

Text S1 CTD measurements

CTD measurements were obtained with a CastAway-CTD (SonTek US patent #8272262). The CTD has an integrated GPS and measures conductivity (accuracy of 0.1 PSU), temperature (accuracy of 0.05 °C), and depth measured from the pressure. Data were collected at the same locations and times as eDNA samples. Continuous measurements were collected from the surface down to 100 meters depth. The resulting temperature/salinity plots (TS plot) were used to determine the likely origin of the water masses found in each branch of the fjord system, using the three categories: Baffin Bay Polar Water (BBPW), Coastal Water (CW) and Subpolar Mode Water (SPMW), as defined by Rysgaard et al. (2020). To clarify the origin of the water masses observed inside the fjord system, we compared our CTD data from summer 2019 to CTD data from the Arctic Station in *Qeqertarsuaq*, Disco Bay (collected 22-07-2019, and downloaded from the Greenland Ecosystem Monitoring database (www.g-e-m.dk), GPS coordinates: 69°15N, 53°34W), and from in front of *Iluissat* at CTD station SJA3 (GPS coordinates: 69°19.078N, 51°17.193W, collected 26-06-2019 by the annual summer cruise by the Greenland Institute of Natural Resources).

Text S2 Stable isotope analysis

Samples for isotope analysis were collected from 5, 25, 50, 75 and 100 meters depth in three replicates per depth with a 2L Standard Water Sampler (Ruttner 2 L, Hydrobios). Additional triplicate samples were collected from icebergs in March 2019 (n=1), July 2019 (n=2), and February 2020 (n=2), as well as from sea ice (n=1) in February 2020. All samples were transferred into 2 mL glass vials and kept at room temperature until analysis. The isotopic compositions ($\delta^{18}\text{O}$, $\delta^2\text{H}$) of the samples were analysed with a Cavity Ringdown Spectrometer, L2130-i Isotopic H₂O (Picarro Inc., USA). Six injections (each injection was of 2.35 μl) were run from each sample. The first three were excluded to remove any residual material from the previous sample and the mean of the three last injections was used as sample analysis. Vapour content, $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values were calculated relative to standards. Four standards were measured at the beginning and end of the sample set. The external standards used to calibrate the results were Vienna Standard Ocean Water 2 ($\delta^2\text{HVSMOW}$, $\delta^{18}\text{OVSMOW}$) (VSMOW2) and Standard Light Antarctic Precipitation 2 ($\delta^2\text{HSLAP2}$, $\delta^{18}\text{OSLAP2}$) (SLAP2). The $\delta^{18}\text{O}$ value is only mentioned in this study as the values for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ are highly correlated ($R^2 = 0.9769$).

Text S3 Water sampling for eDNA

Water samples were collected from the same location and dates as the hydrographic and water isotope samples at 5 and 100 meters depth. Water was sampled in the northern branch (Fig 1, sampling location 1, N 69° 15 W 050° 34) and the southern branch (Fig 1, sampling location 2, N 69° 04, W 050° 46) in March/April 2019, July 2019, and February 2020. The main fjord was only sampled in December 2019, both at the sea ice edge (Fig 1, sampling location 3, N 69° 09, W 050° 28) and at the mouth of the fjord (Fig 1, sampling location 4, N69° 10, W 051). The main fjord was not accessible during the rest of the sampling period due to the presence of tightly packed icebergs. Sampling in March/April 2019 and February 2020 was from the sea ice, while sampling in July 2019 and December 2019 was from a boat.

Water samples were collected at 5 meters and 100 meters depth, using a 2L Standard Water Sampler (Ruttner 2 L, Hydrobios). At each location, 5 sample replicates were collected from each depth. To store the samples, a total of 10 1L Nalgene bottles were used, which were sterilized with DNA AWAY (Surface decontaminant, Thermo Scientific) and after at least 10 minutes cleaned with 70% ethanol. The bottles were stored inside sterile zip-lock bags to avoid contamination during handling and transportation. Single-use nitrile gloves, facemasks, and plastic sleeves were worn during sample handling. Filtration was done in the field in July 2019. During the other sampling campaigns, filtration was performed indoors immediately after returning from the field, as the weather conditions were too cold in the field (temperatures were around minus 20 - 30 °C). Samples were filtered using Sterivex-GP filter units (Merck Millipore, 0.22 µm pore size), using 150 mL sterile syringes, with a separate syringe per sample. Blank field controls consisting of 500 mL of bottled mineral water were filtered along with the samples. A total of 90 field samples, and nine blank field controls (one for each location on each field campaign), were collected. Filters were kept frozen at -20 °C until DNA extraction.

