Supplementary Material to

Comparative metabarcoding and metatranscriptomic analysis of microeukaryotes within coastal surface waters of West Greenland and Northwest Iceland

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1.1 Supplementary Data

Data S1: Table of ASVs and annotated taxonomy of the phytoplankton community during Maria S. Merian cruise MSM21/3 (ARCHEMHAB).

Data S2: Table of gene counts and taxonomic information retrieved from MMETSP.

1.2 Supplementary Figures

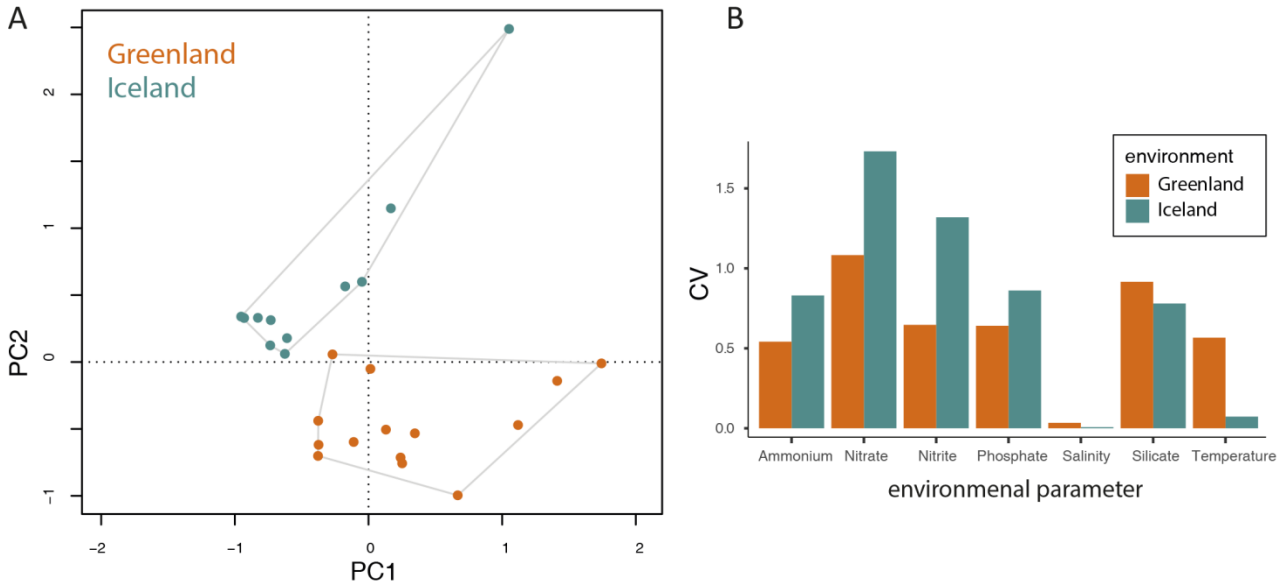

Figure S1: (A) Principal component analysis (PCA) plot of the Euclidean distances of environmental parameters ammonium, nitrate, nitrite, phosphate, salinity, silicate, temperature. Polygons were drawn at 95% confidence limit for the sampling areas of Greenland and Iceland. (B) Coefficient of variation for environmental parameters (ammonium, nitrate, nitrite, phosphate, salinity, silicate, temperature) in Greenland and Iceland.

