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Effects of Glacial Flour on Marine Micro-plankton: Evidences from Natural Communities of Greenlandic Fjords and Experimental Studies

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Meltwater runoff from glaciers carries particles, so-called glacial flour that may affect planktonic organisms and the functioning of marine ecosystems. Protil microplankton is at the base of marine food webs and thus plays an important role in sustaining important ecosystem services. To assess the effect of glacial flour on photoautotrophic, heterotrophic and mixotrophic microplankton, the spatial distribution of these trophic groups was studied in four Greenlandic fjords during summer. The results suggest that the abundance of the autotrophic microplankton was affected by the glacier meltwater due to reduced light penetration and nutrient availability. The abundance of heterotrophic and mixotrophic microplankton were not apparently affected by the glacier meltwater. Incubation experiments were conducted on the natural population and in laboratory cultures of two mixoplanktonic ciliate species. The experiments on the natural population revealed that none of the trophic groups were affected by the suspended material at concentrations up to 50 mg L⁻¹. The experiments on cultures gave no indication that glacial flour was ingested by the mixoplanktonic ciliates. Growth rates of cultured ciliates were not affected by the glacial flour addition. These results suggest that heterotrophic and mixotrophic microplankton are not affected by glacial flour as much as autotrophic microplankton.

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Key words: Melting glaciers; clay particles; planktonic protists; trophic group; planktonic community.

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Introduction

The increase in atmosphere and ocean temperatures in the last decades has led to high mass loss of Greenland ice sheet (Mouginot et al., 2019). Retreating glaciers discharge large volumes of freshwater into the ocean that often carry high loads of sediment derived from the ice-rock abrasions, named glacial flour. This results in the alterations of the physical and chemical properties of the water column and consequently affect marine primary production and important ecosystem services such as carbon sequestration and fisheries (Hopwood et al. 2020). Planktonic organisms of 20–200 μm (i.e. microplankton) are at the base of marine food webs and play a central role in marine biogeochemistry both as primary producers and primary consumers (Azam et al. 1983). Other than being autotrophs or heterotrophs many microplanktonic protists can combine both modes of nutrition, being thus mixotrophs (Mitra et al. 2016). Such organisms have recently been referred to as mixoplankton (Flynn et al. 2019). Mixoplankton can be further split into constitutive mixoplankton (CM) with innate phototrophic capacity that ingest other organisms (e.g., many dinoflagellates), and non-constitutive mixoplankton (NCM) which either retains functional chloroplasts from their prey (e.g., kleptoplastidic dinoflagellates and ciliates) or endosymbionts (symbiont-bearing radiolarian, ciliates and dinoflagellates) (Flynn et al. 2019; Mitra et al. 2016). Due to their dual mode of nutrition, mixoplankton might respond differently than pure heterotrophic and autotrophic organisms to environmental stressors.

The growth of photoautotrophic organisms is related to the availability of light and dissolved inorganic nutrients, which are both affected by glacial meltwater input in marine ecosystems. Generally meltwater is a source of silicate and iron (Hopwood et al. 2020; Meire et al. 2016), which potentially favors diatom growth. Yet, the sediment transported by glacial meltwater limits light penetration into the water column, exerting the opposite effect (Meire et al. 2017; Szeligowska et al. 2021). This might have indirect effects on the growth of heterotrophic organisms as well, because they feed on photosynthetic flagellates and diatoms. However, as mixoplankton can rely both on photosynthesis and ingestion, they might be less affected than purely autotrophic organisms, limited by light and nutrients and than heterotrophic organisms, limited by prey abundances. Mixotrophic nano-flagellates actually proved to be less affected than heterotrophic ones by the addition of glacial flour particles in culture (Sommaruga and Kandolf 2014). On the other end, the common conception about mixoplankton is that this group is inferior to their purely autotrophic and heterotrophic counterparts, because of its lack in specialization toward photosynthesis, nutrient uptake or feeding (Flynn et al. 2019). Highly specialized autotrophs as diatoms, are well adapted to quickly adjust to the variable light conditions due to the vertical mixing of the water masses in which they proliferate (Lavaud 2014). Most of the non-constitutive mixoplankton species are considered unable to do that, as they cannot exert any control on the acquired photosynthetic machineries (Stoecker et al. 2009). Purely heterotrophic species are obviously, not directly affected by the photosynthetic active radiation (PAR), but rather to the quality and quantity of the prey organisms. Most of the constitutive mixoplankton species are obligate phototrophs, which cannot grow in the dark because their ingestion rates are relatively low and only can supplement their energetic requirements (Stoecker et al. 2017). High concentrations of suspended sediment can directly affect the growth of non-selective grazers interfering with uptake of food particles (Arendt et al. 2011; Sommaruga 2014). The ingestion of inert sediment particles can thus potentially affect both heterotroph and mixotrophic microplankton. However, there are evidences in nanoplankton that mixotrophic organisms are insensitive to glacial flour in presence of light, thus this nutritional mode could be favored compared to pure heterotrophy in turbid waters (Sommaruga and Kandolf 2014).