Table S1 Observations made by local informants (n=33) on specific fish and mammal species in the Icefjord.

Species	Observations by local informants
<p>Greenland halibut (<i>Reinhardtius hippoglossoides</i>)</p>	<p>Eleven informants reported that the larger (mature) Greenland halibut likely migrate out of the Icefjord around January-February to spawn, as the informants, during this period, catch smaller Greenland halibut inside the Icefjord. The mature Greenland halibut are back in the Icefjord around March-April emaciated (postspawning state). Four informants explained that the smaller Greenland halibut are less abundant further into the main fjord, and that the fish community becomes gradually less diverse and more dominated by larger Greenland halibut towards the glacier front.</p>
<p>Ringed seal (<i>Pusa hispida</i>)</p>	<p>The only seal species known to reside in the Icefjord year-round. The ringed seals in the fjord system are unique, as all informants describe them as being larger and appearing visually different when compared to the ringed seals that reside on the west coast of Greenland.</p>
<p>Harp seal (<i>Pagophilus groenlandicus</i>)</p>	<p>Forages in areas with open water in the main fjord and around the open water polynya in the southern fjord branch during the winter, foraging on shoals of capelin (<i>Mallotus villosus</i>). Only sighted in areas with open water, and rarely caught in areas covered by sea ice.</p>
<p>Roughead grenadier (<i>Macrourus berglax</i>)</p>	<p>Six informants mentioned that this species has decreased dramatically in abundance during the recent years in the Icefjord and are now rarely caught as bycatch, even though this species was normal as bycatch only a few years back.</p>
<p>Atlantic cod (<i>Gadus morhua</i>)</p>	<p>Five informants mentioned that they did not catch Atlantic cod inside the Icefjord before, but that they have started to appear in low numbers and have increased steadily in abundance over the latest years. The informants explained that this species normally did not occur inside the Icefjord but has started to appear as bycatch.</p>
<p>Arctic char (<i>Salvelinus alpinus</i>)</p>	<p>Migrates to the southern branch during June-July . Here, fishers from <i>Ilimanaq</i> target the species from small boats using gillnets.</p>
<p>Greenland shark (<i>Somniosus microcephalus</i>)</p>	<p>Even though the Greenland shark is an abundant species caught as bycatch in the fisheries around Greenland, none of the informants mentioned catching this species inside the Icefjord and it is therefore considered not to be present inside the Icefjord. Even when this specific species was mentioned in the question, none recalled catching this species inside the Icefjord.</p>
<p>Whales</p>	<p>Informants reported that whales usually only are present at the mouth of the fjord system and are generally not sighted inside the fjord system.</p>

Table S2: Taxa found in the NTC's (PCR blanks) for the Tele02 primers. Taxa that were found in higher read counts in a control sample than in a water sample were removed from the analysis and are here marked in bold. Note that taxa only present in a single PCR replicate of a sample were removed for that sample after this point.

Taxon	Total count	Max.sample	Max.NTC
Bradyrhizobium sp. PSBB068	422	0	211
<i>Delphinapterus leucas</i>	445,034	54,824	42
Enterobacteriaceae	2	0	2
<i>Gadus</i>	3,068,384	348,933	1
<i>Reinhardtius hippoglossoides</i>	3,516,263	247,878	441

Table S3: Taxa found in the extraction blanks (EXT) for the Tele02 primers. Taxa that were found in higher read counts in a control sample than in a water sample were removed from the analysis and are here marked in bold. Note that taxa only present in a single PCR replicate of a sample were removed for that sample after this point.

