



Title	Impacts of meltwater discharge from marine-terminating glaciers on the protist community in Inglefield Bredning, northwestern Greenland
Author(s)	Matsuno, Kohei; Kanna, Naoya; Sugiyama, Shin; Yamaguchi, Atsushi; Yang, Eun Jin
Citation	Marine ecology progress series, 642, 55-65 https://doi.org/10.3354/meps13324
Issue Date	2020-05-28
Doc URL	http://hdl.handle.net/2115/81543
Type	article (author version)
File Information	manuscript received 2020-08-21-2.pdf



[Instructions for use](#)

1 Impacts of meltwater discharge from marine-terminating glaciers on the protist
2 community in Inglefield Bredning, northwestern Greenland

3 Running page head: Meltwater impacts on a protist community

4 Kohei Matsuno^{1,2*}, Naoya Kanna², Shin Sugiyama^{2,3}, Atsushi Yamaguchi^{1,2}, Eun Jin
5 Yang⁴

6 1: Graduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho,
7 Hakodate, Hokkaido, 041-8611, Japan

8 2: Arctic Research Centre, Hokkaido University, Kita-21 Nishi-11 Kita-ku, Sapporo,
9 Hokkaido, 001-0021, Japan

10 3: Institute of Low Temperature Science, Hokkaido University, Kita-19 Nishi-8 Kita-ku,
11 Sapporo, Hokkaido, 060-0819, Japan

12 4: Division of Polar Ocean Science, Korea Polar Research Institute, 26 Songdomirae-ro,
13 Yeonsu-gu, Incheon, 21990, Republic of Korea

14 * Corresponding author

15 E-mail, k.matsuno@fish.hokudai.ac.jp

1 **Abstract**

2 To evaluate the effects of meltwater discharge from marine-terminating glaciers on a
3 fjord protist community in northwestern Greenland during summer, we investigated the
4 distribution, abundance and biomass of the protist community and their relationships
5 with hydrographic parameters. In the standing stock of protists, dinoflagellates (46.4%)
6 and oligotrich ciliates (39.5%) were dominant throughout the study region. According to
7 the vertical distribution, oligotrich ciliates were abundant in the surface layer, mainly
8 due to suitable food conditions (abundance of diatom and nanoflagellates). Near glaciers,
9 relatively high chl *a* concentrations were found in the subsurface layers associated with
10 the low-temperature, high-turbidity and slightly high nutrient levels, indicating that the
11 nutrient inputs from the upwelling glacial meltwater plume increased primary
12 production. Large-sized *Protoperdium* spp. were found only at stations near glaciers
13 where nutrients were abundant, and heterotrophic dinoflagellates showed strong
14 relationships with nanoflagellates. These findings suggest that the upwelling associated
15 with subglacial meltwater discharge can stimulate nanoflagellate production, resulting
16 in increases in ciliate and heterotrophic dinoflagellate production.

17 **Keywords:** Protist community, Meltwater discharge, Marine-terminating glacier,
18 Ciliates, Dinoflagellates

1. INTRODUCTION

Recently, tidewater glaciers in Greenland have been thinning and retreating under the influence of atmospheric and oceanic warming (e.g., Howat & Eddy 2011, Cowton et al. 2018). These glaciers flow directly into the ocean, forming important ice-ocean boundaries in glacial fjords. Near the glacier front in a fjord, subglacial discharge upwells cold and high-nutrient deep-water into euphotic zone (Bendtsen et al. 2015, Meire et al. 2017; Kanna et al., 2018), inducing high primary production throughout summer in the fjord (Arendt et al. 2013, Juul-Pedersen et al. 2015, Meire et al. 2017, Kanna et al. 2018). By contrast, this upwelling does not occur in fjords with land-terminating glaciers, which are characterized by lower productivity (Meire et al. 2017, Middelbo et al. 2018). The amount and type of inflow (surface or subsurface) of freshwater into the fjord may influence fjord water circulation, nutrient availability and subsequent productivity (Mortensen et al. 2011, Lydersen et al. 2014).

Ciliates and heterotrophic dinoflagellates (HDF), which have frequently been observed to ingest bacteria, autotrophic nanoflagellates and diatoms (Gast 1985, Paranjape 1987, Sherr et al. 2003; Calbet et al. 2011), are abundant in Arctic marine systems during summer (Sherr et al. 1997, Rysgaard et al. 1999, Seuthe et al. 2011). Additionally, heterotrophic nanoflagellates (HNF) are the essential link from bacteria to

1 the ciliates and the HDFs. These heterotrophic organisms are preyed upon by
2 mesozooplankton (Levinsen et al. 2000, Levinsen & Nielsen 2002); thus, they play
3 important roles in the microbial loop (Pomeroy 1974). In Arctic marine waters, the
4 species compositions of the protist communities are typically dominated by athecate
5 dinoflagellates and oligotrich ciliates, whereas thecate dinoflagellates and tintinnids
6 show low abundances (Levinsen & Nielsen 2002; Krawczyk et al. 2015). For
7 heterotrophic organisms, their biomasses are influenced by phytoplankton blooms in the
8 Arctic Ocean (Taniguchi 1984, Sherr et al. 1997, Olson & Strom 2002). In the Disko
9 Bay, oligotrich ciliates and HDFs have been found to be the dominant members of the
10 protist community during summer (Levinsen et al. 2000, Levinsen & Nielsen 2002).
11 Their biomasses peak during this time due to decreases in the top-down effect that are
12 driven by the absence of large copepods from the euphotic zone in early spring and late
13 summer (Levinsen & Nielsen 2002).

14 Around Greenland, the features of the protist community have been studied in
15 Disko Bay (Nielsen & Hansen 1995, Levinsen et al. 1999, Levinsen et al. 2000), in
16 Young Sound (Rysgaard et al. 1999, Rysgaard & Nielsen 2006, Krawczyk et al. 2015)
17 and in Godthåbsfjord (Arendt et al. 2010, Calbet et al. 2011). However, beyond these
18 regions, there are few studies and no information is available regarding the protist

1 communities in northwestern Greenland. In addition, the impacts of meltwater
2 discharges from glaciers on bacterial production (Paulsen et al. 2017), primary
3 production (Arendt et al. 2013, Juul-Pedersen et al. 2015, Meire et al. 2017) and
4 zooplankton (Arendt et al. 2016) have been reported in Greenland, and the distributions
5 of protist communities in Greenlandic fjords have been studied (e.g., Nielsen & Hansen
6 1995, Rysgaard et al. 1999). However, the impacts of meltwater discharge on protists
7 are not fully understood.

8 In this study, we revealed the spatial distributions of protists (diatoms,
9 dinoflagellates, ciliates and nanoflagellates) in Inglefield Bredning, a ~100-km long and
10 ~20-km wide glacial fjord in northwestern Greenland. Recent studies in this region have
11 revealed the importance of meltwater discharge from a marine-terminating glacier for
12 biogeochemical processes in fjords (Kanna et al. 2018, Naito et al. 2019). Inglefield
13 Bredning is fed by retreating marine-terminating glaciers (Sakakibara and Sugiyama
14 2018) and glacial changes are possibly affecting marine ecosystems. Based on sampling
15 and hydrographic observations, we evaluated the impacts of meltwater discharges from
16 marine-terminating glaciers on the biomasses of the protist community during summer.