In this study, we aimed to better understand the different effect of glacial flour particles on microplankton, based on observations on communities of impacted areas and on incubation experiments. The study on natural communities was conducted in July 2019 in Disko Bay, on the West Greenland coast in four fjords impacted by runoff of glaciers (Fig. 1).

We hypothesized that

a) The sediment plumes originating from landterminating glaciers create gradients in the physical and chemical properties of the water column along the fjords

b) the abundances of autotrophic and constitutive mixotrophic microplankton will respond to the increased available light and inorganic nutrients along the transects, and species of small size will be relatively more abundant at low inorganic nutrient concentrations.
c) large heterotrophic dinoflagellates (>20 μm) will mainly respond to the increased biomass of diatoms, while non-constitutive mixotrophic filter feeding protists will respond to increases in primary producers < 15 μm.

The addition of glacial flour was tested in incubation experiments on the natural community of an offshore (not impacted) area and in laboratory cultures of mixotrophic ciliates. The aims of these incubations were to:

d) test if the growth of microplankton is affected by the addition of glacial flour

e) assess how non-selective (filter feeding) mixotrophic species are affected by glacial flour particles.

Results

Physical and Chemical Conditions Along Each Fjord

The four fjords were characterized by different physical and chemical conditions in July 2019. In two of the four fjords (transect 2 and 3) the freshwater input induced stratification of the water column. It created a low salinity surface layer along almost all transect 2 (stations from 19 to 44) and at the innermost stations of transect 3 (54, 56 and 58) (Supplementary Material Fig. S5). At these sampling stations salinity ranged from 6 to 18, (Supplementary Material Figs S1, S2, S5; Table 1). Effects of the fresh water inputs were more limited in transects 1 and 4. This was probably due to the hydrology of these two fjords. A thermal stratification was observed in transect 1 and in the innermost stations of transect 3 (54, 56 and 58). At these sites, the surface layer was relatively warmer than the rest of the water column (at least 5 °C warmer in the top 5–10 m). In transect 4, salinity and temperature at surface were at maximum two units lower and 3 °C warmer compared to salinity and temperature measured at 5 m depth (Supplementary Material Figs S1, S2, S5).

Temperature, salinity, turbidity and concentration of inorganic nutrients (DIN, DIP and SiO₂) explained the 72.4% of the cumulative variance (Fig. 2) in the PCA performed on the physical and chemical data from the surface samples of all transects. Surface data of transect 1 clustered in the PCA, thus indicating that no gradients were present in this fjord. Data from the surface samples of transect 2 and 3 arranged along a positive salinity gradient and a negative turbidity gradient from the innermost stations to the outermost stations (Fig. 2; from station 19 to 47 and from 54 to 66). Data from the surface samples of transect 4 display negative trend in dissolved inorganic nitrogen (DIN) and phosphate (DIN), indicating that the outermost stations were relatively more depleted in DIN and DIP compared to the innermost stations. SiO₂ and turbidity resulted to have the same orientation relative to the PC axis and opposed to that of salinity (Fig. 2).
Table 1. DCM depth (m) and surface and DCM temperature (Temp), salinity, turbidity (in FTU but the instrument was not calibrated: arbitrary unit) and concentrations of nitrate, phosphate and silicate (lM) at each station. The stations are numbered from the inner to the outer part of the fjord.

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<th>DIN lM</th>
<th>DIP lM</th>
<th>SiO₂ lM</th>
<th>DCM depth</th>
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Heterotrophs were represented by ciliates and dinoflagellates in all samples. Although radiolarians were occasionally seen, they were rare and their biomass was negligible. Heterotrophic dinoflagellates were consistently more abundant than heterotrophic ciliates, which represented less than one third of the total heterotrophic biomass in most of the samples (Table 2). The most abundant and widespread heterotrophic dinoflagellates belonged to the genera *Gyrodinium* and *Protoperidinium*. Aloricate ciliates were generally more abundant than loricate ciliates (tintinnids), and dominated by the genera *Strombidium*, *Strobilidium* and *Monodinium*. Despite numerically abundant in some samples, the biomass of small heterotrophic ciliates (<20 μm) did not account for a significant proportion of the total heterotrophic biomass in any of the samples (Table 2).