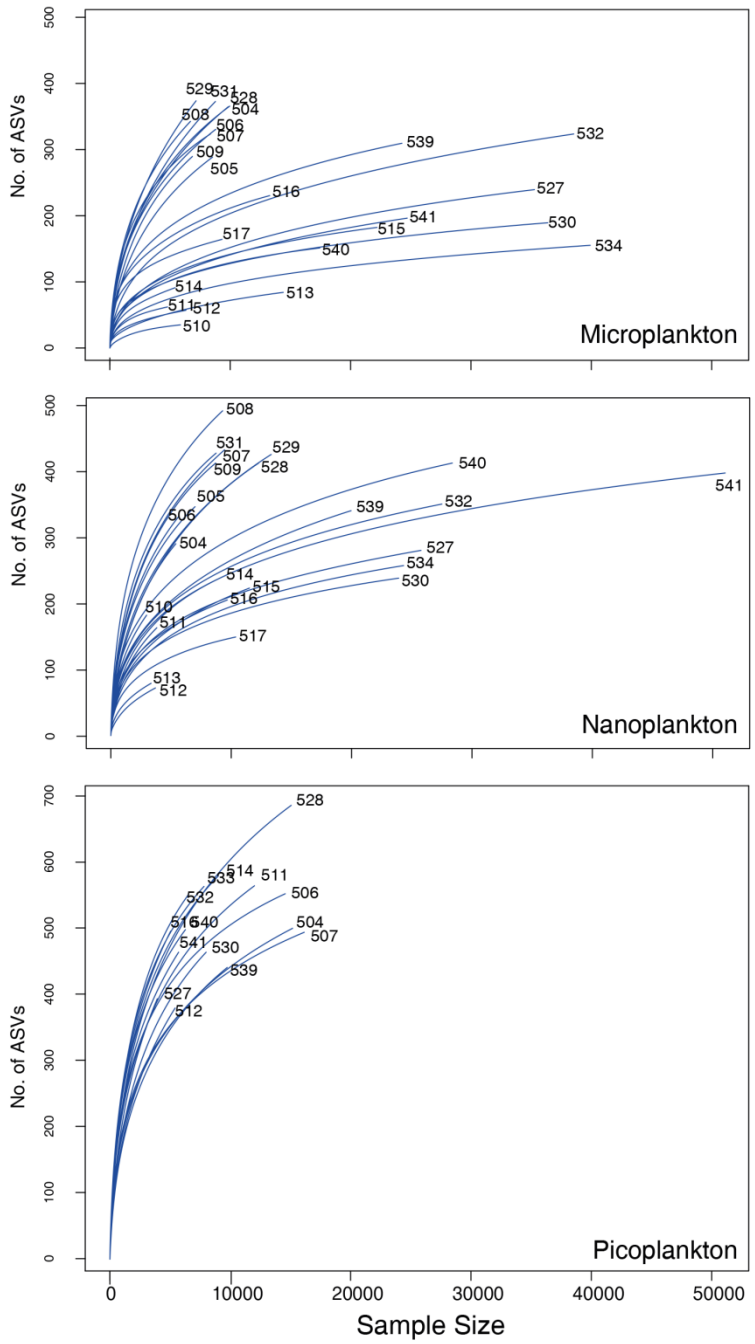

Figure S2: Rarefaction assimilation curves of ASVs for the microplankton (20-50 µm), nanoplankton (3-20 µm), and picoplankton (0.2-3 µm) size-fractions.

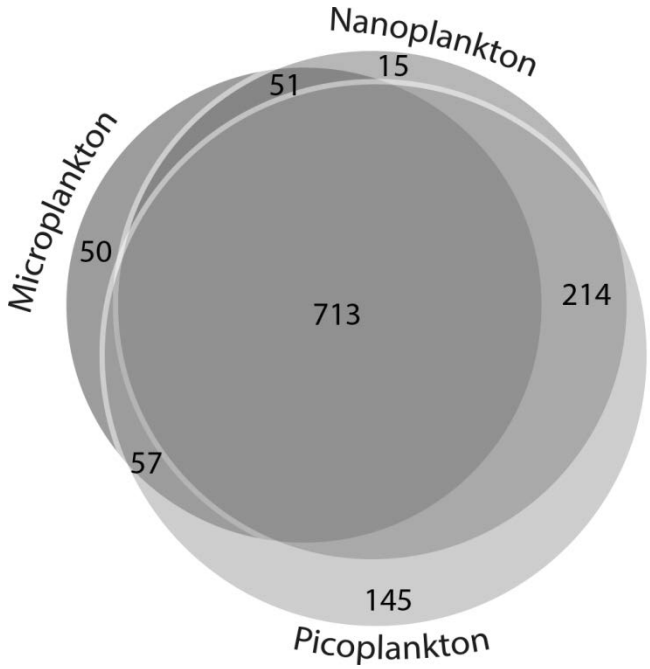

Figure S3: Venn diagram of ASV intersections of the microeukaryotic plankton size-fractions, namely microplankton (50-20 µm), nanoplankton (20-3 µm) and picoplankton (3-0.2 µm) with a total of 1,245 ASVs. ASVs were generated with a 98% similarity threshold.