17 **2. MATERIALS & METHODS**

1 **2.1. Field sampling**

2 Boat-based observations were performed during daytime over 13-17 August 2018 in the
3 northeastern section of Inglefield Bredning (Fig. 1). The study area includes regions that
4 are within several kilometers from Sharp and Hart Glaciers, as well as the region ~10
5 km from Melville Glacier. These glaciers discharge meltwater and icebergs into the
6 fjord through calving fronts that are several kilometers wide (Sakakibara and Sugiyama
7 2018). Temperature, salinity, fluorescence, turbidity (Formazin Turbidity Units: FTU)
8 and dissolved oxygen (DO) were measured with a current, temperature and depth
9 (CTD) profiler (ASTD 102, JFE Advantech, Japan) at six stations. At these stations,
10 water samples (500 mL) were collected by Niskin-X bottles (General Oceanics, Inc.)
11 from 0, 10 m, 20 m, 30 m, 40 m, 50 m, 70 m, and 100 m depth at all six stations except
12 for St. 4, where samples were collected from 0 m - 40 m (Fig. 1). The samples were
13 fixed with 1% glutaraldehyde. For the nutrient and chl *a* measurements, seawater
14 samples were collected from five to eight depths (e.g., 0 m, 10 m, 20 m, 30 m, 40 m, 50
15 m, 70 m, and 100 m, depending on bottom depth). For the chl *a* measurements, 100 mL
16 of seawater from each sample was filtered on a GF/F filter. The filtered sample was
17 immersed in N,N-dimethylformamide (Wako Pure Chemical Industries, Ltd.) under
18 dark conditions for 24 h. The samples for nutrient and chl *a* (extracted in

1 N,N-dimethylformamide) analyses were immediately placed in a freezer (-20°C),
2 transported to Japan without melting, and then stored in a cold laboratory (-20°C) until
3 analysis. The nutrients (nitrate, nitrite, phosphate and silicic acid) in seawater were
4 analyzed using an autoanalyzer (QuAatro, BL TEC, Inc.) after sample filtration using
5 syringe GF/F filters. The measurements of all nutrients were quality controlled by using
6 reference seawater materials (KANSO Technos Co., Ltd.). Chl *a* was measured with a
7 fluorometer (Turner Designs, Inc., 10-AU) using the nonacidification method of
8 Welshmeyer (1994).

9 **2.2. Microscopic analysis**

10 In the laboratory, the 500-mL water samples were stored on a stone table for more than
11 1 day to allow the microprotist cells to settle to the bottom of each bottle. Then, the
12 samples were concentrated to 20 mL using a siphon. Subsamples (0.25-1 mL) were
13 mounted on glass microscope slides with lines and the diatoms, dinoflagellates and
14 ciliates were counted and identified to the species/genus levels under an inverted
15 microscope with 40–400 \times magnification. Nanoflagellates, i.e., flagellates with cell sizes
16 of 5-10 μm , were also counted, but their cell densities might have been underestimated
17 due to their small size. All of the nanoflagellates were classified as

1 autotrophic/mixotrophic/heterotrophic because we did not check the cells having
2 chloroplasts or by not using microscopic epifluorescence. We enumerated 8–2,272
3 protist cells (including nanoflagellates) per sample. As we concentrated each sample
4 from a volume of 500 mL to a volume of 20 mL, the limit of detection was 160 cells L⁻¹
5 for each 0.25-mL subsample and was 40 cells L⁻¹ for each 1-mL subsample.

6 Species identifications were made to the lowest possible level (e.g., species or
7 genus) (Table 1). For species identification, we referred to Hasle and Syvertsen (1997)
8 for diatoms, Fukuyo et al. (1997) and Evagelopoulos (2002) for dinoflagellates, and
9 Maeda (1997) and Taniguchi (1997) for ciliates. Silicoflagellates and cysts of each
10 taxon were omitted from the following analyses due to their low densities. Our
11 preservative (glutaraldehyde) is an adequate fixative for diatoms and dinoflagellates but
12 not for ciliates. Because ciliates are better preserved with acidic Lugol's solution
13 (Stoecker et al. 1994) than with glutaraldehyde, our preservative may have led to biased
14 estimates of community structure and biomass (through the loss of unloricated ciliates).

15 Using the cell sizes (length and width) measured during the counting process,
16 the protist biovolumes were calculated following the method reported by Sun and Liu
17 (2003). Then, the carbon biomasses were estimated using the carbon-volume
18 relationship proposed by Menden-Deuer and Lassard (2000). Finally, the biomasses (μg

1 C L⁻¹) were calculated from the carbon biomass per cell and from the abundances (cells
2 L⁻¹).

3 **2.3. Data analysis**

4 To evaluate the environmental factors controlling the changes in the protist community,
5 we applied Structural Equation Modeling (SEM) analysis (Stomp et al. 2011). For the
6 SEM analysis, the environmental parameters (temperature, salinity, turbidity, silicate
7 and chl *a*) and the biomass of each protist taxon were normalized (average = 0, standard
8 deviation = 1), and regressions among all parameters were calculated. For the path
9 analysis, we set the parameters for three categories (1: temperature, salinity, turbidity,
10 and silicate; 2: chl *a*, diatoms, and nanoflagellates; 3: dinoflagellates, oligotrich ciliates,
11 and tintinnids). Subsequently, parameters with insignificant relationships ($p > 0.05$)
12 were removed from the final model (cf. Matsuno et al. 2016). The SEM analysis was
13 performed using add-in software for MS-Excel
14 (<http://www.ohmsha.co.jp/data/link/978-4-274-06925-3/>).

15 **3. RESULTS**

16 **3.1. Environmental conditions**

1 The surface water temperatures ranged from 0.67 to 4.3°C (Fig. 2). The temperatures
2 between 0 and 20 m depth were lower at St. 4 and 5 than at the other stations. The
3 salinities in the surface layer ranged from 27.5 to 30.2 and, similar to temperature, were
4 lower at stations near the glacier (i.e., St. 4 and St. 5) than at those in the downfjord
5 region (Fig. 2). The turbidity levels showed marked differences among the stations.
6 Near glaciers, high turbidities were observed below 50 m at St. 5 and St. 6 and at
7 approximately 10 m at St. 4 and St. 5. The fluorescence and chl *a* levels showed similar
8 patterns. High chl *a* concentrations were observed near 10 m at the near-glacier stations
9 and relatively high values were observed near 75 m at St. 2, St. 3 and St. 6. The
10 nitrite+nitrate and phosphate levels showed ranges of 0.03-14.1 and 0.18-1.5 $\mu\text{mol kg}^{-1}$,
11 respectively, and high concentrations of both were observed below 60 m at the
12 near-glacier stations (St. 5 and St. 6). The distribution patterns of silicate were similar to
13 the nitrite+nitrate patterns. Comparing the temperature and turbidity at subsurface layer
14 (10 m depth), water at St. 3 and St. 4 was significantly colder and more turbid (1.2°C
15 and 0.82 FTU) than those at the other stations (3.0°C and 0.22 FTU) (*U*-test, $p < 0.01$
16 and $p < 0.05$) (Fig. 3). Low chl *a* concentrations and high nutrient levels were found in
17 the deeper layer (> 60 m depth), whereas in the surface layer, the chl *a* concentrations
18 were highly variable and the nutrient concentrations were low (Fig. S1). The

1 relationship between chl *a* and nitrite+nitrate was significantly negative (Spearman's
2 correlation analysis, $p < 0.05$).

3 **3.2. Spatial distributions of protists**

4 In this study, we identified 33 protist taxa. These included six diatom taxa (five different
5 genera and one species), six dinoflagellate taxa (five different genera and two different
6 species), seven oligotrich ciliate taxa (six different genera and one species), seven
7 tintinnid ciliate taxa (five different genera and six different species), one silicoflagellate
8 taxa, cyst/resting spore for each taxon, and nanoflagellates from different categories
9 based on cell size and shape (Table 1). The total protist abundances and biomasses had
10 ranged of 408–273,019 cells L⁻¹ and 0.07–72.81 µgC L⁻¹, respectively. For the diatoms,
11 their densities ranged from 0 to 526 cells L⁻¹ (representing 0–14% of the total protist
12 density), and the biomass exhibited a range of 0–14.29 µgC L⁻¹ (representing 0–11% of
13 the total protist biomass). The dinoflagellates ranged in density from 0 to 21,270
14 (representing 0–35% of the total protist density) and their biomass ranged from 0 to
15 39.30 µgC L⁻¹ (representing 0–35% of the total protist biomass). The density of
16 oligotrich ciliates ranged from 0 to 4,563 cells L⁻¹ (representing 0–56% of the total
17 protist density) and the oligotrich ciliate biomass ranged from 0 to 23.57 µgC L⁻¹

1 (representing 0–97% of the total protist biomass). The tintinnids ranged in density from
2 0 to 601 cells L⁻¹ (representing 0–30% of the total protist density) and their biomass
3 ranged from 0 to 7.15 µgC L⁻¹ (representing 0–92% of the total protist biomass). For the
4 nanoflagellates, their densities ranged from 106 to 248,505 cells L⁻¹ (representing
5 10–98% of the total protist density), and their biomass range was 0.0007–14.04 µgC L⁻¹
6 (representing 0.009–58% of the total protist biomass). At each station, within the total
7 protist biomass over the whole water column, dinoflagellates and oligotrich ciliates
8 were the dominant taxa, with mean compositions of 46.4 and 39.5%, respectively (Fig.
9 1). The highest total biomasses were observed near the glacier (St. 5) and at St. 1. The
10 different taxa of the protist community exhibited varying spatial patterns. The oligotrich
11 ciliates were abundant in the surface layer at most of the stations (Fig. 4). Large (> 60
12 µm equivalent spherical diameter (ESD)) dinoflagellates (*Protoperidinium* spp.) were
13 found in the surface layer and/or at 30-70 m at all of the near-glacier stations except at
14 St. 6 (e.g., St. 3, St. 4 and St. 5).

