Vol. 83: 65–82, 2019
https://doi.org/10.3354/ame01904

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online June 6

# Vertical export of marine pelagic protists in an ice-free high-Arctic fjord (Adventfjorden, West Spitsbergen) throughout 2011–2012

**Miriam Marquardt[1,2,*], Ragnheid Skogseth[1], Ingrid Wiedmann[2], Anna Vader[1], Marit Reigstad[2], Finlo Cottier[1,3,**], Tove M. Gabrielsen[1,4]**

[1]The University Centre in Svalbard (UNIS), 9171 Longyearbyen, Norway
[2]UiT – The Arctic University of Norway, Breivika, 9037 Tromsø, Norway
[3]The Scottish Association for Marine Science, Dunstaffnage Marine Laboratories, Oban, Argyll PA37 1QA, UK
[4]Department of Natural Sciences, University of Agder (UiA), 4630 Kristiansand S, Norway

ABSTRACT: The ecosystem role of Arctic microbial communities is still largely unknown. Based on a time-series study at the IsA station (West Spitsbergen), the seasonality and contribution of pelagic protists to the vertical flux was investigated at 7 time points during 2011–2012. The hydrography of this high-Arctic fjord was evaluated to identify impacts on the community composition during the different seasons. Protists (<10 µm and >10 µm) were sampled at 4 depths from the water column and from short-time sediment traps, and investigated by 454 next-generation sequencing of the V4 region of the 18S ribosomal DNA. An advective event during winter, exchanging the cold and less saline water mass with warmer and saline Atlantic Water, was potentially responsible for an abrupt shift in the protist composition in March. Small cells (<10 µm) contributed significantly to the vertical flux during autumn and winter, while larger bloom taxa (e.g. diatoms) predominated the water and traps during spring. Parasitic species, such as MALV 1a and *Chytriodinium* sp., were also detected in the traps, possibly being transported along with their hosts. Vertical export of Arctic pelagic protists is not limited to the productive period; however, the contribution of small taxa that are important contributors in this study seems to be seasonally influenced and may alter the flux efficiency. Molecular tools revealed new taxa contributing to the vertical export, but also identified new potential mechanisms exemplified by parasite-host-induced transport, spurring increased attention onto parasitism in the study of carbon cycles and vertical flux.

KEY WORDS: Next-generation sequencing · Vertical flux of protists · Time-series · Size-fractionated protists · Short-time sediment traps · Atlantic-influenced Arctic fjord



## 1. INTRODUCTION

The functional importance of Arctic marine pelagic protists has been increasingly recognized as their biodiversity as well as geographical and seasonal distributions are unraveled (Lovejoy 2014). A few studies have explored the seasonality of Arctic marine protists (Terrado et al. 2009, Seuthe et al. 2011, Marquardt et al. 2016, Kubiszyn et al. 2017), while their fate is still largely unresolved due to methodological constraints. During their brief life spans, these single-celled organisms may be grazed, vertically exported to the sea floor (Turner 2015 and references within), advected by physical processes (Siegel et al. 2016), or recycled back to the dissolved organic pool through lysis by viral attacks (Brussaard et al.

*Corresponding author: miriam.marquardt@uit.no

2013). These processes are potentially influenced by size, density and life history strategies of the protists (Michaels & Silver 1988, Boyd & Newton 1999, Sarthou et al. 2005, Brussaard et al. 2013), the composition and abundance of grazers (Turner et al. 2001, Vargas et al. 2006, Pasternak et al. 2008), and the hydrography of the system (Greene & Pershing 2007, Hamilton et al. 2008, Kubiszyn et al. 2014).

At high latitudes, the annually most prominent vertical export of protists and organic matter tends to follow the spring peak of autotrophic biomass (Olli et al. 2002, Reigstad et al. 2008, 2011, Rynearson et al. 2013, Lalande et al. 2014). These mass sedimentation events represent a high-quality food input to the sea floor, fuelling the benthic ecosystem (Renaud et al. 2008, Wassmann & Reigstad 2011). Large diatom cells often dominate the spring blooms in northern cold waters (68–80° N) with cell numbers up to 6–10 $\times$ $10^6$ cells $l^{-1}$ (Degerlund & Ellertsen 2010), and this is regarded as one of the main drivers of the vertical carbon flux (Billett et al.1983, Beaulieu 2002, Amacher et al. 2009, Rynearson et al. 2013).

Several mechanisms contribute to the intensified vertical export: resting spores of diatoms are heavily silicified (Sicko-Goad et al. 1989, Kuwata et al. 1993), they lack flagella, and, because they excrete transparent exopolymer particles (TEP), the formation of aggregates with a high sinking velocity is promoted (Alldredge & Gottschalk 1989, Kiørboe & Hansen 1993, Passow & Alldredge 1995). Ballasting by inorganic substances may further enhance the sinking of these aggregates (Iversen & Robert 2015). In addition to diatoms, the haptophyte *Phaeocystis pouchetii* is also known for its mass blooms in the northern hemisphere (Wassmann et al. 1999, Schoemann et al. 2005, Hodal et al. 2012, Assmy et al. 2017). *P. pouchetii* has been assumed to play a minor role in the downward flux of carbon because of its small cell size and the buoyancy of the colonies (Beaulieu 2002, Reigstad & Wassmann 2007, Wolf et al. 2016), but recent findings suggest that inorganic ballast (gypsum) released from the sea ice may enhance the sinking velocity of *P. pouchetii* colonies (Wollenburg et al. 2018). Small-sized protists (<10 µm) such as *P. pouchetii* may also become repackaged into fast-sinking fecal pellets after grazing, and thus contribute to the vertical export (Olli & Heiskanen 1999, Waite et al. 2000, Richardson & Jackson 2007, Durkin et al. 2015). However, it is still assumed that large cells (>20 µm), which have faster sinking velocities, contribute relatively more to the vertical carbon export (Michaels & Silver 1988, Boyd & Newton 1999, Sarthou et al. 2005).

Water mass composition can be an important driver structuring the protist communities and the vertical flux. In Kongsfjorden, a high-Arctic fjord of West Spitsbergen, several events have been reported where Atlantic water intrusions strongly influenced the system in combination with freshwater input from the adjacent glaciers (Svendsen et al. 2002, Cottier et al. 2005) that severely affected the protist community composition in different seasons (Hegseth & Tverberg 2013, Kubiszyn et al. 2014, Piquet et al. 2014). Protists have limited mobility and are therefore largely influenced by circulation patterns and hydrographic conditions (Greene & Pershing 2007, Hamilton et al. 2008). Further, due to their short life cycles and high reproduction rates, protists are very likely highly affected by climate-driven changes (Foissner & Hawksworth 2009).