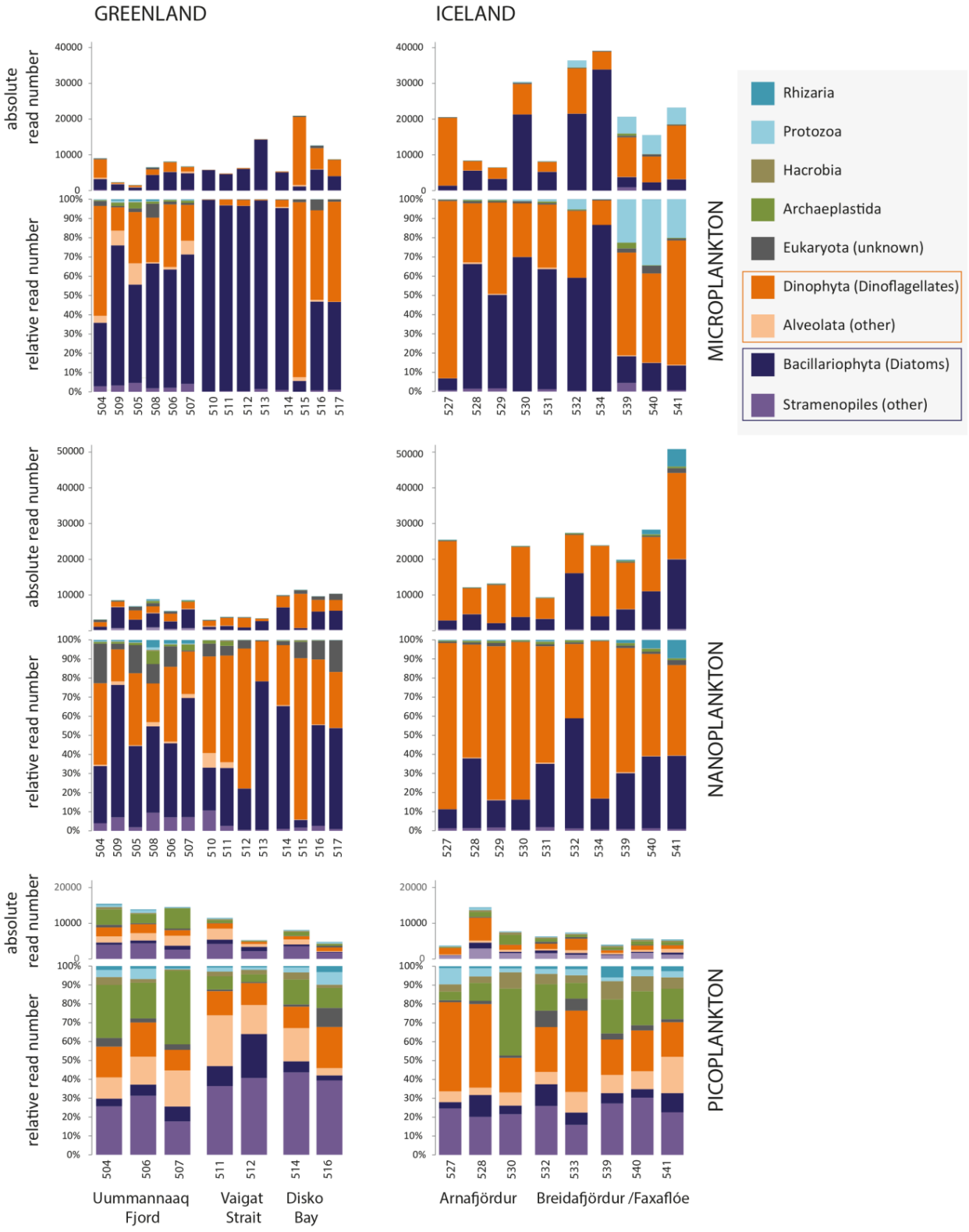

Figure S4: Absolute (top) and relative (bottom) abundance of taxonomic groups within Greenland and Iceland coastal water samples for the microeukaryotic plankton size fractions microplankton (20-50 µm), nanoplankton (3-20 µm), and picoplankton (3-0.2 µm). Histogram bar labels correspond to station numbers for the respective study areas.