15 **3.3. SEM analysis**

16 The SEM analysis revealed a negative correlation between chl *a* and salinity (path
17 coefficient (pc) = -0.66), i.e., the chl *a* levels increased as the salinity decreased (Fig. 5).

1 The diatom biomass showed positive correlations with turbidity and with the biomass of
2 oligotrich ciliates ($pc=0.48$ and 0.39 , respectively). Nanoflagellates had positive
3 correlations with temperature, nitrite+nitrate, dinoflagellates and oligotrich ciliates; the
4 correlations with temperature and dinoflagellates were particularly high ($pc=0.92$ and
5 0.76 , respectively) (Fig. 5). The oligotrich ciliates showed positive correlations with
6 diatoms ($pc=0.39$) and nanoflagellates ($pc=0.37$), whereas they were negatively
7 correlated with temperature ($pc=-0.48$), salinity ($pc=-0.85$) and turbidity ($pc=-0.37$).

8 **4. DISCUSSION**

9 **4.1. Protist biomass in the fjords around Greenland**

10 The heterotrophic protists, which mainly comprise HNFs, ciliates and HDFs, consume a
11 broad range of prey, ranging from bacteria to diatoms (Gast 1985, Paranjape 1987, Sherr
12 et al. 2003; Calbet et al. 2011). Heterotrophic protists, in turn, are important prey items
13 for mesozooplankton (Levinsen et al. 2000, Levinsen & Nielsen 2002). Thus, the
14 heterotrophic protists play significant roles in the pelagic food webs of the Arctic Ocean
15 (and in many other ocean systems) (Sherr et al. 1997, Rysgaard et al. 1999, Seuthe et al.
16 2011).

17 In Arctic waters, protist communities are dominated by athecate dinoflagellates

1 and by oligotrich ciliates, whereas thecate dinoflagellates and tintinnids show low
2 abundances (Levinsen & Nielsen 2002). Similar patterns are observed in Disko Bay
3 (Nielsen & Hansen 1995, Levinsen et al. 1999, Levinsen et al. 2000) and in Young
4 Sound (Rysgaard et al. 1999) in Greenland. Despite the suboptimal preservation of
5 oligotrich ciliates, our findings regarding protist community structures and biomass are
6 comparable to those of previous studies.

7 We summarize the mean biomass of each heterotrophic species for our site and
8 other well-studied sites around Greenland (Table 2). Our biomass values are higher than
9 those for Young Sound (northeast Greenland) and lower than those for Disko Bay (west
10 Greenland). Two geophysical factors influence protist biomass: glacier type (marine- vs.
11 land-terminating) and sea-ice coverage. Inglefield Bredning is characterized by the
12 intrusion of marine-terminating glaciers. Upwelling subglacial discharges from those
13 glaciers affected the water properties (e.g., temperature, salinity and turbidity) and
14 caused relatively high chl *a* concentrations at St. 4 and St. 5. Regarding sea-ice
15 coverage, the fjord is covered by sea ice until late summer (typically July) (Sugiyama et
16 al. 2015). Accordingly, the protist biomass in the present study was intermediate
17 between that of the highly productive Disko Bay (short sea-ice cover duration and
18 marine-terminating glaciers) and low-productive Young Sound (long sea-ice cover

1 duration and land-terminating glaciers) during summer.

2 **4.2. Relationships between environmental parameters and the protist community**

3 The temperature and salinity distributions revealed a cold and fresh surface water layer
4 near glaciers. Oligotrich ciliates dominated in this layer, and the SEM analysis revealed
5 strongly negative correlations between these ciliates and both temperature and salinity.
6 These findings suggest that fresh surface water is a suitable habitat for oligotrich ciliates.
7 Higher temperatures enhance the growth of ciliates in polar regions (Hansen & Jensen
8 2000). Matsuno et al. (2014) reported a positive relationship of ciliates with temperature
9 in the western Arctic Ocean during autumn. Thus, the relationship between ciliates and
10 temperature in this study was opposite to that reported in previous studies. This result
11 means that the environmental parameters (especially temperature) are not critical factors
12 that affect ciliates in this study region during summer.

13 Ciliate dominance in the region is possibly controlled by the available carbon
14 sources. Ciliates ingest bacteria, phytoflagellates and small diatoms (Gast 1985,
15 Paranjape 1987, Sherr et al. 2003). In this study, diatoms and nanoflagellates had
16 positive relationships with ciliates, and the prey abundances were higher under warmer,
17 higher-nutrient conditions, which indicates that hydrographic conditions influence prey

1 concentrations. Levinsen et al. (2000) suggested that the threshold concentration at
2 which the growth rate equals zero for ciliates and HDFs is $18 \mu\text{gC L}^{-1}$. Assuming that all
3 of the oligotrich ciliates, tintinnids and dinoflagellates in this study were heterotrophic
4 organisms, the average contribution of the ciliates to the carbon biomass of the
5 heterotrophic organisms in this study was 53.5%. The threshold concentration for
6 ciliates is estimated to be $9.63 \mu\text{gC L}^{-1}$ ($=18*0.535$). In this study, the maximum
7 biomass of potential prey (i.e., diatoms and nanoflagellates) for ciliates was $14.07 \mu\text{gC}$
8 L^{-1} at 0 m at St.1, and the average biomass throughout the study region was $0.936 \mu\text{gC}$
9 L^{-1} . Since the average prey concentration was much lower than the threshold
10 concentration needed for ciliate growth, ciliate growth would have been limited by the
11 low prey concentrations (Levinsen et al. 2000).

12 We did not examine the bacterial abundances in this study. Bacterial production
13 is reported to be high ($0.13 \mu\text{gC L}^{-1} \text{ day}^{-1}$) in the surface layer of Young Sound
14 (northeastern Greenland) during summer despite low dissolved organic carbon (DOC)
15 concentrations from river inflows (Paulsen et al. 2017). High bacterial productivity may
16 contribute to the dominance of ciliates in the surface layer, supporting HNF linkage
17 between bacteria and ciliates (Nielsen & Hansen 1995). Seasonally, the maximum
18 ciliate biomasses were seen in surface water after diatom blooms and under decreased

1 grazing pressure from copepods following the migration of large copepods (*Calanus*
2 spp.) to the deeper layers (Levinsen et al. 1999). Despite no information on the grazing
3 pressure by copepods in this study, the ciliate abundances are believed to be controlled
4 by bottom-up effects, not only by sufficient food concentrations (diatoms and
5 nanoflagellates) but also by HNF thriving via high bacterial production.