The high-Arctic Isfjorden-Adventfjorden time series station (IsA), established in autumn 2011, is located within the Isfjorden system on the west coast of Spitsbergen (Fig. 1), and is an excellent model system for studying the fate of pelagic protists as the station is sampled and monitored regularly throughout the year. The IsA station is usually dominated by cold and fresh Arctic Water (ArW), formed locally or advected with the coastal current originating from the East Spitsbergen Current (ESC), but is periodically influenced by warm and saline Atlantic Water (AW) originating from the West Spitsbergen Current (WSC; Nilsen et al. 2008). Several fjords at the west coast of Svalbard experience increased intrusion of AW (Pavlov et al. 2013, Nilsen et al. 2016, Tverberg et al. 2019) with concomitant large fluctuations and reduction in their seasonal sea-ice cover (Onarheim et al. 2014, Muckenhuber et al. 2016, Nilsen et al. 2016). Adventfjorden has, in recent years, been ice-free year round (www.met.no, ice-free: 2006–2007, 2010, 2012–2014). This scenario is expected to become more common in the future due to climatic warming (Muckenhuber et al. 2016, Nilsen et al. 2016). In summer and autumn, Adventfjorden is affected by strong glacial runoff with large volumes of sediment-loaded water from the 2 adjoining rivers: the Advent and Longyear Rivers (Węsławski et al. 1999; Fig. 1).

The present study was part of an investigation of the IsA time-series station that included zooplankton assessments (Stübner et al. 2016, Brandner et al. 2017), studies of protist seasonality based on microscopy (micro-sized protists: Kubiszyn et al. 2016), high-throughput sequencing (pico- and nano-sized protists: Marquardt et al. 2016), and quantification of the vertical flux (Wiedmann et al. 2016). As it is difficult to assess the composition of small-sized pelagic

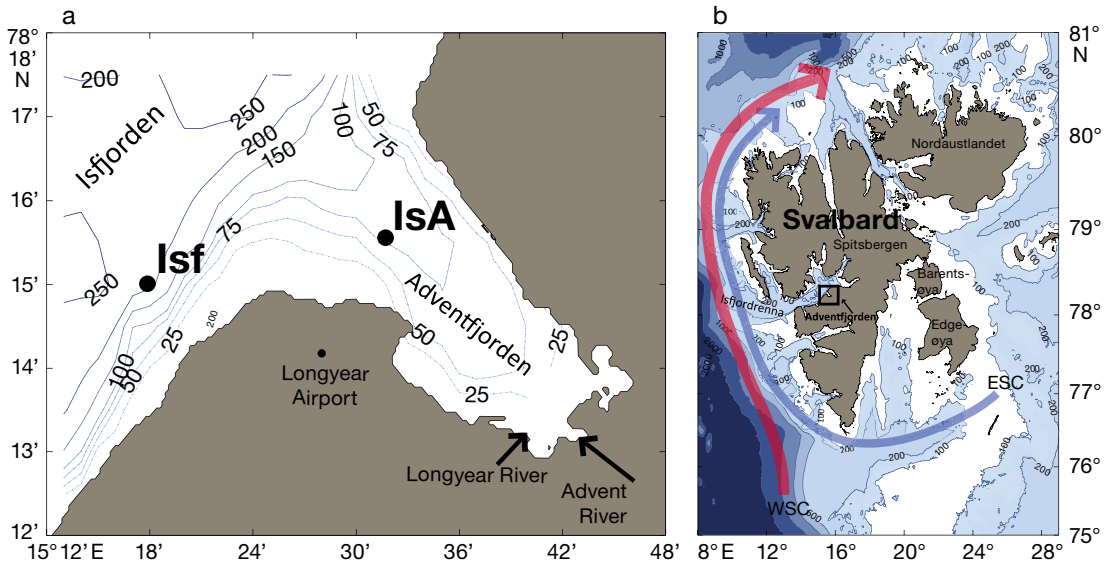



Fig. 1. (a) Map of Adventfjorden showing the Isfjorden-Adventfjorden (IsA) sampling site, the CTD station in Isfjorden proper (Isf), the Longyear Airport, the Longyear River and the Advent River. (b) Bathymetry around Svalbard with Isfjordrenna and Adventfjorden indicated. The West Spitsbergen Current (WSC) and the East Spitsbergen Current (ESC) are shown by the red and blue arrows, respectively. The bathymetric data are from the International Bathymetric Chart of the Arctic Ocean (IBCAO version 3; Jacobsen et al. 2012)

protists to the vertical biomass flux using microscopy, due to their size and lack of morphological characteristics when incorporated into aggregates or fecal pellets, we used DNA signatures to identify the contribution of small pelagic protists to the downward flux. In this study, we combined the 454 pyrosequencing data to identify 2 size fractions of pelagic protists (0.45–10 and >10 µm) at different depths (5, 15, 25 and 60 m) to assess their contribution to the vertically exported material from December 2011 to September 2012. Focusing particularly on the contribution of the small size fraction, we also describe the hydrography at the station to evaluate the potential importance of advection and layering on the community composition of the pelagic protists.

## 2. MATERIALS AND METHODS

Samples were collected from December 2011 to December 2012 (see Marquardt et al. 2016) at the IsA time series station close to Longyearbyen on the west coast of Spitsbergen (78° 15.6′ N, 15° 31.8′ E, 85 m depth; Fig. 1). Short-time sediment traps were deployed 7 times from 14 December 2011 to 19 September 2012, prioritizing spring, autumn and winter periods due to lack of ship time during summer months. On the same dates of deployment, water was collected from 4 depths (Table 1). We refer to the winter sampling days (December–January) as Winter I (WI), Winter II (WII) and Winter III (WIII), the spring sampling days (April–May) as Spring I (SI), Spring II (SII) and Spring III (SIII), and the one in autumn (September) as Autumn I (AI; Table 1, in accordance with Wiedmann et al. 2016).

