=12	Human eDNA +ve n=12	42	92	100
	Chicken eDNA +ve n=9	56	89	100
	Cow eDNA +ve n= 5	40	100	100
Niagara beaches n=12	Human eDNA +ve n=12	67	100	100
	Chicken eDNA +ve n=9	56	100	100
	Cow eDNA +ve n= 9	78	100	100
Niagara sand n=12	Human eDNA +ve n=12	8	100	100
	Chicken eDNA +ve n=9	11	100	100
	Cow eDNA +ve n= 6	17	100	100
	Pig eDNA +ve n=2	50	100	100
Toronto rivers n=12	Human eDNA +ve n=12	83	67*	100
	Chicken eDNA +ve n=10	90	71*	100
	Cow eDNA +ve n=8	100	50*	100
	Pig eDNA +ve n=1	100	100	100
Total samples	Human eDNA +ve n=48	50	91*	100
	Chicken eDNA +ve n=37	59	91*	100
	Cow eDNA +ve n= 28	64	88*	100
	Pig eDNA +ve n=3	67	100	100

* n=9 rather than 12 due to insufficient amount of DNA for PCR.