6 **4.3. Effects of glacial meltwater discharge on protists**

7 Heterotrophic dinoflagellates dominate the microzooplankton biomass in the Arctic
8 Ocean during summer (Andersen 1988, Nielsen & Hansen 1995, Sherr et al. 1997).
9 Levinsen et al. (1999) reported that HDFs represented on average 70% of the integrated
10 microzooplankton biomass when the diatoms formed subsurface blooms. After the
11 diatoms declined, the large HDFs became less abundant. In the present study, the diatom
12 abundances were low throughout the study region, suggesting that the HDF abundances
13 were likely low due to food limitations (Levinsen et al. 1999). The SEM analysis
14 revealed that the dinoflagellates had a significant positive relationship with the
15 nanoflagellates but not with diatoms. This result suggests that the HDF population was
16 maintained by grazing nanoflagellates after the diatom blooms (Levinsen & Nielsen
17 2002). According to Jakobsen and Hansen (1997), the ability of HDFs to cope with

1 starvation is greater than that of planktonic ciliates because under starvation conditions,
2 dinoflagellates can extend their minimum generation time to beyond that of the ciliates.
3 Thus, HDFs can survive due to their flexible grazing strategy (changing prey types in
4 response to availability) and their strong tolerance to starvation in fjords during the
5 summer.

6 Freshwater discharges from the bottom of marine-terminating glaciers induce
7 strong upwelling near the glacier fronts, which transports nutrient-rich deep-layer water
8 into the euphotic layer (Bendtsen et al. 2015, Meire et al. 2017; Kanna et al., 2018). In
9 this study, upwelling water was represented by a highly turbid and low-temperature
10 subsurface layer observed near glaciers. Due to the nutrient input into the euphotic layer,
11 the chl *a* concentrations and primary production increased within the relatively thin
12 subsurface layer (Calbet et al. 2011, Meire et al. 2017). As with previous studies, the chl
13 *a* concentrations in the subsurface layer (10 m) were slightly higher at the near-glacier
14 stations (i.e., St. 4 and St. 5) than at the other stations (0.99 vs 0.67, *U*-test, $p = 0.39$).
15 The nutrient concentrations were uniformly distributed among the stations, but
16 relatively high concentrations were found at St. 4 and St. 5 (0.85 vs 0.14, *U*-test, $p =$
17 0.13). These observations suggest that the relatively high chl *a* levels were maintained
18 by nutrients upwelling near the glacier. Previous studies have indicated that the euphotic

1 depth is very important for chl *a* distribution. During summer in Disko Bay, the
2 euphotic zone is situated at around 30 m (Levinsen et al. 1999). Although we did not
3 measure vertical profiles of light intensity in the water column, high chl *a*
4 concentrations were found in the highly turbid layer. This observation suggests that high
5 turbidity did not prevent primary production by phytoplankton, at least in the subsurface
6 layer. We did not measure primary production and do not have field data from other
7 seasons. Nevertheless, our study results suggest that the nutrient concentrations in the
8 upwelling water near the glacier play a critical role in primary production in Inglefield
9 Bredning during summer.

10 Nanoflagellates are important components of the food web in fjords because
11 they are grazed upon by larger heterotrophic species, as mentioned above. They are
12 abundant in the summer fjords, particularly where plumes occur in front of glaciers (e.g.,
13 Middelbo et al. 2018). In Young Sound, phytoplankton smaller than 10 μm were
14 dominant near the glacier throughout the year (Middelbo et al. 2018, 2019, Holding et al.
15 2019). Near the glacier, strong stratification due to fresh surface water prevents
16 upwelling of nutrients to the surface layer. Thus, small phytoplankton dominate in a
17 low-nutrient surface layer (Daufresne et al. 2009; Li et al. 2009; Gardner et al. 2011).
18 Compared to micro-sized autotrophs (e.g., diatoms), the small cells of nanoflagellates (<

1 10 μm) and even smaller picophytoplankton ($< 2 \mu\text{m}$) are adapted to low-light
2 intensities and are capable of generating efficient primary production (Taguchi 1976,
3 Raven 1998, Holding et al. 2019). The SEM analysis revealed that the nanoflagellates
4 had positive relationships with temperature and nitrite+nitrate. Since the trophic type of
5 the nanoflagellates were unidentifiable in this study, two feasible reasons could explain
6 regarding to heterotrophic and autotrophic nanoflagellates, respectively. Firstly,
7 high-temperature conditions potentially increase the growth rate of HNFs (Choi &
8 Peters 1992). Because of that, the strong relationship between temperature and
9 nanoflagellates in the SEM means that the HNFs potentially increase in the high
10 temperatures. From the different aspect of the nanoflagellates, secondary, the
11 nitrite+nitrate levels potentially stimulated the primary production by autotrophic
12 nanoflagellates (Middelbo et al 2018, 2019, Holding et al. 2019).

13 On the other hand, large-sized *Protoperidium* spp. were found only at stations
14 near glaciers where nutrients were supplied by upwelling water. Large *Protoperidium*
15 spp. (mean 104 μm ESD) can graze on small dinoflagellates (e.g., *Gymnodinium* spp.,
16 mean 31 μm ESD) and on small ciliates (e.g., *Strombidium* spp., mean 41 μm ESD)
17 (Levinsen & Nielsen 2002); the small dinoflagellates and ciliates graze on
18 nanoflagellates (Jakobsen & Hansen 1997). Based on these facts, we speculate that the

1 nanoflagellates (we assume autotrophic and mixotrophic nanoflagellates here) used the
2 upwelling nitrite+nitrate and that the resulting production was transported to the large
3 *Protoperidium* spp. via the small dinoflagellates and ciliates. While as the heterotrophic
4 nanoflagellates, the HNFs have a strong link to bacteria and to the small dinoflagellates
5 and ciliates (Nielsen & Hansen 1995); bacterial production was supported by the labile
6 carbon supplied by meltwater from glaciers (Paulsen et al. 2017, 2019). Therefore, both
7 autotrophic and heterotrophic nanoflagellates could be increased by the upwelling
8 nutrients and the high bacterial production, resulting in the abundance of large HDFs
9 only near glaciers. We conclude that the upwelling subglacial discharge not only affects
10 the chl *a* concentrations but can also stimulate nanoflagellate production, ultimately
11 increasing production via HDFs and ciliates.

12 **Acknowledgements**

13 We thank Toku Oshima, Kim Peterson, Sakiko Daorana, Takuto Ando, Yoshiki Fujisi
14 and Izumi Asaji, who assisted with the field observations. This study was financially
15 supported by the Arctic Challenge for Sustainability (ArCS) project led by Drs. Kumiko
16 Azuma and Toru Hirawake. Part of this study was supported by a Grant-in-Aid for
17 Scientific Research grants 19H03037 (B), 18K14506 (Early Career Scientists), and

1 17H01483 (A) from the Japanese Society for the Promotion of Science (JSPS). Part of
2 this study was supported by the KOPRI Asian Polar Science Fellowship Program 2019.
3

1 **References**

- 2 Andersen P (1988) The quantitative importance of the “microbial loop” in the marine
3 pelagic: a case study from the north Bering/Chukchi Seas. Arch Hydrobiol
4 31:243–251
- 5 Arendt KE, Nielsen TG, Rysgaard S, Tønnesson K (2010) Differences in plankton
6 community structure along the Godthåbsfjord, from the Greenland Ice Sheet to
7 offshore waters. Mar Ecol Prog Ser 401:49–62
- 8 Arendt KE, Juul-Pedersen T, Mortensen J, Blicher ME, Rysgaard S (2013) A 5-year
9 study of seasonal patterns in mesozooplankton community structure in a
10 sub-Arctic fjord reveals dominance of *Microsetella norvegica* (Crustacea,
11 Copepoda). J Plankton Res 35:105–120
- 12 Arendt KE, Agersted MD, Sejr MK, Juul-Pedersen T (2016) Glacial meltwater
13 influences on plankton community structure and the importance of top-down
14 control (of primary production) in a NE Greenland fjord. Estuar Coast Shelf Sci
15 183:123–135
- 16 Bendtsen J, Mortensen J, Lennert K, Rysgaard S (2015) Heat sources for glacial ice
17 melt in a West Greenland tidewater outlet glacier fjord: The role of subglacial
18 freshwater discharge. Geophys Res Lett 42:4089–4095