### 2.1. Hydrographical conditions, currents and winds

A mooring was deployed at IsA from 28 September 2011 to 6 September 2012 (position 78° 15.636′ N, 15° 32.094′ E at 85 m depth; Fig. 1). It was equipped with HOBO temperature loggers at 20, 30, 37 and 65 m and with Seabird SBE37 conductivity, temperature and depth (CTD) sensors at 25 and 77 m. An RDI 300 kHz acoustic Doppler current profiler (ADCP) was mounted at 76 m depth sampling at 4 m bins from 70 to 10 m depths. Due to a long-term automatic sediment trap approximately 10 m above the ADCP, the data from the 3 lowermost bins were corrupted. Temperature loggers recorded every 15 min and the upper CTD sensor logged every hour during the full deployment period. The ADCP and lower CTD sensor logged every 20 min and stopped on 7 August 2012 and 31 July 2012, respectively. On each field sampling date, additional hydrographical data from a hand-held vertical CTD profiler (SAIV A/S, Bergen, Norway) were obtained at IsA (Marquardt et al. 2016). The water masses were characterized according to Nilsen et al. (2008). Wind and air temperature data from the Longyear Airport (78° 14′ N, 15° 28′ E; Fig. 1) were downloaded from the Norwegian Meteorological In-

Table 1. Overview of sampling dates, deployment time for sediment traps and volume of water filtered for DNA samples (S: small DNA/chlorophyll *a* [chl *a*] filter <10 µm; L: large DNA/chl *a* filter >10 µm) at the Isfjorden-Adventfjorden (IsA) station (78° 15.67′ N, 15° 32.10′ E). BD: below detection; ND: not determined. Total chl *a* concentrations from all 4 depths have been published in Stübner et al. (2016), and fractionated chl *a* estimates from 25 m have been reported in Marquardt et al. (2016)

| Date<br>Season<br>Deployment time (h:min) | Sediment trap deployed (m) | Water sampling deployed (m) | Water volume filtered for DNA (ml)<br>S \| L | Chl *a* (<10 µm) | Chl *a* (>10 µm) |
|---|---|---|---|---|---|
| 13/14 December 2011 | 20 | | 479 | 0.030[a] | 0.072[a] |
| Winter I | 30 | | 365 | 0.097[a] | 0.019[a] |
| 24:15 | 40 | | 365 | 0.034[a] | 0.076[a] |
| | 60 | | 345 | 0.058[a] | 0.066[a] |
| | | 5 | 3500 \| ND | 0.035[b] | 0.006[b] |
| | | 15 | 3600 \| ND | 0.034[b] | 0.006[b] |
| | | 25 | 3500 \| ND | 0.036[b] | 0.006[b] |
| | | 60 | 3400 \| ND | 0.034[b] | 0.007[b] |
| 17/18 January 2012 | 20 | | 450 | BD | 0.223[a] |
| Winter II | 30 | | 515 | 0.017[a] | 0.162[a] |
| 22:00 | 40 | | 660 | BD | 0.206[a] |
| | 60 | | 194* | 0.027[a] | 0.289[a] |
| | | 5 | 4300 \| 4300 | 0.051[b] | 0.007[b] |
| | | 15 | 4200 \| 4200 | 0.051[b] | 0.012[b] |
| | | 25 | 4200 \| 4200 | 0.051[b] | 0.014[b] |
| | | 60 | 4200 \| 4200 | 0.051[b] | 0.018[b] |
| 27/28 January 2012 | 20 | | 450 | 0.028[a] | 0.117[a] |
| Winter III | 30 | | 400 | BD | 0.127[a] |
| 25:00 | 40 | | 400 | BD | 0.148[a] |
| | 60 | | 470 | 0.079[a] | 0.189[a] |
| | | 5 | 3750 \| 4000 | 0.031[b] | 0.013[b] |
| | | 15 | 4050 \| 4050 | 0.040[b] | 0.029[b] |
| | | 25 | 3830 \| 4000 | 0.037[b] | 0.024[b] |
| | | 60 | 4050 \| 4050 | 0.028[b] | 0.013[b] |
| 26/27 April 2012 | 20 | | 660 | 10.839[a] | 10.143[a] |
| Spring I | 30 | | 655 | 9.796[a] | 6.608[a] |
| 24:25 | 40 | | 650 | 8.274[a] | 8.462[a] |
| | 60 | | 555 | 9.798[a] | 6.445[a] |
| | | 5 | 4000 \| 4000 | 1.379[b] | 1.853[b] |
| | | 15 | 4000 \| 4000 | 0.559[b] | 1.664[b] |
| | | 25 | 3800 \| 4000 | 0.160[b] | 1.460[b] |
| | | 60 | 4200 \| 4200 | BD | 1.437[b] |
| 10/11th May 2012 | 20 | | 500 | BD | 15.500[a] |
| Spring II | 30 | | 500 | 3.727[a] | 14.102[a] |
| 23:25 | 40 | | 500 | 0.616[a] | 14.948[a] |
| | 60 | | 500 | 3.374[a] | 13.820[a] |
| | | 5 | 3800 \| 4000 | 0.806[b] | 2.553[b] |
| | | 15 | 3700 \| 4000 | 0.700[b] | 3.250[b] |
| | | 25 | 3900 \| 4000 | 0.758[b] | 3.450[b] |
| | | 60 | 3850 \| 4050 | 0.569[b] | 2.514[b] |
| 30/31 May 2012 | 20 | | 500 | 0.405[a] | 2.018[a] |
| Spring III | 30 | | 500 | 2.207[a] | 3.033[a] |
| 23:45 | 40 | | 500 | 0.949[a] | 4.306[a] |
| | 60 | | 500 | 0.491[a] | 6.515[a] |
| | | 5 | 4230 \| 4230 | 0.508[b] | 0.153[b] |
| | | 15 | 3820 \| 4000 | 0.432[b] | 0.184[b] |
| | | 25 | 3780 \| 4000 | 0.723[b] | 0.666[b] |
| | | 60 | 3890 \| 4000 | 0.668[b] | 0.983[b] |
| 18/19 September 2012 | 20 | | 1000 | 0.177[a] | 0.544[a] |
| Autumn I | 30 | | 1025 | BD | 0.703[a] |
| 23:05 | 40 | | 2005 | 0.119[a] | 0.694[a] |
| | 60 | | 1075 | BD | 0.680[a] |
| | | 5 | 3940 \| 4000 | 0.342[b] | 0.051[b] |
| | | 15 | 2540 \| 3000 | 0.303[b] | 0.060[b] |
| | | 25 | 3920 \| 4000 | 0.224[b] | 0.057[b] |
| | | 60 | 3560 \| 4000 | 0.187[b] | 0.059[b] |

[a]mg $m^{-2}$ $d^{-1}$; [b]mg $m^{-3}$

stitute (www.eklima.met.no). Time-series analysis, hydrography and current plots were produced in MATLAB R2013b (8.2.0.701, The MathWorks).