Mixotrophs were generally less abundant than heterotrophs (about ¼ of the heterotrophic biomass on average) and mostly represented by mixotrophic ciliates. Mixotrophic dinoflagellates only exceeded mixotrophic ciliate biomass in: a) surface samples of transect 3, where dinoflagellates belonging to the genera *Alexandrium* and *Heterocapsa* were abundant; b) two stations in transect 1 (10 DCM and 12 surface), where *Dinophysis* and *Heterocapsa* contributed to the relatively high biomass of mixotrophic dinoflagellates. Mixotrophic ciliates belonging to the genera *Laboea*, *Strombidium* and *Mesodinium* almost equally contributed to the biomass of mixotrophic ciliates in all samples. However, *Mesodinium rubrum/major* accounted for most the mixotrophic ciliate biomass on a few occasions (Table 2).

Combining the microplankton community of all transects, autotrophs were negatively related to temperature \(r = -0.396; p = 0.006\), meaning that they were relatively more abundant in cold waters. Mixotrophs were positively related to temperature \(r = 0.600; p < 0.0001\) and negatively related to DIN \(r = -0.7128; p < 0.0001\) and DIP \(r = -0.506; p < 0.0001\), meaning that they were relatively more abundant warm waters where dissolved inorganic nutrients were depleted. Similarly, heterotrophs were positively related to temperature \(r = 0.379; p = 0.009\) and negatively related to DIN \(r = -0.603; p < 0.0001\) and DIP \(r = -0.497; p = 0.0004\). Abundance of mixotrophs (μg L⁻¹) resulted positively correlated with chl a in the size fraction < 15 μm \(r = 0.493; p = 0.0007\), while abundance of heterotrophs did not show any correlation with chl a values.

In transect 1, where no gradients were observed, only autotrophs where significantly correlated with environmental parameters. Autotrophs biomass was negatively related to depth, temperature and turbidity \(p = 0.02; p = 0.03; p = 0.02\) and \(r = -0.759; r = -0.756; r = -0.805\) and positively correlated with DIN and DIP \(p = 0.02\) and \(p = 0.007\) and \(r = 803\) and \(r = 0.900\) respectively), thus were relatively more abundant at the DCM (see Fig. 3 and Table 1). In this transect almost
Table 2. Biomass (µgC L⁻¹) of the most representative protists groups (dinoflagellates, ciliates and diatoms) at selected stations at (a) surface and (b) DCM. Protists were grouped according to their trophic mode. (Gyrod. = *Gyrodinium*; Protop. = *Protoperidinium*; Small spp= ~20 µm; Heteroc. = *Heterocapsa*; Dinoph = *Dinophysis*; Mesod. = *Mesodinium*).

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<th>Station</th>
<th>Heterotrophs (µgC L⁻¹)</th>
<th>Ciliates</th>
<th>Mixotrophs (µgC L⁻¹)</th>
<th>Autotrophs (µgC L⁻¹)</th>
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<td></td>
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<td>Protop. spp</td>
<td>Total</td>
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<td>2.3</td>
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<tr>
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<td>-</td>
<td>-</td>
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<td>0.9</td>
<td>1.3</td>
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<td>17.7</td>
<td>15.9</td>
<td>0.2</td>
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80% of the chl \( a \) was found in the \(< 15 \, \mu m\) size fraction (Fig. 4).

In transect 2, where salinity and turbidity gradients were observed, heterotrophs, mixotrophs and autotrophs were negatively correlated with turbidity \((p = 0.0005; p = 0.005; p = 0.002\) and \(r = -0.818; r = -0.704; r = -0.776\) respectively) and heterotrophs and autotrophs were positively correlated with salinity \((p = 0.02; p = 0.03\) and \(r = 0.629\) and \(r = 0.600\)). Indeed, microplankton biomass in the outermost stations (e.g., stations 42–47) was at least double compared to microplankton biomass in the innermost stations (e.g., stations 19–33) at both surface and DCM (Fig. 3). In this transect, chl \( a \) in the \(< 15 \, \mu m\) size fraction represented \(\sim 70\%\) of the total chl \( a \) in the innermost stations and \(\sim 30\%\) in the outermost stations (Fig. 4).