- 1 Calbet A, Riisgaard K, Saiz E, Zamora S, Stedmon C, Nielsen TG (2011) Phytoplankton
2 growth and microzooplankton grazing along a sub-Arctic fjord (Godthåbsfjord,
3 west Greenland). *Mar Ecol Prog Ser* 442:11–22
- 4 Choi JW, Peters F (1992) Effects of temperature on two psychrophilic ecotypes of a
5 heterotrophic nanoflagellate, *Paraphysomonas imperforate*. *Appl Environ*
6 *Microbiol* 58:593–599
- 7 Cowton TR, Sole AJ, Nienow PW, Slater DA, Christoffersen P (2018) Linear response
8 of east Greenland’s tidewater glaciers to ocean/atmosphere warming. *Proc Natl*
9 *Acad Sci* 115:7907–7912
- 10 Daufresne M, Lengfellner K, Sommer U (2009) Global warming benefits the small in
11 aquatic ecosystems. *Proc Natl Acad Sci USA* 106:12788–12793
- 12 Evagelopoulos A (2002) Taxonomic notes on *Proto-peridinium* (Peridinales,
13 Dinophyceae) species in the Thermaikos Bay (North Aegean Sea, Greece).
14 *Mediterr Mar Sci* 3:41–54
- 15 Fukuyo Y, Inoue H, Takayama H (1997) Class Dinophyceae. In: Chihara M, Murano M
16 (eds) An illustrated guide to marine plankton in Japan. Tokai University Press,
17 Tokyo, p 31–112
- 18 Gardner JL, Peters A, Kearney MR, Joseph L, Heinsohn R (2011) Declining body size:

- 1 a third universal response to warming? Trends Ecol Evol 26:285–291
- 2 Gast V (1985) Bacteria as a food source for microzooplankton in the Schlei Fjord and
3 Baltic Sea with special reference to ciliates. Mar Ecol Prog Ser 22:107–120
- 4 Hansen BW, Jensen F (2000) Specific growth rates of protozooplankton in the marginal
5 ice zone of the central Barents Sea during spring. J Mar Biol Assoc UK
6 80:37–44
- 7 Hasle GR, Syvertsen EE (1997) Marine diatoms. In: Tomas CR (ed) Identifying marine
8 phytoplankton. Academic Press, San Diego, p 5–385
- 9 Holding JM, Markager S, Pedersen TJ-, Paulsen ML, Møller EF, Meire L, Sejr MK
10 (2019) Seasonal and spatial patterns of primary production in a high-latitude
11 fjord affected by Greenland Ice Sheet run-off. Biogeosciences 16:3777–3792
- 12 Howat IM, Eddy A (2011) Multi-decadal retreat of Greenland's marine-terminating
13 glaciers. J Glaciol 57:389–396
- 14 Jakobsen HH, Hansen PJ (1997) Prey size selection, grazing and growth response of the
15 small heterotrophic dinoflagellate *Gymnodinium* sp. and the ciliate *Balanion*
16 *comatum* – a comparative study. Mar Ecol Prog Ser 158:75–86
- 17 Juul-Pedersen T, Arendt KE, Mortensen J, Blicher ME, Søgaard DH, Rysgaard S (2015)
18 Seasonal and interannual phytoplankton production in a sub-Arctic tidewater

1 outlet glacier fjord, SW Greenland. *Mar Ecol Prog Ser* 524:27–38

2 Kanna N, Sugiyama S, Ohashi Y, Sakakibara D, Fukamachi Y, Nomura D (2018)

3 Upwelling of macronutrients and dissolved inorganic carbon by a subglacial

4 freshwater driven plume in Bowdoin Fjord, northwestern Greenland. *J Geophys*

5 *Res* 123:1666–1682

6 Krawczyk DW, Arendt KE, Juul-Pedersen T, Sejr MK, Blicher ME, Jakobsen HH

7 (2015) Spatial and temporal distribution of planktonic protists in the East

8 Greenland fjord and offshore waters. *Mar Ecol Prog Ser* 538:99–116

9 Levinsen H, Nielsen TG (2002) The trophic role of marine pelagic ciliates and

10 heterotrophic dinoflagellates in arctic and temperate coastal ecosystems: A

11 cross-latitude comparison. *Limnol Oceanogr* 47:427–439

12 Levinsen H, Nielsen TG, Hansen BW (1999) Plankton community structure and carbon

13 cycling on the western coast of Greenland during the stratified summer

14 situation. II. Heterotrophic dinoflagellates and ciliates. *Aquat Microb Ecol*

15 16:217–232

16 Levinsen H, Nielsen TG, Hansen BW (2000) Annual succession of marine pelagic

17 protozoans in Disko Bay, West Greenland, with emphasis on winter dynamics.

18 *Mar Ecol Prog Ser* 206:119–134

- 1 Li WKW, McLaughlin FA, Lovejoy C, Carmack EC (2009) Smallest algae thrive as the
2 Arctic ocean freshens. *Science* 326:539–539
- 3 Lydersen C, Assmy P, Falk-Petersen S, Kohler J, Kovacs KM, Reigstad M, Steen H,
4 Strøm H, Sundfjord A, Varpe Ø, Walczowski W, Weslawski JM, Zajaczkowski
5 M (2014) The importance of tidewater glaciers for marine mammals and
6 seabirds in Svalbard, Norway. *J Mar Syst* 129:452–471
- 7 Maeda M (1997) Suborder Oligotrichida. In: Chihara M, Murano M (eds) An illustrated
8 guide to marine plankton in Japan. Tokai University Press, Tokyo, p 397–420
- 9 Matsuno K, Ichinomiya M, Yamaguchi A, Imai I, Kikuchi T (2014) Horizontal
10 distribution of microprotist community structure in the western Arctic Ocean
11 during late summer and early fall of 2010. *Polar Biol* 37:1185–1195
- 12 Matsuno K, Abe Y, Yamaguchi A, Kikuchi T (2016) Regional patterns and controlling
13 factors on summer population structure of *Calanus glacialis* in the western
14 Arctic Ocean. *Polar Sci* 10:503–510
- 15 Meire L, Mortensen J, Meire P, Juul-Pedersen T, Sejr MK, Rysgaard S, Nygaard R,
16 Huybrechts R, Meysman FJ (2017) Marine-terminating glaciers sustain high
17 productivity in Greenland fjords. *Glob Change Biol* 23:5344–5357
- 18 Menden-Deuer S, Lassar EJ (2000) Carbon to volume relationships for dinoflagellates,

- 1 diatoms, and other protest plankton. *Limnol Oceanogr* 45:569–579
- 2 Middelbo AB, Sejr MK, Arendt KE, Møller EF (2018) Impact of glacial meltwater on
3 spatiotemporal distribution of copepods and their grazing impact in Young
4 Sound NE, Greenland. *Limnol Oceanogr* 63:322–336
- 5 Middelbo AB, Møller EF, Arendt KE, Thyrring J, Sejr MK (2019) Spatial, seasonal and
6 inter-annual variation in abundance and carbon turnover of small copepods in
7 Young Sound, Northeast Greenland. *Polar Biol* 42:179–193
- 8 Mortensen J, Lennert K, Bendtsen J, Rysgaard S (2011) Heat sources for glacial melt in
9 a sub-Arctic fjord (Godthåbsfjord) in contact with the Greenland Ice Sheet. *J*
10 *Geophys Res* 116, C01013, doi:10.1029/2010JC006528
- 11 Naito A, Abe Y, Matsuno K, Nishizawa B, Kanna N, Sugiyama S, Yamaguchi A (2019)
12 Surface zooplankton size and taxonomic composition in Bowdoin Fjord,
13 north-western Greenland: A comparison of ZooScan, OPC and microscopic
14 analyses. *Polar Sci* 19:120–129
- 15 Nielsen TG, Hansen BW (1995) Plankton community structure and carbon cycling on
16 the western coast of Greenland during and after the sedimentation of a diatom
17 bloom. *Mar Ecol Prog Ser* 125:239–257
- 18 Nielsen TG, Ottosen LD, Hansen BW (2007) Structure and function of the pelagic