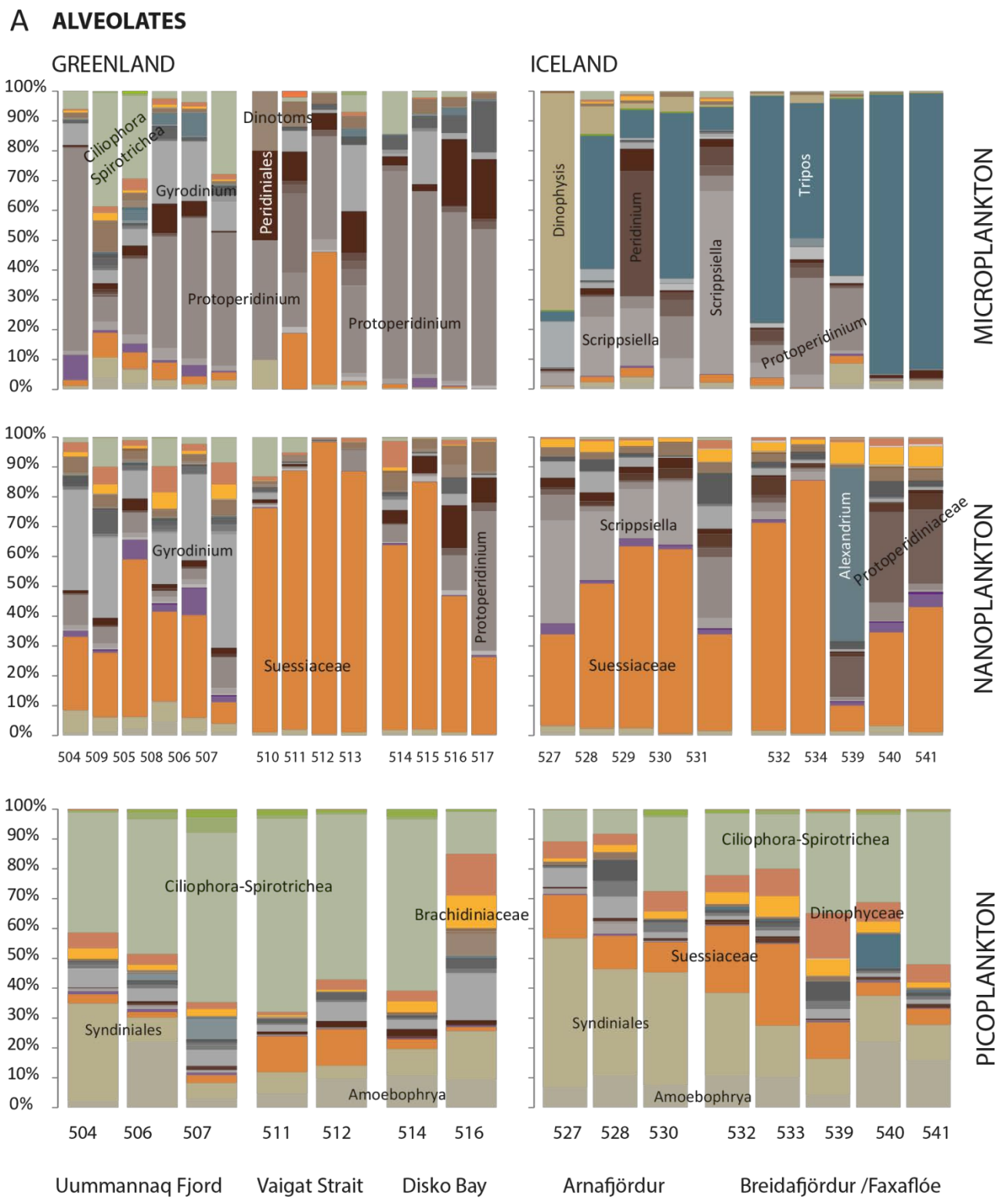

Figure S5: Relative abundance of taxonomic groups within the **(A)** Alveolates and **(B)** Stramenopiles (% of total reads) for the microplankton (20-50 µm), nanoplankton (3-20 µm), and picoplankton (3-0.2 µm) size fractions. Histogram bar labels correspond to station numbers for the respective study areas.