On the contrary, in transect 3, where salinity and turbidity gradients were also observed, none of the microplankton trophic groups were significantly related to turbidity. However, autotrophs were still positively correlated with salinity \((p = 0.04\) and \(r = 0.616\)) and negatively with depth \((p = 0.02\) and \(r = -0.593\)), indeed were relatively more abundant at the DCM in the innermost stations (Fig. 3). The chl \( a \) in the \(< 15 \, \mu m\) size fraction did not exceed the 50\% of the total chlorophyll in any of the stations (Fig. 4).

In transect 4, gradients were observed in temperature and in dissolved inorganic nutrients. The only significant correlations were the negative correlations of autotrophs with DIN \((p < 0.0001\) and \(r = -0.952\)), DIP \((p = 0.001; r = -0.900\)) and SiO\(_2\) \((p = 0.003; r = -0.855\)). The chl \( a \) in the \(< 15 \, \mu m\) size fraction was almost null along the entire transect. This was due to the fact that biomass was mainly constituted by chain forming diatoms in the genus *Chaetoceros*. Transect 4 differed from the other transects in that the total biomass was 6 to 10 time higher (about 600 \(\mu g\) Cl\(^{-1}\)) and mainly made by diatoms along the entire transect (Fig. 3).

**Development of the Protist Community During the On-board Incubation Experiment**

The biomass of organisms \(> 15 \, \mu m\) was significantly higher at T0 in treatment 1 (addition of 30\% V/V of glacial flour containing water) compared to the control \((p = 0.003)\) and treatment 2 \((p = 0.002)\) (addition of 50 mg/L of glacial flour). The higher total biomass in treatment 1, where glacial flour containing water was added, was mainly ascribable to the heterotrophic dinoflagellates (Fig. 5). The chl \( a < 15 \, \mu m\) biomass at T1 was also significantly higher in treatment 1 compared to the control \((p = 0.0003)\) and treatment 2 \((p = 0.0003)\) (Supplementary Material Fig. S6).

Only dinoflagellates (both heterotrophs and mixotrophs) grew in all treatments (Figs 5, 6). Diatom biomass slightly increased in all treatments until day 3, then decreased again from day 3 to day 5 (Fig. 5), thus diatom growth rates calculated over the 5-days of incubation (Fig. 6) were not really different from zero in the control and treatment 1, despite that some growth during the experiment. Mixotrophic ciliates had negative growth rates in all treatments, including the control treatment (Fig. 6). Mixotrophic ciliate biomass displayed a rapid drop especially in the first 3 days in treatment 1 (Fig. 5). Finally, the biomass of heterotrophic ciliates did not significantly vary during the experiment in any of the treatments (Fig. 5) so growth rate was not different from zero in any of the experimental treatments (Fig. 6).

**Development of Cultures During the Laboratory Incubation Experiment**

The growth rate of both ciliate species was not statistically different between the experimental treatments that contained glacial flour compared to the control treatment that did not contain glacial flour (Fig. 7). Sediment particles of 2–15 \(\mu m\) in size decreased in number in the same proportion in the experimental treatment and in the control, where glacial flour was added to the algal prey only (C2) (Supplementary Material Fig. S7).

Flow cytometry scatter plots and microscopy showed clear flocculation of glacial flour particles to form larger aggregates, which were too large for ciliates to ingest (data not shown). The algal prey density showed a similar trend in the experimental treatment and the control mixed culture (C1). Removal due to ciliate ingestion was evident by comparing algal density to the algal monoculture control (C2). The chlorophyll \( a \) content of ciliates in the experimental treatment was not different from chlorophyll \( a \) content of ciliates incubated without glacial flour (C1) (Supplementary Material Fig.S8).
Figure 3. Biomass (µg C L\(^{-1}\)) of heterotrophs (in black), mixotrophs (in grey) and autotrophs (striped) and depth of the DCM (dots) at all stations along each transect.
Distribution of Protist Groups Along the Four Transects

Gradients associated with the glacial meltwater inputs were distinguishable in the physical and chemical properties of the water column in three of the four fjords. However, the nature of such gradients was different in the different fjords, due to the different geology and hydrographic conditions.