### 2.2. Suspended water samples (DNA, chl *a*)

Seawater was sampled using Niskin bottles (KC Denmark) at 4 standard depths (5, 15, 25 and 60 m). Immediately after collection, water for DNA analyses (4 l from each depth depending on availability, in accordance with Marquardt et al. 2016) was prefiltered by gravity through a 10 µm nylon mesh (KC Denmark) and then on 0.45 µm Durapore filters (Millipore) using vacuum filtration. All filters were snap-frozen and stored at −80°C until further analyses (Vader et al. 2015). These filters will be referred to as the large (>10 µm) and small (0.45–10 µm) suspended fractions. Seawater from the same depths was filtered and analyzed for fractionated chlorophyll *a* (chl *a*) biomass (total or >10 µm) and analysed fluorometrically by standard methods (Holm-Hansen & Riemann 1978).

### 2.3. Vertical flux characterization (DNA, chl *a*)

An anchored short-term sediment trap array was deployed at IsA to study the vertical flux of size-fractionated chl *a* biomass and protist diversity (Table 1). Paired, empty trap cylinders (KC Denmark, 7.2 cm inner diameter, 45 cm high, 1.8 l) without baffles or poison were deployed empty at IsA, which allowed the cylinders to fill themselves with ambient seawater of the deployment depth (20, 30, 40 and 60 m). These depths were chosen to sample material sinking from the euphotic to the aphotic zone and investigate the vertical flux, but at the same time minimize sampling of re-suspended bottom material. The deployment time of the traps ranged from 22 to 25 h, and is given in Table 1.

Material collected in the sediment trap cylinders was transferred into carboys after trap array recovery and stored cold and dark until filtration within a few hours. Sub-samples from each sampling depth (duplicates or triplicates of 150 ml) were filtered to determine the fractionated chl *a* concentration as described for size-fractionated suspended samples in Section 2.2. For the traps, 300–500 ml (on one occasion, 1000 ml) of water was used for DNA filtration, depending on availability as other parameters had to be collected from the total volume of 1.8 l. Cells were harvested by direct filtration onto 0.45 µm Durapore filters (Millipore) using vacuum filtration. No prefiltration was performed to cover total exported eukaryotes. This filter will be referred to as the total exported (>0.45 µm) fraction.

### 2.4. Molecular analyses and data processing

DNA extractions from suspended and exported samples (80 samples in total; Table 1 and Table S1 in the Supplement) were conducted using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol, with an additional bead-beating step (Vader et al. 2015). The large suspended fraction from WI was lost in transition. PCR of the V4 region of the 18S rRNA gene and preparation of the samples for 454 amplicon sequencing were done according to Marquardt et al. (2016). Six to 10 samples were pooled for each 1/8th lane of the 3 sequencing plates prepared for this project. Sequencing was performed on a Roche 454 GS-FLX Titanium platform at the IBIS/Plateforme d'Analyses Génomiques de l'Université Laval.

The amplicon data (combined samples) were analyzed with MacQIIME (version 1.7.0, Caporaso et al. 2010). Quality filtering, demultiplexing, chimera checking and clustering (similarity level of 97%; Table S1) were performed as described in Marquardt et al. (2016). The large suspended fraction (>10 µm) from 60 m in WII had only 448 reads, and was excluded prior to normalizing all samples to an equal sequencing depth (3400 reads), reducing the total number of samples to 79. The representative sequence of each operational taxonomic unit (OTU) was queried against both a curated reference database of Arctic marine eukaryotes (Comeau et al. 2011) and the PR2 database (Chevenet et al. 2006, 2010, Guillou et al. 2013) using BlastN (e-value 0.00001; Altschul et al. 1990). Taxonomy was assigned based on agreement between the 2 databases and a sequence similarity better than 98%.

### 2.5. Applied statistics

Multivariate analyses (clustering with bootstrap n = 1000, and NMDS with $\log_{10}$[n+1]-transformed data; not shown) were performed using the vegan package (Oksanen et al. 2013) in R (R version 3.2.1, R Core Team 2015) and PAST (version 3.01; Hammer et al. 2001). A global one-way analysis of similarities (ANOSIM; permutations: 999; distance matrix: Bray-Curtis; Clarke 1993) was computed in R to test for significant differences in the community composition

using raw, unweighted (presence/absence) and $\log_{10}$-(n+1)-transformed data. Tested groups included seasons (winter, spring and autumn), the 3 different categories of samples (suspended small, suspended large and total exported), type (suspended; trap), depth (5, 15, 20, 25, 30, 40 and 60 m), shallow versus deep (5–40 and 60 m; based on the hydrographic density profiles that indicated 2 layers) and phosynthetically active radiation (euphotic zone: 5–20 m, aphotic zone: 25–60 m, according to Kubiszyn et al. 2016).

To identify the most abundant OTUs in suspended and exported samples, the upper water samples of the water column (5–25 m) as well as the trap samples (20–40 m) were pooled (Table 2) after consideration of NMDS/CLUSTER and ANOSIM tests, which indicated no significant differences between depths, neither in the suspended nor in the sediment trap material.