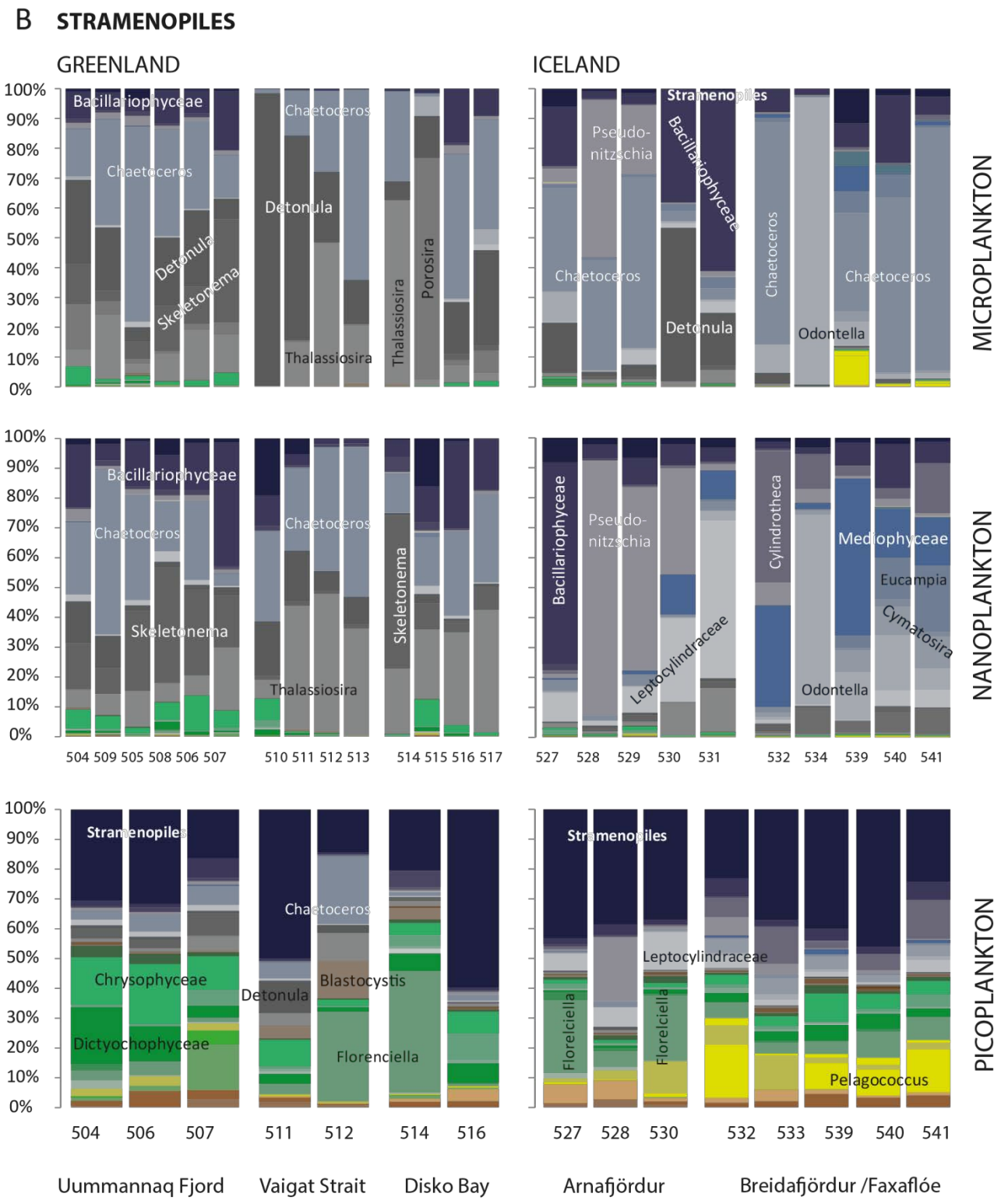

Figure S5: Relative abundance of taxonomic groups within the **(A)** Alveolates and **(B)** Stramenopiles (% of total reads) for the microplankton (20-50 µm), nanoplankton (3-20 µm), and picoplankton (3-0.2 µm) size fractions. Histogram bar labels correspond to station numbers for the respective study areas.