- 1 ecosystem in Young Sound, NE Greenland. Meddr Grønland Bioscience
2 58:88–107
- 3 Olson MB, Strom SL (2002) Phytoplankton growth, microzooplankton herbivory and
4 community structure in the southeast Bering Sea: insight into the formation
5 and temporal persistence of an *Emiliana huxleyi* bloom. Deep Sea Res II
6 49:5969–5990
- 7 Paranjape MA (1987) Grazing by microzooplankton in the eastern Canadian arctic in
8 summer 1983. Mar Ecol Prog Ser 40:239–246
- 9 Paulsen ML, Nielsen SEB, Müller O, Møller EF, Stedmon CA, Juul-Pedersen T,
10 Markager S, Sejr MK, Delgado-Huertas A, Larsen A, Middelboe M (2017)
11 Carbon bioavailability in a high Arctic fjord influenced by glacial meltwater,
12 NE Greenland. Front Mar Sci 4, 176, doi: 10.3389/fmars.2017.00176
- 13 Paulsen ML, Müller O, Larsen A, Møller EF, Middelboe M, Sejr MK, Stedmon C
14 (2019) Biological transformation of Arctic dissolved organic matter in a NE
15 Greenland fjord. Limnol Oceanogr 64:1014–1033
- 16 Pedersen SA, Ribergaard MH, Simonsen CS (2005) Micro- and mesozooplankton in
17 Southwest Greenland waters in relation to environmental factors. J Mar Syst
18 56:85–112

- 1 Pomeroy LR (1974) The ocean's food web: a changing paradigm. *Bioscience*
2 24:499–503
- 3 Raven JA (1998) Small is beautiful: the picophytoplankton, *Funct Ecol* 12:503–513
- 4 Rysgaard S, Nielsen TG, Hansen BW (1999) Seasonal variation in nutrients, pelagic
5 primary production and grazing in a high-Arctic coastal marine ecosystem,
6 Young Sound, Northeast Greenland. *Mar Ecol Prog Ser* 179:13–25
- 7 Rysgaard S, Nielsen TG (2006) Carbon cycling in a high-arctic marine
8 ecosystem—Young Sound, NE Greenland. *Prog Oceanogr* 71:426–445
- 9 Sakakibara D, Sugiyama S (2018) Ice front and flow speed variations of
10 marine-terminating outlet glaciers along the coast of Prudhoe Land,
11 northwestern Greenland. *J Glaciol* 64:300–310
- 12 Seuthe L, Iversen K R, Narcy F (2011) Microbial processes in a high-latitude fjord
13 (Kongsfjorden, Svalbard): II. Ciliates and dinoflagellates. *Polar Biol*
14 34:751–766
- 15 Sherr EB, Sherr BF, Fessenden L (1997) Heterotrophic protist in the central Arctic
16 Ocean. *Deep Sea Res II* 44:1665–1682
- 17 Sherr EB, Sherr BF, Wheeler PA, Thompson K (2003) Temporal and spatial variation in
18 stocks of autotrophic and heterotrophic microbes in the upper water column

- 1 of the central Arctic Ocean. *Deep Sea Res I* 50:557–571
- 2 Stoecker DK, Gifford DJ, Putt M (1994) Preservation of marine planktonic ciliates:
3 losses and cell shrinkage during fixation. *Mar Ecol Prog Ser* 110:293–299
- 4 Stomp M, Huisman J, Mittelbach GG, Litchman E, Klausmeier CA, (2011) Largescale
5 biodiversity patterns in freshwater phytoplankton. *Ecology* 92:2096–2107
- 6 Sugiyama S, Sakakibara D, Tsutaki S, Maruyama M, Sawagaki T (2015) Glacier
7 dynamics near the calving front of Bowdoin Glacier, northwestern Greenland.
8 *J Glaciol* 61:223–232
- 9 Sun J, Liu D (2003) Geometric models for calculating cell biovolume and surface area
10 for phytoplankton. *J Plankton Res* 25:1331–1346
- 11 Taguchi S (1976) Relationship between photosynthesis and cell size of marine diatoms,
12 *J Phycol* 12:185–189
- 13 Taniguchi A (1984) Microzooplankton biomass in the Arctic and subarctic Pacific
14 Ocean in summer. *Mem Natl Inst Polar Res (special issue)* 32:63–80
- 15 Taniguchi A (1997) Suborder Tintinnina. In: Chihara M, Murano M (eds) An illustrated
16 guide to marine plankton in Japan. Tokai University Press, Tokyo, p 421–483
- 17 Welshmeyer NA (1994) Fluorometric analysis of chlorophyll *a* in the presence
18 chlorophyll *b* and phaeopigments. *Limnol Oceanogr* 39:1985–1992
- 19

1 Table 1. Species of protists observed in Inglefield Bredning, northwestern Greenland,
 2 during 13-17 August 2018.

Diatoms	Tintinnids
<i>Cocconeis</i> spp.	<i>Codonella galea</i>
<i>Cylindrotheca closterium</i>	<i>Favera azorica</i>
<i>Navicula</i> spp.	<i>Parafavera denticulata</i>
<i>Odontella</i> spp.	<i>Parafavera hadai</i>
Pennate diatoms	<i>Parafavera jorgenseni</i>
<i>Thalassiosira</i> spp.	<i>Ptychocylis obtusa</i>
Dinoflagellates	<i>Tintinnopsis</i> spp.
<i>Amphidinium</i> spp.	Silicoflagellates
<i>Dinophysis</i> spp.	<i>Dictyoca</i> spp.
<i>Gymnodinium</i> spp.	Other
<i>Gyrodinium</i> spp.	Ciliate cysts
<i>Protoperidinium depressum</i>	Diatom resting spores
<i>Protoperidinium divergens</i>	Dinoflagellates cysts
Ciliates	Nanoflagellates (slender, 5-10 µm)
<i>Laboera strobila</i>	Nanoflagellates (spherical, < 5 µm)
<i>Leegardiera</i> spp.	Nanoflagellates (spherical, 5-10 µm)
<i>Lohmanniella</i> spp.	
<i>Mesodinium</i> spp.	
Scuticociliates	
<i>Strobilidium</i> spp.	
<i>Strombidium</i> spp.	

3

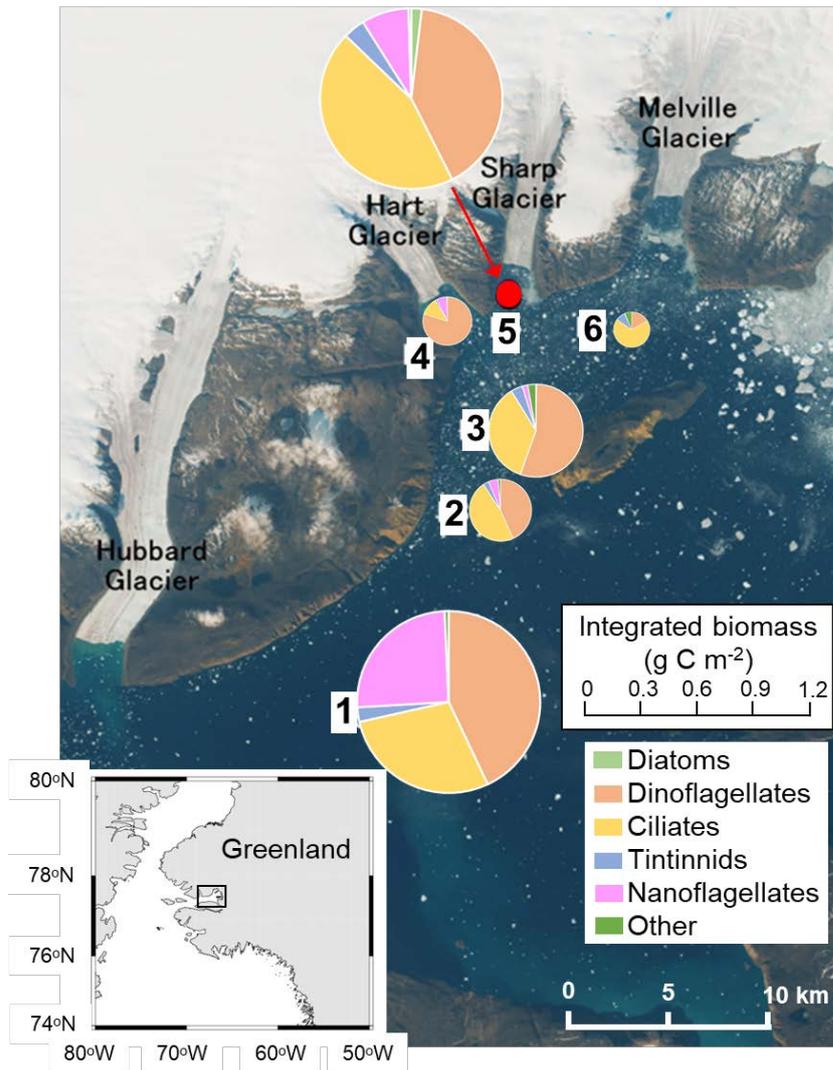
4

1 Table 2. A summary of the biomass standing stock of ciliates, dinoflagellates and
 2 nanoflagellates (NF) around Greenland. MTG: marine-terminating glacier, LTG:
 3 land-terminating glacier. Mean biomass shown integrated biomass for each taxon.