## 3. RESULTS

### 3.1. Hydrography and environmental conditions

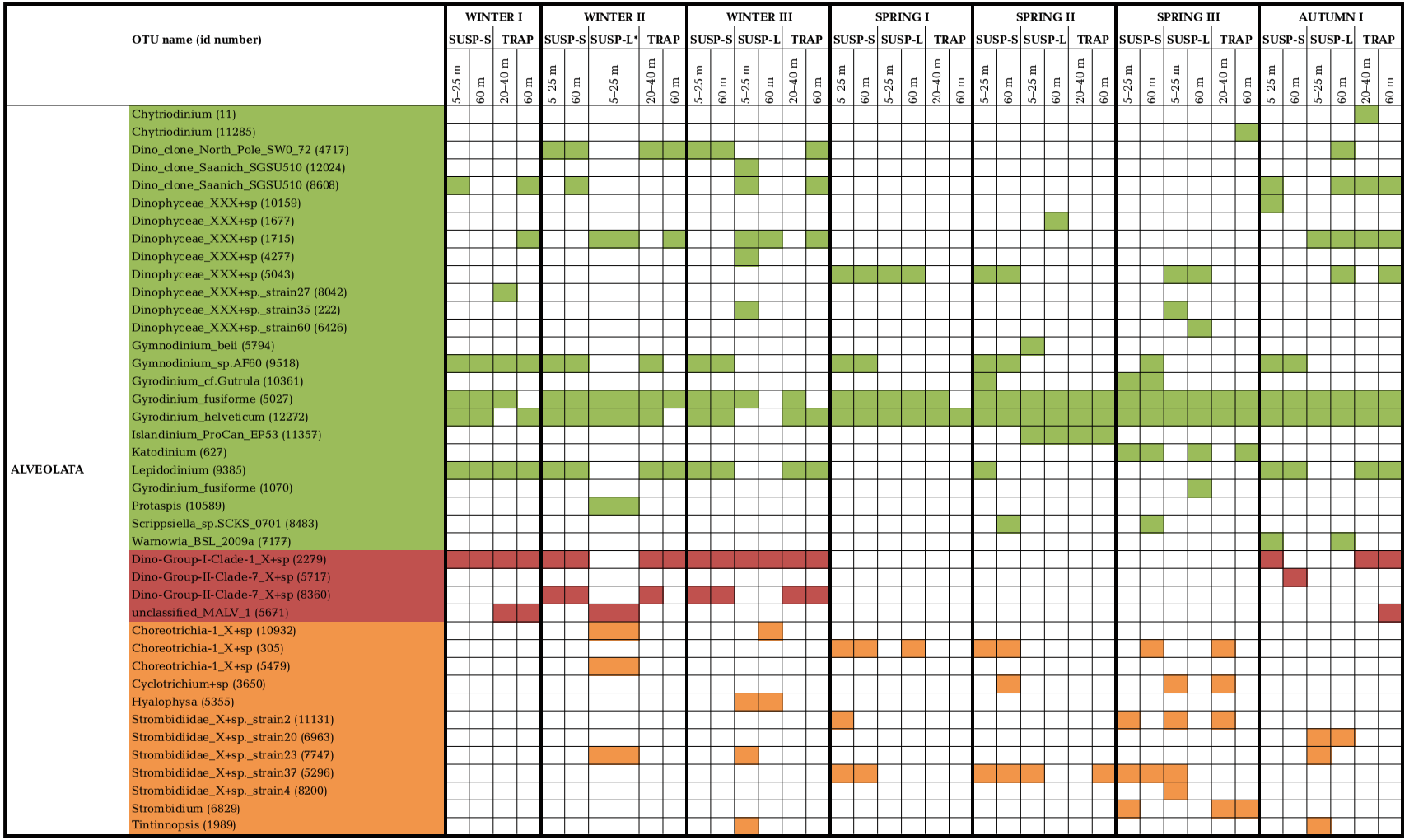
The dominant wind direction at the Longyear Airport (see Fig. 1) was from east to southeast (out-fjord direction) with a mean wind speed of 5 m $s^{-1}$, reaching up to 18 m $s^{-1}$ (Fig. 2a). The salinity and temperature from the sub-surface moored instruments (Fig. 2b,c) showed the same time trends as the earlier reported CTD profiles from the IsA station (Marquardt et al. 2016, Stübner et al. 2016). However, the higher temporal resolution of the moored instruments allowed precise determination of the timing of water mass inflow events (Fig. 2d). From deployment in September until mid-November, the water column was occupied with Surface Water (SW; temperature [$T$] > 1°C and salinity [S] < 34) in the upper 25 m and Intermediate Water (IW; $T$ > 1°C and 34 < S < 34.7) below, the latter most likely being the result of mixing between SW and underlying Transformed Atlantic Water (TAW; $T$ > 1°C and S > 34.7) in Isfjorden. The water column cooled from the surface and became more saline when the freshwater run-off from the Longyear and Advent Rivers (see Fig. 1) ceased due to freezing, and more saline water from below became entrained into the surface layer. From mid-November until the beginning of June, the water column was more homogeneous, with salinity differences of 0–0.2. Also, the stratification depths changed with seasons, with the surface layer reaching down to 10–20 m depths in summer and 30–50 m depth in autumn/early winter. Below 70 m, denser water was present during all seasons (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/a083p065_supp.pdf).

In accordance with the air temperature, gradual cooling of the water column continued in November and December, leading to transformation of the whole water column into Local Water (LW; $T$ < 1°C and S > 34). However, winter cooling was frequently interrupted by inflow of warmer (>1°C) and more saline water, first in mid-November and then in the beginning of December, when warm water was measured at all depths and the salinity increased. During 2 subsequent inflow events in February, warm water was present only at the bottom, before being detected by all sensors again from March until mid-April. From 19 April, less cold, but more saline LW reappeared throughout the water column, probably the result of out-flow of modified LW that had been pushed further into the fjord during the March–April inflow. Due to solar heating, the temperature increased abruptly after the end of May, first in the surface water and then gradually in the rest of the water column. Higher air temperatures (>0°C), increased solar radiation and the onset of melting and river runoff led to a warmer and less saline water column, which by 10 June was occupied with IW, most likely heated and freshened LW. Sediment-loaded freshwater run-off into the fjord mixed with underlying IW or LW created stronger surface stratification with low salinity SW in the upper 25 m. This was heated, reaching temperatures above 6°C by late July. As seen in Fig. 2, out-fjord wind events (i.e. late August) pushed out the SW, driving an upwelling of colder and more saline water from below, while in-fjord wind events (i.e. late July) pushed the SW into Adventfjorden, increasing the surface layer thickness. As mooring data only were acquired until early September, CTD profile time series from IsA (Marquardt et al. 2016) were examined and showed that in September, cooling started at the surface while heat penetrated downwards by mixing. In mid-September, the surface layer was absent, most likely due to an upwelling situation (strong out-fjord wind), an incident that co-occurred with our final sampling in September.