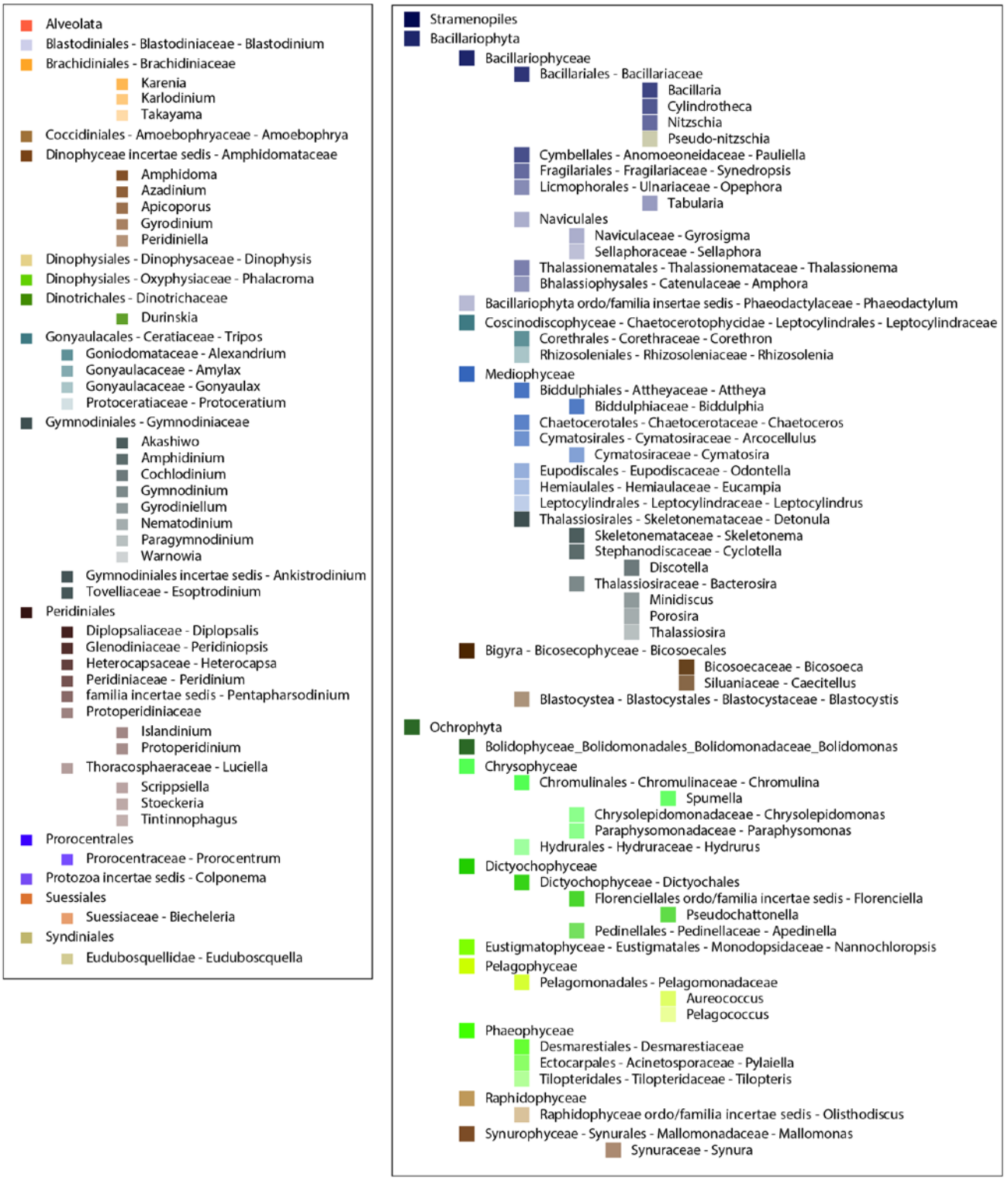

Figure S5: Color Code. Relative abundance of taxonomic groups within the **(A)** Alveolates and **(B)** Stramenopiles (% of total reads) for the microplankton (20-50 µm), nanoplankton (3-20 µm), and picoplankton (3-0.2 µm) size fractions.

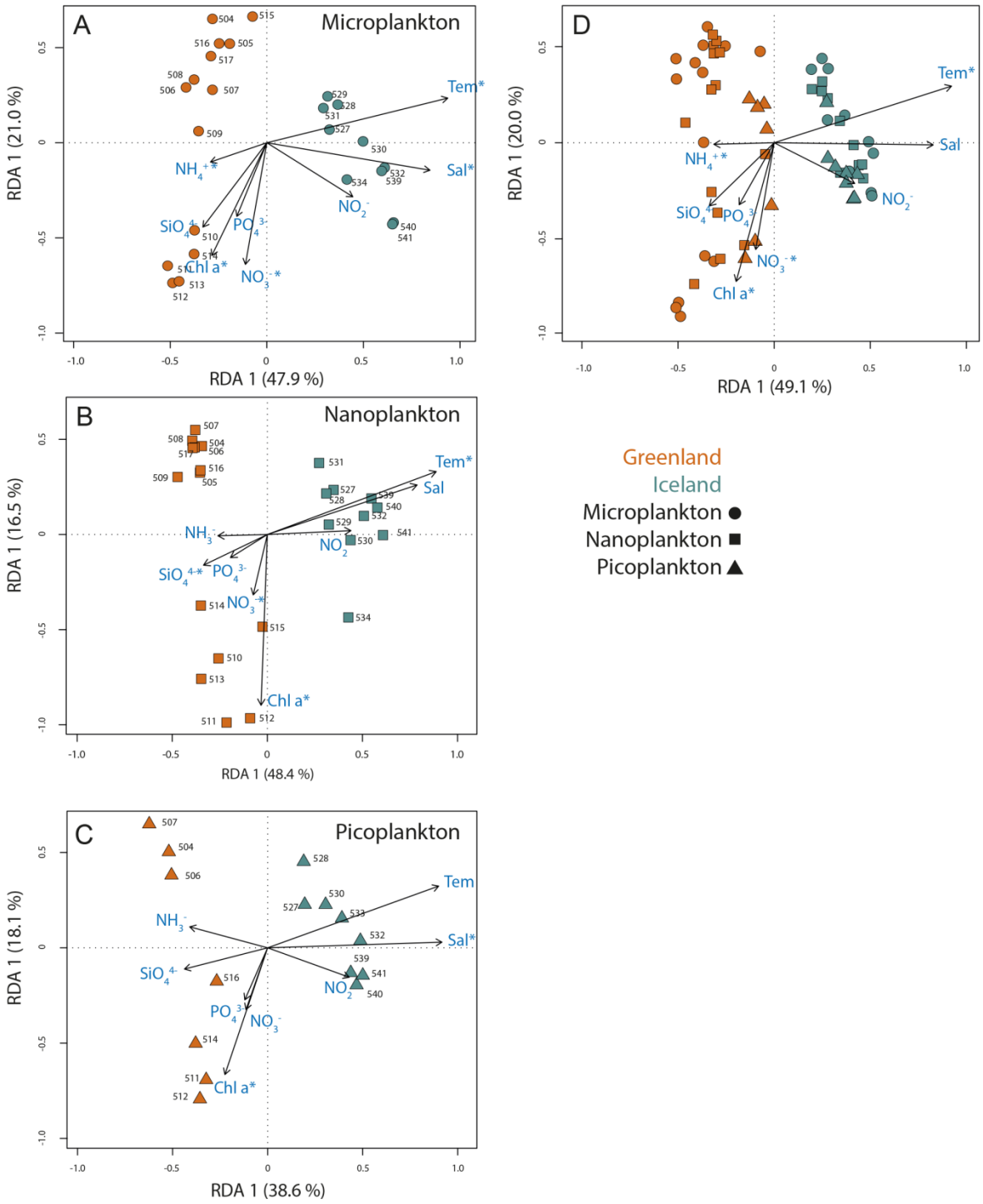

Figure S6: Redundancy analysis (RDA) plots of Hellinger-transformed surface samples from for the microeukaryotic plankton size-fractions microplankton (A, 20-50 µm), nanoplankton (B, 3-20 µm), picoplankton (C, 0.2-3 µm), and all size-fractions together (D) correlated with environmental variables ammonium (NH_3^-), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), salinity (Sal), silicate (SiO_4^{4+}), and temperature (Tem). The percentage of explained variance by each RDA axis is written in parenthesis within axis labels. Asterisks represent statistical significance (ANOVA test: $p \leq 0.05$) of environmental parameters.