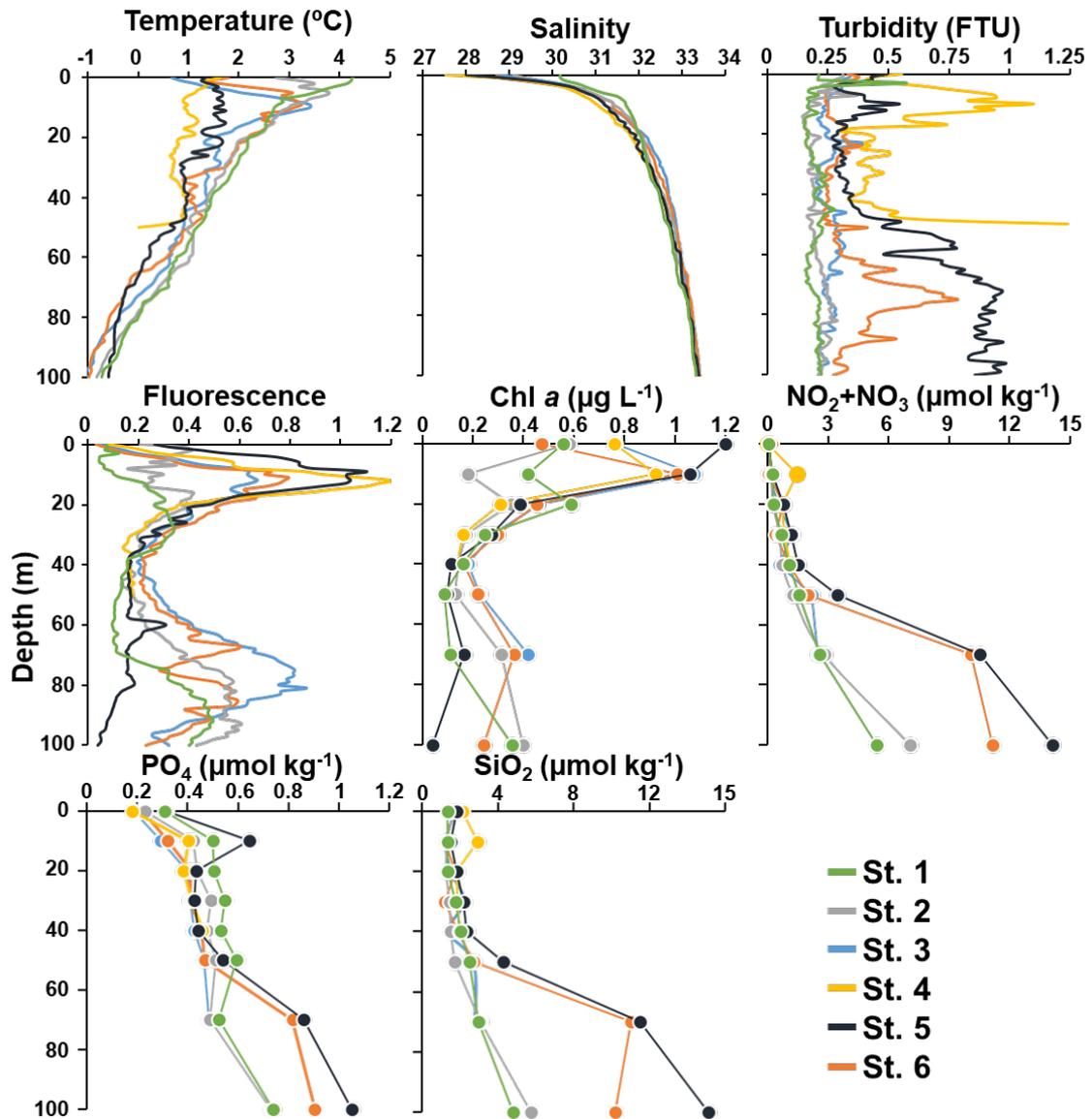
Area	Glacier type	Depth	Period	Integrated biomass (mgC m ⁻²)			Reference
				Ciliates	Dinoflagellates	NF	
W Greenland (Disko Bay)	MTG	0-30 m	July	330	393	246 ^a	Nielsen & Hansen 1995
		0-28 m	August	270	500		Levinsen & Nielsen 2002
		0-30 m	July-September	286	486		Levinsen et al. 1999
		0-200 m	August	620	910		Levinsen et al. 2000
SW Greenland	MTG	0-200 m	June	243	386	134 ^b	Pedersen et al. 2005
NE Greenland (Young Sound)	LTG	0-35 m	June	98	40	48 ^b	Nielsen et al. 2007
		0-35 m	August	20-143	20-70		Rysgaard et al. 1999
NW Greenland (Inglefield Bredning)	MTG	0-100 m	August	241	294	74 ^c	This study

4 a: autotrophic/mixotrophic, b: heterotrophic, c: autotrophic/mixotrophic/heterotrophic

5



1
 2 Fig. 1. Horizontal distributions of the integrated carbon biomass and species
 3 compositions of the protist community in the water column (0-100 m for all stations
 4 except St. 4 (0-40 m)) in Inglefield Bredning, northwestern Greenland, obtained during
 5 13-17 August 2018. The numbers indicate the station names. The background image is
 6 from Modified Copernicus Sentinel data (13 August 2018), processed with Sentinel
 7 flow (<https://github.com/juseg/sentinelflow>). The inset shows the location of the study
 8 site.

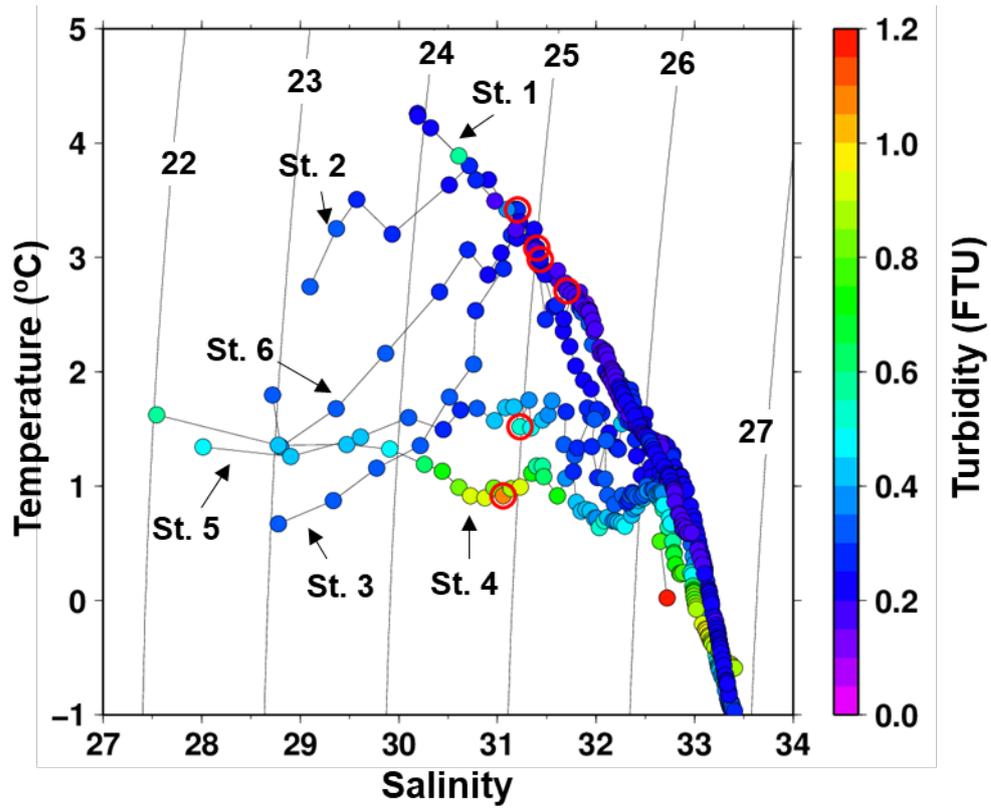


1

2 Fig. 2. Vertical profiles of environmental parameters in Inglefield Bredning,

3 northwestern Greenland, obtained during 13-17 August 2018. The dots in the plots of

4 chlorophyll *a* and nutrients are data obtained from water samples.