### 3.2. Small and large protists in the water column

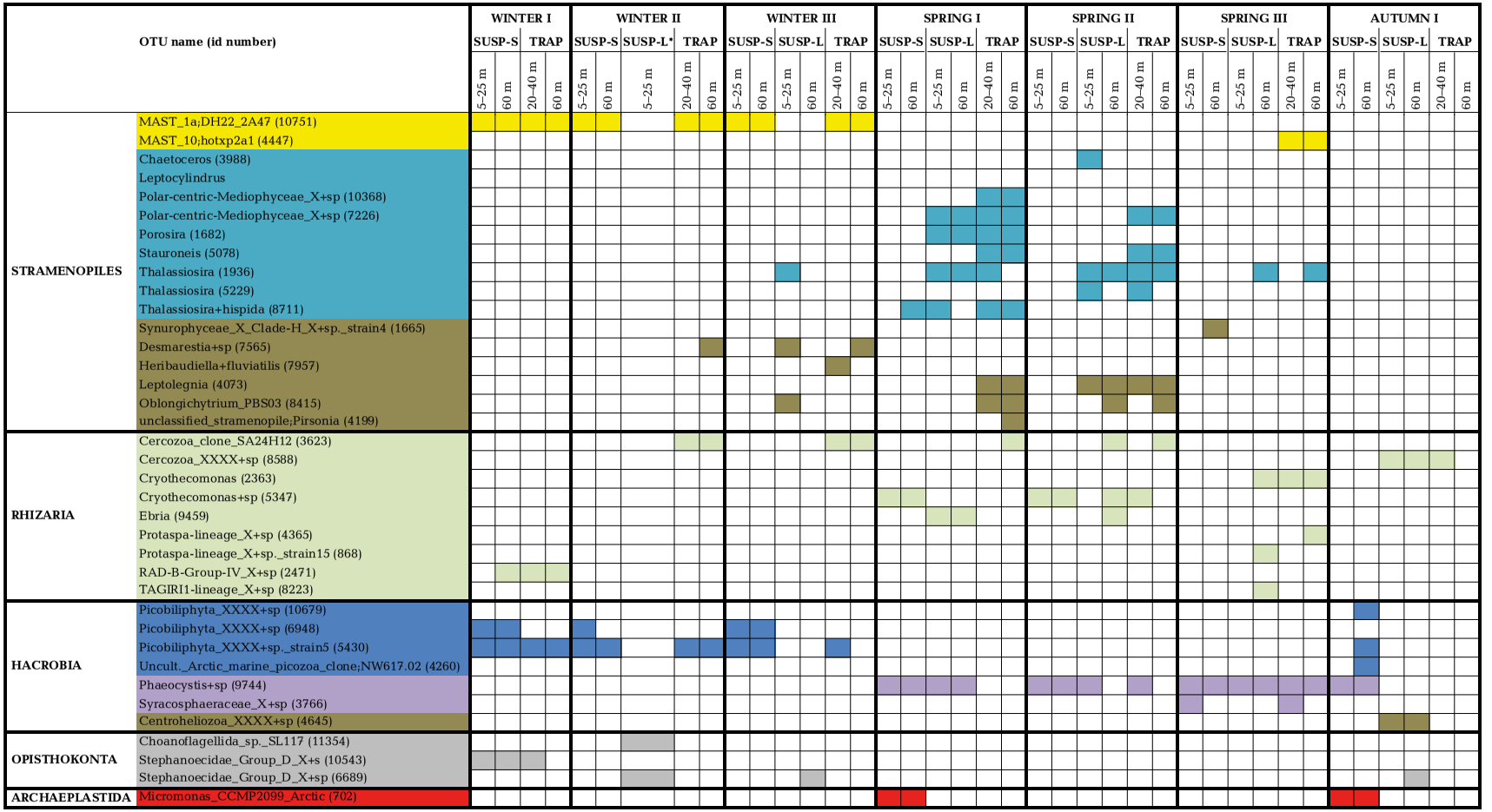
Although the same taxonomic groups (Dinophyceae, marine alveolates [MALVs], Ciliophora, Rhizaria, marine stramenopiles [MASTs], Bacillariophyceae; Fig. 3) were present in the small and large protist communities, the OTUs that made up the respective

Table 2. Overview of the most abundant operational taxonomic units (OTUs) (highest total DNA reads, sequencing depth 3400 reads) per sampling date and depth (samples partly pooled: 5–25 m, 20–40 m, 60 m) in the 3 different sample types (SUSP-S: suspended small; SUSP-L: suspended large; TRAP: total exported eukaryotes). The presence of a certain OTU at a certain date/depth is indicated by colors — Dinophyceae: green; MALVs: wine red; Ciliophora: orange; MASTs: yellow; Bacillariophyceae: turquoise; Eukaryote others: olive green; Rhizaria: light green; Picozoa: blue; Haptophyta: purple; Choanoflagellida: grey; Chlorophyta: red. *Winter II 60 m sample removed from analyses due to too few reads




(Table continued on next page)

Table 2 (continued)