The sediment plume was only evident in two out of the four fjords, at stations that were relatively close to the glacier input. A large fraction of glacial sediments settled within a few kilometers from the input, in accordance with previous observations in the area (Meire et al. 2017; Murray et al. 2015). Turbidity caused by suspended particles seemed to have only affected the microplankton community in one out of the four fjords (transect 2), where the biomass of autotrophs had an inverse trend compared to the turbidity gradient towards the open waters. In two out of the four fjords (transect 2 and 3) the freshening of the upper part of the water column led to the stratification of water column. This might affect microplankton by reducing the vertical mixing and creating a nutrient-poor surface layer that limits the growth of autotrophic organisms (Holding et al. 2019; Hopwood et al. 2020). This effect was less evident in transect 4 due to the presence of marine-terminating glaciers that could have led to the upwelling of nutrient rich bottom water (Meire et al. 2017). Indeed, diatoms dominated the protist communities in transect 4. The negative correlation found between autotrophs and dissolved inorganic nutrients in this transect was also ascribable to the biological removal.

The heterotrophic and mixotrophic protists were associated with relatively warmer and nutrient-depleted waters. Their relative abundance, in terms of biomass, exceeded that of the autotrophs at the innermost stations of transect 1 and 3. On such stations chla was mainly found in the < 15 μm fraction, suggesting that smaller phototrophic organisms, which have a higher surface to volume ratio compared to phototrophic microplankton, were favored.
in nutrient limiting conditions (Stolte and Riegman 1995). Moreover, many photosynthetic nanoplankton species (<20 μm in size) other than diatoms, are known to be mixotrophs and to sustain their metabolism feeding on bacteria (Stoecker et al. 2017), whose growth may be boosted by the presence of suspended particles (Szeligowska et al. 2021). A similar predominance of chl in the small size fraction has previously been observed in the inner location of other West Greenlandic fjords (Arendt et al. 2010, 2016). The predominant grazing activity on primary producers in such locations is likely attributed to the microplankton rather than copepods, which instead have a major grazing impact in the coastal zone (Arendt et al. 2010 and 2016). Similarly, the mixotrophic microplankton seemed to be associated with the smaller size fraction of primary producers (i.e., the chl fraction < 15 μm).

Heterotrophic dinoflagellates were also found on more offshore stations where most of the chl was due to chain forming diatoms (functionally

**Figure 5.** Development of the biomass (μg C L⁻¹) of each functional group during the incubation experiment.
Figure 6. Growth rates of each functional group in the on-board experiment, calculated over the five-day incubation.

Figure 7. Development of the mixed cultures of non-constitutive mixotrophic ciliates (*Strombidium* cf. *basimorphum* and *Strombidium* cf. *conicum*) in a treatment where ciliates were incubated with both their prey (*Teleaulax amphioxeia*) and 50 mg L$^{-1}$ of glacier flour (treatment), and in a treatment where the ciliates were incubated with prey only (control).
> 15 μm). Dinoflagellates of the genera Protoperidinium and Gyrodinium have already been recorded to be dominant in summer in Greenland and associated to diatom blooms (Krawczyk et al. 2015; Levinsen et al., 2000). Heterotrophic ciliate biomass, instead, was very low or even absent in those samples. This is likely explained by differences in the feeding mechanisms in these two groups. The ciliate species found in this survey were mostly filter feeders. Thus, their grazing potential was limited to particles which size did not exceed that of their feeding apparatus (Jonsson 1986). The feeding mechanisms of dinoflagellates are more diverse. Many thecate species, like Protoperidinium and the Diplosalis group use a pallium to catch and digest their prey. Most of the athecate species like Gyrodinium spp use direct engulfment, while many thecate and thecate species, like Phalacroma and many small heterotrophic species use peduncles (feeding tubes). These are all feeding mechanisms that allow the organisms to ingest prey items exceeding their own size (Hansen and Calado 1999; Jacobson and Anderson 1986).

Mixotrophic microplankton were relatively more abundant in areas where density stratification was determined by both salinity and temperature. This is especially evident on transect 3, where stratification was induced by both salinity and temperature. Peaks in the relative abundance of mixotrophic microplankton were formed by constitutive mixotrophic species (Heterocapsa spp. and Alexandrium spp.), while non-constitutive mixoplankton never dominated the microplankton communities. The reasons for that may be found in biotic factors such as the top-down control from metazoan grazers and specific interaction among microorganisms. Mixotrophic ciliates, in particular, are a preferred prey of copepods (Stoecker and Lavrentyev 2018), while at least some constitutive mixotrophic dinoflagellates produce toxins (Burkholder et al. 2008) as a defense from predation (Kubanek et al. 2007; Xu and Kierboe 2018).