Taxon	Total count	Max.sample	Max.EXT
<i>Anarhichas</i>	170,492	44,065	641
<i>Anisarchus medius</i>	42,569	16,881	155
<i>Boreogadus saida</i>	9,186,871	642,608	88,335
<i>Clupea harengus</i>	7,028	7,020	8
Cottidae	10,015	2,297	311
<i>Cystophora cristata</i>	67,427	12,412	361
<i>Delphinapterus leucas</i>	445,034	54,824	1,717
<i>Eumesogrammus praecisus</i>	7,987	2,458	143
<i>Gadus</i>	3,068,384	348,933	8,999
<i>Hippoglossoides platessoides</i>	194,176	23,817	174
<i>Lagopus muta</i>	84,117	7,221	396
<i>Larus</i>	12,334	2,826	23
<i>Leptoclinus maculatus</i>	29,600	12,883	69
Liparis	244	0	208
<i>Liparis fabricii</i>	3,769	3,025	279
Lumpenidae	2,312	697	385
<i>Lycodes</i>	138,639	14,121	6,577
<i>Mallotus villosus</i>	825,979	58,817	19,821
<i>Monodon monoceros</i>	114,185	4,995	787
<i>Myoxocephalus</i>	483,225	37,471	1,364
<i>Pagophilus groenlandica</i>	1,847,331	82,043	2,001
<i>Pusa hispida</i>	692,724	35,573	1,146
<i>Rangifer tarandus</i>	148,111	10,664	53
<i>Reinhardtius hippoglossoides</i>	3,516,263	247,878	2,665
<i>Salvelinus alpinus</i>	1,337,903	74,860	28,016

Table S4: Taxa found in the field blanks (FB) for the Tele02 primers. Taxa that were found in higher read counts in a control sample than in a water sample were removed from the analysis and are here marked in bold. Note that taxa only present in a single PCR replicate of a sample were removed for that sample after this point.

Taxon	Total count	Max.sample	Max.FB
<i>Amblyraja radiata</i>	660	130	3
<i>Ammodytes</i>	3,038	2,523	19
<i>Anarhichas</i>	170,492	44,065	69
Anatidae	5,528	2,496	303
<i>Anisarchus medius</i>	42,569	16,881	36
<i>Arctogadus glacialis</i>	47,862	6,258	363
<i>Balaenoptera physalus</i>	1,654	724	6
<i>Boreogadus saida</i>	9,186,871	642,608	21,066
<i>Cephus grylle</i>	1,363	410	47
<i>Cololabis saira</i>	86,926	174	24,120
<i>Corvus</i>	89	80	9
<i>Cystophora cristata</i>	67,427	12,412	135
<i>Delphinapterus leucas</i>	445,034	54,824	5,035
<i>Eumesogrammus praecisus</i>	7,987	2,458	9
<i>Gadus</i>	3,068,384	348,933	24,959
<i>Lagopus muta</i>	84,117	7,221	911
<i>Larus</i>	12,334	2,826	15
Lumpenidae	2,312	697	26
<i>Lycodes</i>	138,639	14,121	1,039
<i>Mallotus villosus</i>	825,979	58,817	1,943
<i>Monodon monoceros</i>	114,185	4,995	1,082
<i>Myoxocephalus</i>	483,225	37,471	1,789
<i>Ovibos moschatus</i>	3,087	607	3
<i>Pagophilus groenlandica</i>	1,847,331	82,043	16,844
Phasianidae	16,874	3,248	1,613
<i>Pusa hispida</i>	692,724	35,573	139
<i>Rangifer tarandus</i>	148,111	10,664	845
<i>Reinhardtius hippoglossoides</i>	3,516,263	247,878	2,469
<i>Salvelinus alpinus</i>	1,337,903	74,860	253
<i>Stichaeus punctatus</i>	21,185	8,604	8

Table S5: Taxa found in the NTC's (PCR blanks) for the Elas02 primers. Taxa that were found in higher read counts in a control sample than in a water sample were removed from the analysis and are here marked in bold. Note that taxa only present in a single PCR replicate of a sample were removed for that sample after this point.