1.3 Supplementary Tables

Table S1 Transcriptome indices according to Martinez and Reyes-Valdes (2008). With p_{ij} = relative transcriptome frequency for the i^{th} gene (i = 1, 2, …, g) in a functional category j^{th} (j = 1, 2, …, t).

	Transcriptome index	Formula
(1)	Transcriptome diversity Hj	$H_j = -\sum_{i=1}^{g} p_{ij} log_2(p_{ij})$
(2)	Average gene frequency p_i	$p_i = \frac{1}{t}\sum_{j=1}^{i} p_{ij}$
(3)	Gene specificity S_i	$S_i = \frac{1}{t}(\sum_{j=1}^{t} \frac{p_{ij}}{p_i} log_2 \frac{p_{ij}}{p_i})$
(4)	Functional specialization δ_j	$\delta_i = \sum_{i=1}^{g} p_{ij} S_i$
(5)	Kullback–Leibler divergence D_j	$D_j = (-\sum_{i=1}^{g} p_{ij} log_2(p_i)) - H_j$

Table S2 454-pyrosequencing reads and ASVs after quality processing and removing of singletons, doubletons, potential fungal and metazoan sequences (defined by QIIME) for the microeukaryotic plankton size-fractions, namely microplankton (20-50 µm), nanoplankton (3-20 µm), and picoplankton (0.2-3 µm).

	Microplankton		Nanoplankton		Picoplankton	
Station	reads	ASVs	reads	ASVs	reads	ASVs
504	9080	337	3042	264	15431	446
509	2342	252	8572	385		
508	1556	246	6850	326		
505	6575	324	8820	451		
506	8170	307	5515	308	13867	511
507	6807	306	8606	405	14519	446
510	5801	27	2961	177		
511	4785	62	3809	161	11506	523
512	6318	58	3705	73	5279	360
513	14382	81	3342	79		
514	5392	85	9958	234	8134	524
515	20940	161	11493	218	4736	450
516	12615	221	9629	202		
517	8726	155	10354	147		
527	20546	204	25478	263	3796	364
528	8409	324	12146	385	14457	632
529	6517	344	13212	400		
530	30485	164	23726	229	7712	434
531	8245	343	9362	403		
532	36411	278	27382	337	6299	509
533					7388	520
534	39126	139	23906	250		
539	20631	271	19869	324	3966	411
540	15556	129	28280	400	5703	468
541	23183	174	50843	381	5465	431

Table S3 Number of ASVs correlating with environmental parameter: ammonium, nitrate, nitrite, phosphate, salinity, silicate, and temperature for Greenland and Iceland coastal water samples.

environmental parameter	Greenland	Iceland
Temperature	34	32
Salinity	32	44
Nitrate	19	36
Nitrite	14	10
Phosphate	14	23
Silicate	15	16
Ammonium	5	6

Table S4 Mantel test to determine the environment impact on the community structure independent from geographic distances.

	Microplankton		Nanoplankton		Picoplankton	
Factors to correlate	Mantel statistic	*p*-value	Mantel statistic	*p*-value	Mantel statistic	*p*-value
Community to environment (partial)	0.3324	0.001	0.2686	0.003	0.06992	0.248
Community to geographic distance (partial)	0.5617	0.001	0.6276	0.001	0.6891	0.001
Community to environment	0.3884	0.001	0.34	0.001	0.2567	0.018
Community to geographic distance	0.5887	0.001	0.6498	0.001	0.712	0.002
Environment to geographic distance	0.214	0.009	0.2165	0.007	0.2947	0.011

Table S5 Calculations of diversity indices (inverse Simpson's index, Simpson's index, Shannon diversity and Pielou's evenness J) for the microeukaryotic plankton size-fractions, namely microplankton (20-50 µm), nanoplankton (3-20 µm), and picoplankton (0.2-3 µm).