The only identifiable non-constitutive mixotrophic dinoflagellate species were Dinophysis spp., which are prey specialist grazers that can only acquire phototrophy by feeding on the non-constitutive mixotrophic ciliate, Mesodinium rubrum species complex (Hansen et al. 2013). Not surprisingly, Dinophysis spp were only found in samples where the mixotrophic Mesodinium spp were also present. Non-constitutive mixotrophic ciliates in the Mesodinium rubrum species complex are also prey specialist grazers that can only acquire chloroplasts via feeding on cryptophytes within the Teleaulax/Plagiogioselminus/Geminigera clade (Hansen et al. 2013). Differently from many other mixotrophic ciliates, Mesodinium rubrum can take up and utilize inorganic nutrients for growth and go through up to 4 cell divisions without prey (Kim et al. 2017; Tong et al. 2015). Mesodinium spp only dominated the mixotrophic ciliate biomass in few of our samples. Indeed, Mesodinium biomass is usually low under non-bloom conditions, which tend to occur in localized patches (Crawford 1989), as is evident from the distribution of this ciliate in these fjords. Except for a few locations, prey generalist mixotrophic ciliates were equally or more abundant than Mesodinium, as typical in polar waters (Leles et al. 2017; Levinsen and Nielsen 2002; Stoecker et al. 2009). The total biomass of mixotrophic ciliates was in the low range of what it could be in summer in more open waters of the same area, but their relative abundance compared to the total ciliate biomass (30-70%) was comparable to previous records (Levinson et al. 2000; Levinson and Nielsen 2002; Putt 1990).

The Glacial Flour Addition in the Incubation Experiments

The glacial flour addition in the incubation experiments did not seem to have any effects on any of the microplankton functional groups. This suggests that sediment particles themselves, in the concentrations used here, do not directly interfere with these organisms. Mixotrophic ciliates were the only group that seemed affected by the incubation during the experiment on the natural community. At first, this could indicate an effect of the ingestion of sediment particles, which could have displaced the sequestered chloroplasts, thereby causing a negative effect on their growth. Tests in laboratory cultures done after the cruise, however, did not demonstrate such an effect. In fact, the two non-constitutive mixoplanktonic ciliate species tested in the laboratory culture experiments did not seem to ingest sediment particles, and they were able to maintain the same chl a content (pg cell^-1), when grown with sediment as in controls without sediment. In addition, the negative growth of mixotrophic ciliates in the control treatment during the on-board incubation with the natural population suggests that the negative effect observed may be due to reasons other than the ingestion of suspended particles.
Such reasons include competition for prey with other functional groups and direct predation by other microorganisms (e.g., dinoflagellates in the genera *Gyrodinium*, *Gymnodinium* and *Dinophysis*) or an effect due to the mixing of water in the bottles during the incubation. Ciliates, especially, *Mesodinium* and oligotrotrich ciliates have previously been shown to be affected by incubation in bottles (Hansen et al. 2019).

**Conclusion**

The way in which the meltwater runoff affected the physical and chemical properties of the water column depended on the geological and hydrological characteristics of the specific fjord. Autotrophic microplankton abundance seems to be more influenced by the glacial inputs compared with the heterotrophic and mixotrophic microplankton, that are not directly affected by turbidity and inorganic nutrients availability. The glacial flour addition had no effect on any trophic group during the incubation at the concentrations used in our experiments. Microplanktonic filters feeders (i.e., ciliates) do not seem to ingest glacial flour particles as their chl a content and growth rates were unaffected by the presence of particles in suspension. However, even if phagotrophic microplankton (either heterotroph or mixotroph) were unaffected by glacial flour, their abundances never reached values comparable to those reached by autotrophs in non-impacted areas. This suggests that in coastal areas the water runoff from land terminating glaciers may lead to a shift from fast growing photoautotrophic microplankton communities to less productive communities dominated by heterotrophic and mixotrophic microplankton species. The reasons for that are ascribable to the increased turbidity associated with the freshening of surface waters, that cause a reduced supply of nutrients from deeper water masses resulting in nutrient depleted surface layer.