Taxon	Total count	Max.sample	Max.NTC
<i>Bradyrhizobium sp. PSBB068</i>	82	0	41
<i>Delphinapterus leucas</i>	240,773	27,076	370
<i>Reinhardtius hippoglossoides</i>	1,384,176	64,412	7,004

Table S6: Taxa found in the extraction blanks (EXT) for the Elas02 primers. Taxa that were found in higher read counts in a control sample than in a water sample were removed from the analysis and are here marked in bold. Note that taxa only present in a single PCR replicate of a sample were removed for that sample after this point.

Taxon	Total count	Max.sample	Max.EXT
<i>Anarhichas</i>	63,360	13,915	105
<i>Anisarchus medius</i>	12,184	3,119	93
<i>Boreogadus saida</i>	3,281,050	173,231	32,829
<i>Clupea harengus</i>	8	2	6
Cottidae	5,586	1,581	165
<i>Cyclopterus lumpus</i>	1,200	274	145
<i>Cystophora cristata</i>	17,070	3,388	76
<i>Delphinapterus leucas</i>	240,773	27,076	240
<i>Gadus</i>	949,465	69,573	1,058
<i>Hippoglossoides platessoides</i>	69,711	3,450	55
<i>Lagopus muta</i>	38,340	2,615	233
<i>Larus</i>	22,945	7,986	10
<i>Liparis</i>	84	0	60
<i>Liparis fabricii</i>	326	263	45
Lumpenidae	1,502	323	284
<i>Lycodes</i>	50,694	3,338	961
<i>Mallotus villosus</i>	412,840	58,008	2,380
<i>Monodon monoceros</i>	78,001	4,738	184
<i>Myoxocephalus</i>	273,941	19,994	289
<i>Pagophilus groenlandica</i>	846,559	27,488	837
<i>Pusa hispida</i>	351,739	20,307	356
<i>Rangifer tarandus</i>	91,818	11,673	18
<i>Reinhardtius hippoglossoides</i>	1,384,176	64,412	1,106
<i>Salvelinus alpinus</i>	634,379	41,056	11,943
<i>Stichaeus punctatus</i>	7,907	2,433	3

Table S7: Taxa found in the field blanks (FB) for the Elas02 primers. Taxa that were found in higher read counts in a control sample than in a water sample were removed from the analysis and are here marked in bold. Note that taxa only present in a single PCR replicate of a sample were removed for that sample after this point.

Taxon	Total count	Max.sample	Max.FB
<i>Acidovorax</i> sp. KKS102	4	0	4
<i>Amblyraja hyperborea</i>	3,331	695	2
<i>Amblyraja radiata</i>	923	181	2
<i>Ammodytes</i>	1,249	1,057	14
<i>Anarhichas</i>	63,360	13,915	23
Anatidae	2,625	895	218
<i>Anisarchus medius</i>	12,184	3,119	22
<i>Arctogadus glacialis</i>	21,644	1,636	93
<i>Boreogadus saida</i>	3,281,050	173,231	12,258
<i>Cepphus grylle</i>	847	345	21
<i>Cololabis saira</i>	37,255	81	10,627
<i>Corvus</i>	78	74	4
<i>Cystophora cristata</i>	17,070	3,388	68
<i>Delphinapterus leucas</i>	240,773	27,076	1,919
<i>Eumesogrammus praecisus</i>	4,859	2,496	11
<i>Gadus</i>	949,465	69,573	9,621
<i>Lagopus muta</i>	38,340	2,615	359
<i>Larus</i>	22,945	7,986	17
Lumpenidae	1,502	323	24
<i>Lycodes</i>	50,694	3,338	464
<i>Mallotus villosus</i>	412,840	58,008	787
<i>Monodon monoceros</i>	78,001	4,738	660
<i>Myoxocephalus</i>	273,941	19,994	674
<i>Ovibos moschatus</i>	2,972	854	6
Phasianidae	6,841	1,099	1,096
<i>Pagophilus groenlandica</i>	846,559	27,488	5,127
<i>Pusa hispida</i>	351,739	20,307	113
<i>Rangifer tarandus</i>	91,818	11,673	326
<i>Reinhardtius hippoglossoides</i>	1,384,176	64,412	1,517
<i>Salvelinus alpinus</i>	634,379	41,056	104