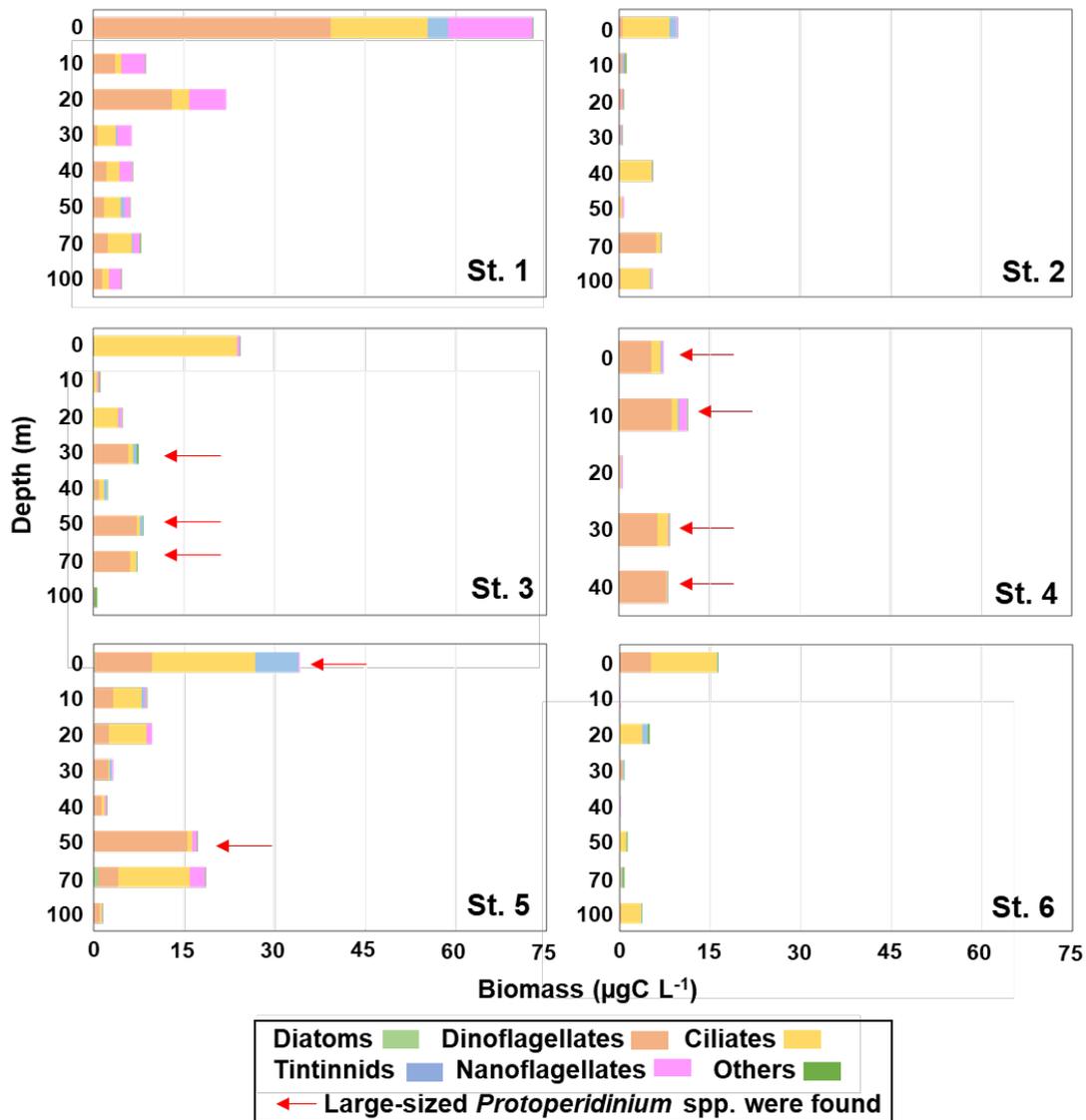


1

2 Fig. 3. T-S diagram with turbidity in Inglefield Bredning, northwestern Greenland,

3 during 13-17 August 2018. Red circles indicate the value at 10 m depth.

4



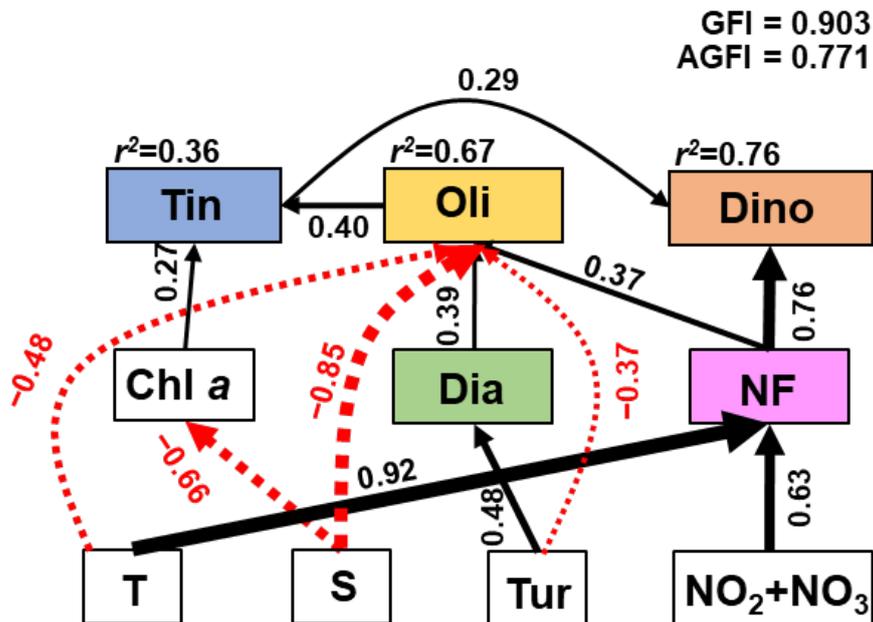
1

2 Fig. 4. Vertical distributions of the protist community in Inglefield Bredning,

3 northwestern Greenland, during 13-17 August 2018. The red arrows indicate the

4 large-sized *Protoperidinium* spp were found.

5



1

2 Fig. 5. Results of the structural equation modeling (SEM) of protist biomasses and
 3 environmental factors. The numbers along the pathways represent the standardized path
 4 coefficients. The arrows with solid and dashed lines indicate positive and negative
 5 effects, respectively. The arrow thicknesses vary with the path coefficient values. The
 6 overall fit of the model was evaluated using the goodness-of-fit index (GFI) and the
 7 adjunct goodness-of-fit index (AGFI). T: temperature, S: salinity, Tur: turbidity,
 8 NO₂+NO₃: nitrite and nitrate, Chl *a*: chlorophyll *a*, Dia: diatoms, NF: nanoflagellates,
 9 Dino: dinoflagellates, Oli: oligotrich ciliates, and Tin: tintinnids.