**Methods**

**Sampling:** Samples were collected along transects from the inner part of the fjord to the mouth (Fig. 1B). At each sampling station, profiles of temperature, salinity, fluorescence and turbidity were collected using a SBE19plus CTD. Water was collected from sub-surface (1 m depth) and at the deep chlorophyll maximum (DCM; variable depth) using 5 and 10 L Niskin bottles and siphoned off with a silicon tube to reduce organism loss due to mechanical disturbance. For organisms’ identification and count, two samples of 200 mL were collected from each depth in 250 mL amber glass bottles; One sample was fixed using a Lugol’s solution (1% final.
concentration), while the other sample was fixed with a glutaraldehyde solution (2% final concentration). For chlorophyll analysis, 1 L of water was collected and split in two equal subsamples of 500 mL from which chlorophyll concentration of two different size classes were obtained as described below (chlorophyll analysis). Water samples were also collected for dissolved macronutrients (nitrate, phosphate, and silicate).

**On-board incubation experiment of the natural community:** Simultaneously to the sampling in Transect 1, an incubation experiment was conducted to determine the direct effects of particles addition on the planktonic community resident in the nearest offshore area not impacted by the sediments plume. At Station 1, just outside the fjord (Fig. 1B), 30 L of surface water were collected; other 20 L of surface water were collected at Station 2, the innermost fjord station (Fig. 1B) where a visibly high amount of glacial flour was present in suspension. Two different treatments were set-up (Fig. 8). In treatment 1, the glacial flour containing water was added to the water collected outside the fjord in a volume corresponding to 30% of the total final volume (Fig. 8C). In treatment 2, the glacial flour containing water was filtered through Whatman GF/F glass microfiber filters to remove most of the suspended particles, and used to dilute the water collected outside the fjord in the same proportion as in treatment 1. Then 50 mg L⁻¹ of glacial flour were added to the suspension (Fig. 8D). A control treatment was set-up in the same way as treatment 2, but without the addition of any glacial flour (Fig. 8B). This served to account for the effect on the organisms of the mixing of the two water masses with different salinities and potentially different nutrients concentrations. The glacial flour added in treatment 2 was previously collected from sediment samples from different locations of the same area. Sediment samples were mixed into a common representative sample. This sample was dried, sieved through a 200 μm net mesh, and weighted by Micro-Analytical Balance.

Each experimental treatment was distributed into fifteen borosilicate glass bottles of 500 mL (VWR 215–1594), which were mounted on a plankton wheel and incubated at 5 °C. Light was provided with cool white LED at an irradiance of 110 μmol photons m⁻² s⁻¹. Triplicate bottles from each treatment were withdrawn from the remaining volume (T0) for enumeration of organisms and chlorophyll measurement. The incubation lasted five days and triplicate samples (individual bottles) were withdrawn every 24 h. From each replicate, a 150 mL subsample was fixed in Lugol's solution (1% final concentration). For enumeration of ciliates in mixed cultures, to verify that ciliates were ingesting it. All treatments were set in 1.2 L volume and then split in 15 replicates, each of 50 mL, in tissue culture flasks that were mounted on a plankton wheel and incubated in the same condition used for cultures maintenance (15 °C, ~70 μmol photons m⁻² s⁻¹ in a 14:10 light: dark cycle). In all treatments, the initial algal density was 2.0x10⁴ cells mL⁻¹. In the experimental treatment and the C1 treatment the initial ciliate density was of 10 cells mL⁻¹. The incubation lasted five days, and subsamples (3 replicates from each treatment) were withdrawn daily and used for cells and particles enumeration. Ciliate chlorophyll a content was measured after three and 4 days from the beginning of the incubation as described below (see Chlorophyll a analysis).

**Organisms and particles enumeration:** Planktonic protists with a cell diameter of > 15 μm were enumerated in the transects samples and on the-board incubation experiment on 50 mL of the Lugol's samples, using sedimentation chambers (Hydrobios) in accordance with Holmer (1985). Cells were counted on an inverted light microscope Olympus BX 40 equipped with the camera Olympus DP73 at 200x magnification. For enumeration of ciliates in the incubation experiment in laboratory cultures, 5 to 20 mL samples were fixed in Lugol's solution (final concentration 1%), and counted using sedimentation chambers (as above) on an inverted light microscope (Olympus CKX53) at 50 × magnification. A minimum of 200 individuals was counted for each replicate. Algal prey and sediment particles in this experiment were counted using a CytoFLEX flow cytometer (Beckman Coulter, USA) calibrated and set to discriminate and count particles based on fluorescence (photosynthetic pigments) and forward and side angle light scatter, proxy for particle size and complexity (Olson et al. 1991). Glacier flour particles were not enumerated on fixed samples from transects and the on-board incubation experiment because fixatives interfered with the method.

