Table S1. Summary table of sampling design by year, including: dates of sampling periods, sites and transects sampled in each period, sampling depths, tidal phase at time of sampling, summary of additional sampling, and sample replication (i.e., number of 1 L samples collected) from each hole.

Year	Dates	Sites and Transects	Depths	Tides	Additional Sampling	Sample Replicates
2018	February 7 - 8	- US1 - Transects 1 – 6 - DS1	- All holes at surface	- All on a falling tide	- Sampling in March - Sampling at depth	- Three at all site and transect holes
	March 5 - 7	- US1 – 2 - Transects 1 – 6 - DS1 – 3	- All holes at surface - 4C, 4D, 5C and 5D at middle			
2019	February 4 - 12	- US1 – 3 - Transects 1 – 9 - SW	- All holes at surface - 4C, 4D, 5C and 5D at middle and bottom	- All on a falling tide - US1 – 3, Transects 2, 6 and 9 on a rising tide	- Sampling on a rising tide - Sampling at depth	- Two at all site and transect holes
2020	February 10 - 12	- US1 - Transects 1 – 8	- All holes at surface	- All on a falling tide - Transect 6 hourly between high tides (tidal time series)	- 4 extra holes at transect 5 - Tidal time series - Molecular tracers	- Two at US1, Transects 1-5, 7 and 8 holes - One at transect 6 holes, hourly

List S1
DFO-GULF environmental DNA (eDNA) extraction protocol V1.01

Please note that this is the version of extraction protocol used when processing the samples presented in this publication. Please contact the authors for the most recent version of the extraction protocol.

1) Spray the exterior of all sample tubes with bleach 0.5 %.
2) Remove the filter from the tube and place on a weigh boat. Cut filter in half. Return one half to the ethanol tube (as a backup) and leave the other half to air dry until ethanol has evaporated.
3) Once dried, place the half-filter in a 2 mL tube. Roll the filter so that the surface containing the material is facing inward.
4) Prepare a mix of 360 µL of T1 lysis buffer (Macherey-Nagel, PA, USA) and 50 µL of Proteinase K for each sample to process. Add 410 µL of the mix to each tube. Mix by inversion so that the buffer is in contact with the entire filter.
5) Incubate at 56 ± 3 °C on with light shaking overnight to digest the material on the filters.

6) Add 400 µL of Buffer B3 (Macherey-Nagel, PA, USA) to the tube containing the filter. Mix by light vortex or vigorous shaking.
7) Incubate at 70 ± 3 °C on the heat block for 10 minutes.
8) Add 420 µL of absolute ethanol 100 % to the tube with the filter. Mix by light vortex or vigorous shaking.
9) Transfer the liquid to a MN spin-column (Macherey-Nagel, PA, USA). While collecting the supernatant, squeeze the filter with the pipette tip to push all the liquid out of the filter material.
10) Centrifuge at 11 000 g for 1 min. Discard the flow-through and return the spin-column to the collection tube.
11) Repeat steps 9 and 10 two or three times until all liquid has passed through the spin-column.
12) After the last DNA-binding spin, place the spin-column in a new collection tube.
13) Add 500 µL of Buffer BW (Macherey-Nagel, PA, USA) to the spin column. Centrifuge at 11 000 g for 1 min. Discard the flow-through and return the spin-column to the collection tube.
14) Add 600 µL of Buffer B5 (Macherey-Nagel, PA, USA) to the spin-column. Centrifuge at 11 000 g for 1 min. Discard the flow-through and return the spin-column back in the collection tube.
15) Centrifuge empty-spin column at 11 000g for 1 min to dry the membrane.
16) Transfer the spin-column to a clean labeled 1.5 mL tube. Add 50 µL of Buffer BE (Macherey-Nagel, PA, USA), pre-warmed to 70 ± 3 °C. Let stand 1 min at room temperature, centrifuge at 11 000 g for 1 min. Repeat the Buffer BE application for a final volume of approximately 100 µl of eluate.