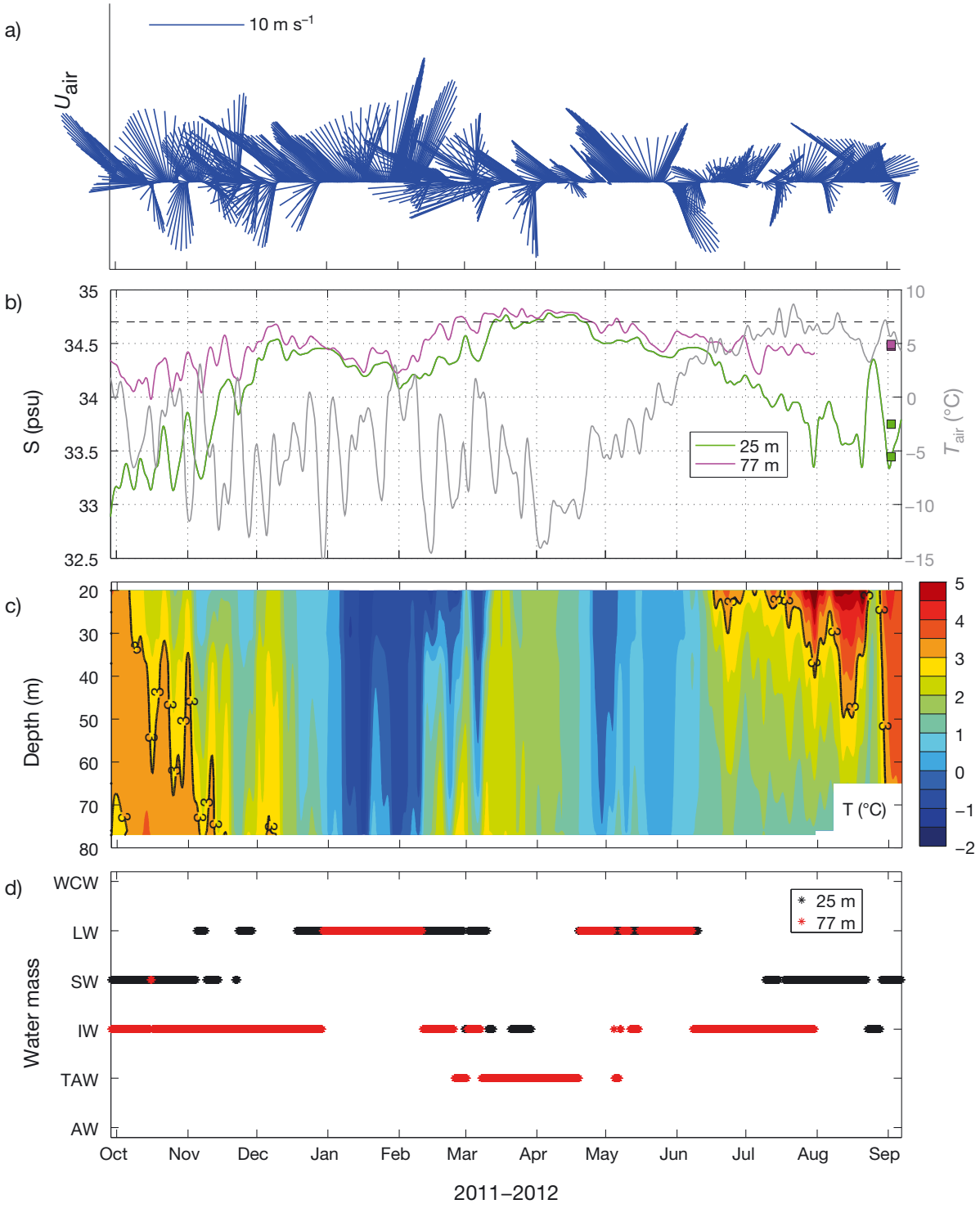



Fig. 2. (a) Stickplot of the wind velocity ($U_{air}$) from Longyear Airport. Sticks are pointing where the wind is heading. North is up, East is right. The length of the 10 m $s^{-1}$ unit is indicated. (b) Time evolution of the salinity (S) at 25 and 77 m depths together with air temperature ($T_{air}$, grey line) from Longyear Airport. (c) Temperature ($T$) at 20, 25, 30, 37, 65, 76 and 77 m depths from moored instruments nearby the Isfjorden-Adventfjorden sampling site (IsA) from 28 September 2011 to 6 September 2012. (d) Vertical distribution of water mass with time at IsA from interpolated temperature and salinity time series. WCW: Winter Cooled Water; LW: Local Water; SW: Surface Water; IW,: Intermediate Water; TAW: Transformed Atlantic Water; AW: Atlantic Water. The black dashed line in (b) is S = 34.7 and the black contour line in (c) is $T$ = 3°C, and together they indicate when TAW ($T$ > 1°C, S > 34.7) is present. All data have been low-pass filtered with a 3.5 d window

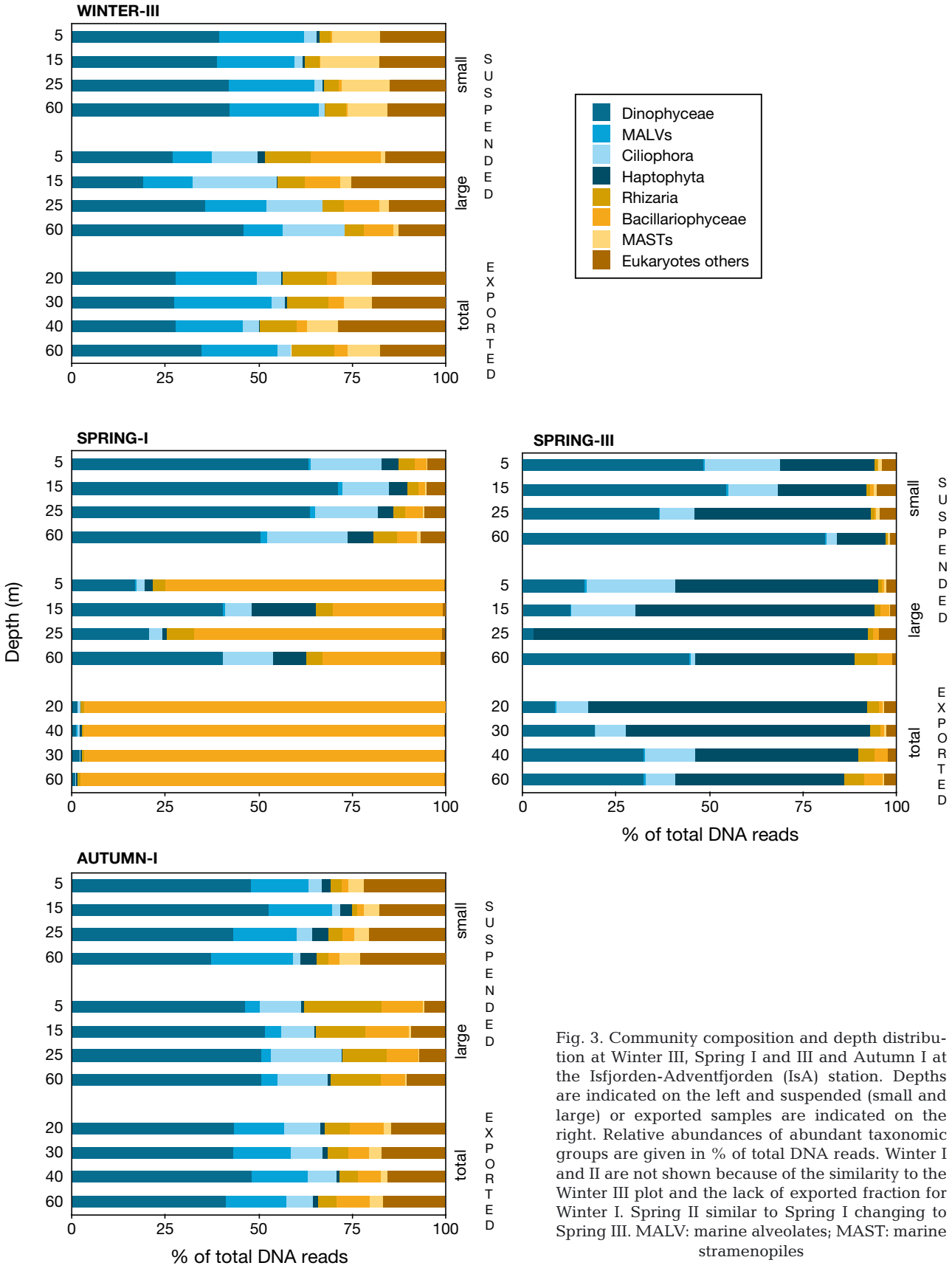



Fig. 3. Community composition and depth distribution at Winter III, Spring I and III and Autumn I at the Isfjorden-Adventfjorden (IsA) station. Depths are indicated on the left and suspended (small and large) or exported samples are indicated on the right. Relative abundances of abundant taxonomic groups are given in % of total DNA reads. Winter I and II are not shown because of the similarity to the Winter III plot and the lack of exported fraction for Winter I. Spring II similar to Spring I changing to Spring III. MALV: marine alveolates; MAST: marine stramenopiles