	Microplankton				**Nanoplankton**				**Picoplankton**			
Station	invsimp	simp	shannon	J	invsimp	simp	shannon	J	invsimp	simp	shannon	J
504	13.394	0.925	3.593	0.617	13.878	0.928	3.674	0.659	33.774	0.970	4.470	0.733
505	21.114	0.953	4.247	0.771	13.599	0.926	3.618	0.625				
506	19.395	0.948	3.832	0.669	20.298	0.951	3.814	0.666	51.142	0.980	4.739	0.760
507	18.396	0.946	3.766	0.658	11.568	0.914	3.721	0.620	10.618	0.906	3.913	0.641
508	20.602	0.951	3.864	0.668	28.017	0.964	4.293	0.702				
509	15.280	0.935	3.737	0.676	7.434	0.865	3.424	0.575				
510	1.075	0.070	0.231	0.070	4.977	0.799	2.868	0.554				
511	2.265	0.558	1.488	0.361	3.738	0.732	2.304	0.453	58.990	0.983	4.790	0.765
512	4.806	0.792	1.892	0.466	2.063	0.515	1.348	0.314	15.281	0.935	3.890	0.661
513	3.921	0.745	1.842	0.419	4.793	0.791	2.085	0.477				
514	2.771	0.639	1.739	0.391	6.511	0.846	2.826	0.518	20.435	0.951	4.575	0.731
515	4.438	0.775	2.424	0.477	2.336	0.572	1.896	0.352				
516	20.391	0.951	3.644	0.675	12.617	0.921	3.196	0.602	48.413	0.979	4.786	0.783
517	16.901	0.941	3.467	0.688	9.146	0.891	2.748	0.551				
527	2.137	0.532	1.573	0.296	8.366	0.880	2.944	0.528	21.225	0.953	4.138	0.702
528	11.265	0.911	3.271	0.566	6.107	0.836	2.921	0.491	34.515	0.971	4.628	0.718
529	12.696	0.921	3.522	0.603	4.774	0.791	2.963	0.495				
530	4.699	0.787	2.257	0.443	4.234	0.764	2.545	0.468	19.929	0.950	4.128	0.680
531	6.299	0.841	3.019	0.517	12.023	0.917	3.579	0.597				
532	4.030	0.752	2.235	0.397	6.113	0.836	2.708	0.465	46.830	0.979	4.800	0.770
533									54.245	0.982	4.938	0.790
534	1.442	0.306	0.942	0.191	2.263	0.558	1.808	0.327				
539	8.424	0.881	3.109	0.555	17.083	0.941	3.545	0.613	72.029	0.986	5.011	0.833
540	4.683	0.786	2.161	0.445	18.965	0.947	3.775	0.630	65.544	0.985	4.934	0.802
541	3.040	0.671	1.813	0.351	14.165	0.929	3.379	0.569	55.743	0.982	4.770	0.786

Table S6 Calculations of diversity indices (inverse Simpson's index, Simpson's index, Shannon diversity and Pielou's evenness J) for the diatoms and dinoflagellates in the microplankton size-fraction (20-50 µm).

		dinoflagellates				**diatoms**			
	Station	invsimp	simp	shannon	J	invsimp	simp	shannon	J
Greenland	504	5.361	0.813	2.499	0.504	8.674	0.885	2.950	0.664
	505	26.207	0.962	3.850	0.844	8.591	0.884	2.750	0.645
	506	7.753	0.871	2.953	0.613	6.254	0.840	2.702	0.655
	507	7.492	0.867	2.849	0.612	10.824	0.908	3.007	0.670
	508	11.640	0.914	3.277	0.676	10.035	0.900	2.980	0.669
	509	22.130	0.955	3.784	0.856	9.528	0.895	2.893	0.653
	510	6.250	0.840	1.887	0.970	1.067	0.063	0.198	0.071
	511	7.069	0.859	2.445	0.759	2.110	0.526	1.284	0.381
	512	3.631	0.725	1.898	0.590	4.467	0.776	1.719	0.510
	513	12.165	0.918	2.858	0.877	3.775	0.735	1.745	0.480
	514	5.199	0.808	2.189	0.672	2.482	0.597	1.461	0.384
	515	3.693	0.729	2.126	0.453	2.324	0.570	1.333	0.420
	516	7.417	0.865	2.811	0.613	12.787	0.922	2.929	0.659
	517	7.533	0.867	2.695	0.617	9.004	0.889	2.720	0.670
Iceland	527	1.821	0.451	1.143	0.253	7.204	0.861	2.572	0.623
	528	5.382	0.814	2.649	0.543	5.916	0.831	2.319	0.511
	529	5.179	0.807	2.673	0.536	6.758	0.852	2.586	0.579
	530	3.746	0.733	2.140	0.486	2.544	0.607	1.323	0.332
	531	4.321	0.769	2.504	0.511	2.923	0.658	1.931	0.431
	532	2.081	0.519	1.588	0.329	1.840	0.456	1.237	0.279
	534	4.417	0.774	2.177	0.506	1.089	0.082	0.277	0.073
	539	3.369	0.703	2.124	0.452	9.914	0.899	2.664	0.659
	540	1.586	0.370	1.000	0.251	3.726	0.732	1.778	0.489
	541	1.420	0.296	0.817	0.192	1.531	0.347	1.009	0.270