**Protist community analysis:** Protists were identified based on Hoppenrath et al. (2009) and Hasle et al. (1996). The linear dimension (cellular length and width) of the planktonic protists in the transect samples and the on-board incubation experiment were measured using CellSense software. Cellular biovolumes were calculated using geometric formulas for spheres, cylinders, prolate spheroids or cones according to Hillebrand et al. (1999), and measured using CellSense software. Cellular biovolumes were measured using sedimentation chambers (Hydrobios) in accordance with Holmer et al. (1999) and converted into cellular carbon content according to Menden-Deuer and Lessard (2000); this allowed calculations of the biomass (μg C L⁻¹) of the individual protistan functional groups. Protists were assigned to functional groups (heterotrophs, mixotrophs and phototrophs) according to unequivocal literature records (Schneider et al. 2020) or further analysis of the glutaraldehyde-preserved samples. Glutaraldehyde-preserved organisms were collected on polycarbonate filters (pore size 2 μm) by filtering 50 to 100 mL of the glutaraldehyde fixed sample. Filters were stained with Calcofluor (Andersen and Kristensen 1995) and DAPI (Porter and Feig 1980), and inspected with an epifluorescence microscope (Olympus BX 50) equipped with UV, Green and Blue excitation filters prior and after the count of the Lugol sample. This filter set allowed the detection of DAPI, Calcofluor, chlorophyll and phycocyanin pigments allowing a deeper characterization of the organism morphotypes observed in the Lugol samples. All samples were enumerated by the same person to eliminate observer bias. Triplicate samples from the incubation experiment were averaged for each time point.
Chlorophyll a analysis: The total chlorophyll a (total chla) content of the waters samples as well as the chla content in the size fraction < 15 μm (fractionated chla) were analyzed. For total chla analysis, biomass was directly collected via filtration on Whatman glass microfiber filters GF/F, while for the fractioned chla, samples were first sieved through a 15 μm net mesh. Filters were stored at −80 °C until further processing. Chla samples were extracted in 5 mL 96% ethanol for 24 h in the dark at 4 °C and quantified using a Turner Trilogy Fluorometer using the chla non-acidification insert.

Ciliate chlorophyll a content in the laboratory experiment on cultures was measured on three replicates, each consisting of twenty cells individually picked with a drawn micropipette from each experimental bottle. Ciliates were rinsed twice in clean media and added to 2 mL of 96% ethanol. Samples were kept for 24 h in the dark at 4 °C and chla was quantified as above.

Dissolved inorganic nutrients analysis: Subsamples (10 mL) for nutrients were filtered through 0.45 μm filters (Q-Max GF syringe filters) and directly frozen at −20 °C until analysis. Nutrients were measured using standard colorimetric methods on a Seal QuAAtro autoanalyzer.

Statistical analysis: The environmental variables (temperature, salinity, turbidity, DIN, DIP, SiO2) of the surface samples of the four transects were analyzed in a principal component analysis (PCA) to visually represent the physical and chemical gradients produced by the melt water along each fjord. This analysis and plot were done using the software PRIMER-E v. 6. The correlation between environmental variables and the abundance of the different microplankton trophic groups was explored by a Spearman correlation matrix. The confidence interval was set to the 95%, correlation with p < 0.05 were considered significant. Statistical tests were conducted to assess the significance of differences observed in the biomass of each functional group between treatments and between sampling points in the incubation experiment. Differences between treatments were assessed using ordinary-one way ANOVA followed by Tukey’s multiple comparisons test at significance level 0.05. For this analysis, data were tested for normality (Shapiro–Wilk test) using the software Past v.4. The same tests were conducted to compare ciliate chlorophyll a content in the incubation experiment on cultures. These analyses were performed using the software GraphPad prism 6.

Conflict of Interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript. The submission is original work and is not under review at any other publication.

CRediT authorship contribution statement

Maira Maselli: Conceptualization, Investigation, Formal analysis, Visualization, Writing – original draft. Lorenz Meire: Investigation, Writing – review & editing. Patrick Meire: Investigation, Writing – review & editing. Per Juel Hansen: Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision.

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This manuscript was submitted to the journal PROTIST to honor Professor Bland Finlay. Bland’s research interests were centered around the global distribution of protists in fresh and marine waters and on functional biology of protists. Very little is currently known about protists in the transition zones of fresh and marine waters in the Arctic, and how glacier flour affects the protist communities in such fjords. Thus, the paper is in many ways inspired by the work of Bland.

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Appendix A. Supplementary Material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.proot.2022.125928.

References


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