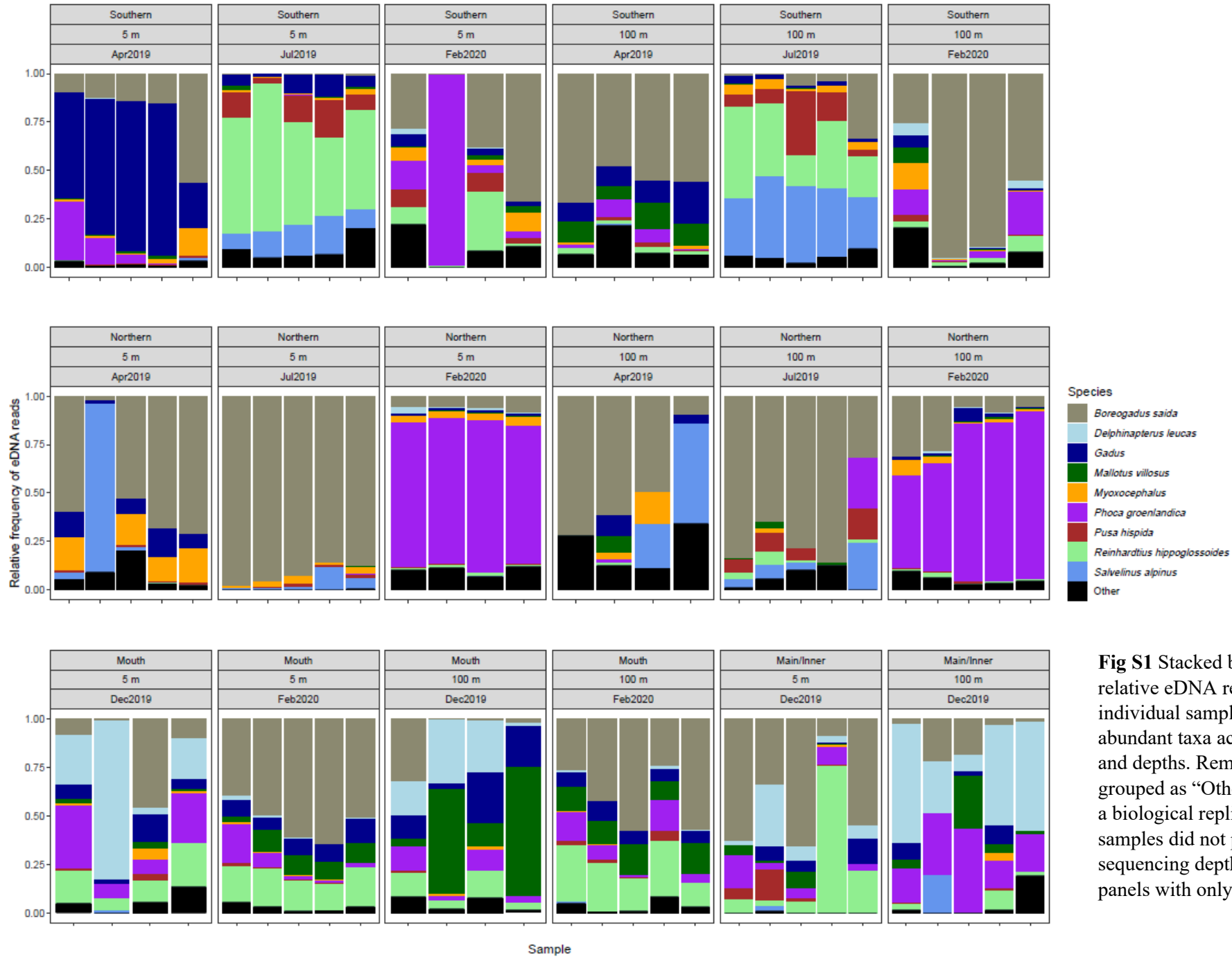


Fig S1 Stacked bar plot showing relative eDNA read frequencies of individual samples of the 9 most abundant taxa across all areas, seasons and depths. Remaining species are here grouped as “Other”. Each bar represents a biological replicate. Note that seven samples did not provide adequate sequencing depth, resulting in seven panels with only four samples.