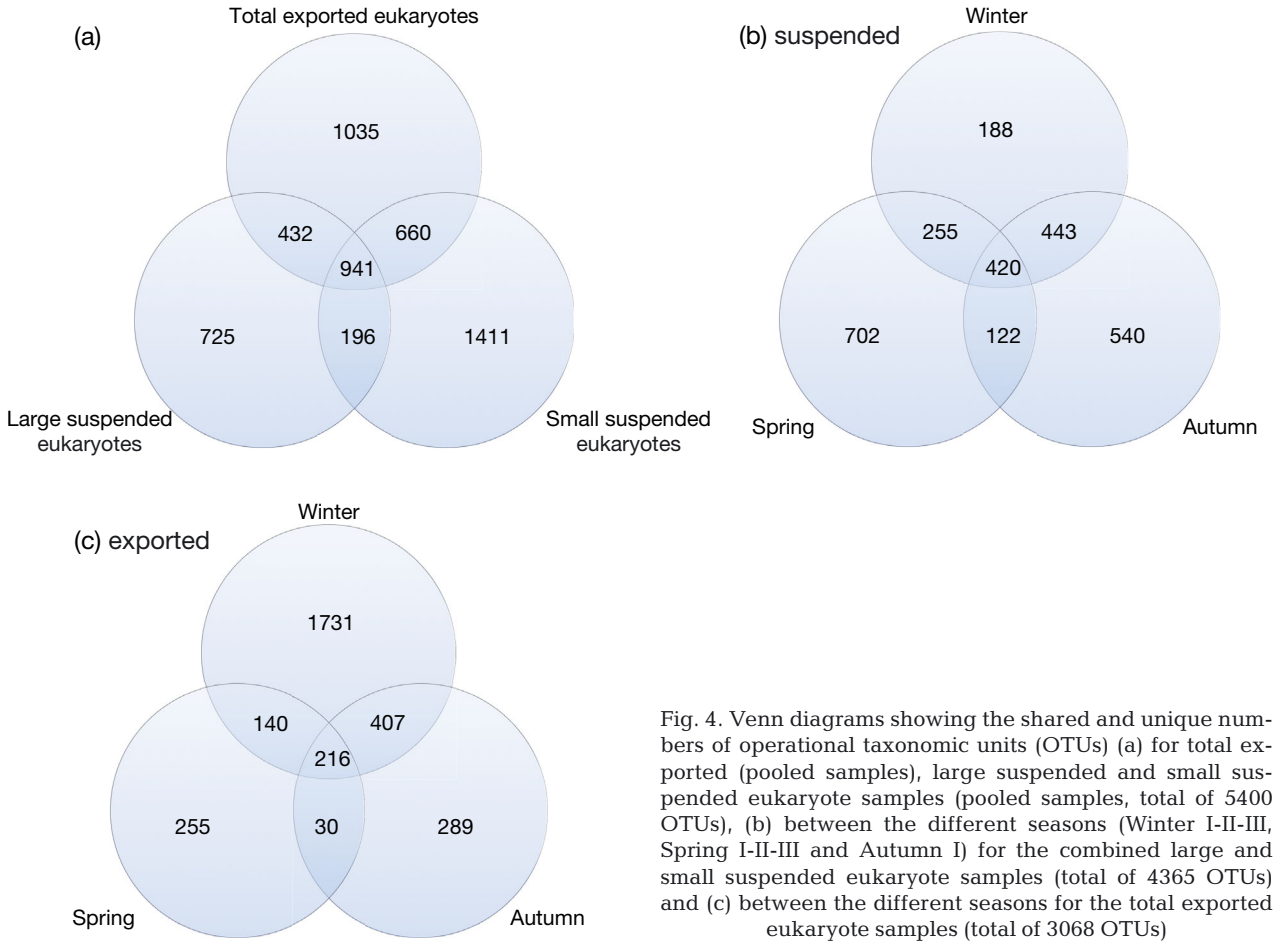



Fig. 4. Venn diagrams showing the shared and unique numbers of operational taxonomic units (OTUs) (a) for total exported (pooled samples), large suspended and small suspended eukaryote samples (pooled samples, total of 5400 OTUs), (b) between the different seasons (Winter I-II-III, Spring I-II-III and Autumn I) for the combined large and small suspended eukaryote samples (total of 4365 OTUs) and (c) between the different seasons for the total exported eukaryote samples (total of 3068 OTUs)

communities varied greatly. Out of 5400 OTUs in the combined dataset, only 196 were uniquely shared between the small and large communities, i.e. were not also shared with the exported samples (Fig. 4a). Comparing the different taxa that were unique to the 2 suspended size fractions, the OTU richness of MALV and Dinophyceae were noticeably higher within the small fraction, whereas there were more Metazoa and Bacillariophyta OTUs within the large fraction (data not shown).

Considering read abundances instead of OTU richness, the differences between the small and large suspended samples were especially apparent during spring when, for example, Bacillariophyceae and Haptophyta were very abundant in the large fraction (Fig. 3). An overview of the 10 most abundant OTUs (Table 2) showed that especially centric diatoms such as *Thalassiosira*, *Porosira* and *Chaetoceros* were numerous at this time (SI and SII). Other Stramenopiles in the large fraction included the parasitic oomycetes *Leptolegnia* and Oblongichtyrium_PB503, while MAST OTUs were never abundant in the large fraction. Two Dinophyceae OTUs, *Gyrodinium helvecticum* and *Gyrodinium fusiforme*, were numerous in nearly all samples, within both the large and the small communities, throughout all seasons. Other Dinophyceae were abundant in only one of the fractions, e.g. *Lepidonium* and *Gyrodinium* sp. AF60 in the small and Dinophyceae strain 35 and *Protaspis* in the large fractions (Table 2).

The large fraction contained several abundant ciliate taxa including *Hyalophysa* and *Tintinnopsis*. Different strains of Choreotricha and Strombidiidae-type ciliates were abundant in either the small (i.e. Strombidium) or the large (i.e. Choreotricha-1 [10932]) fractions. Picozoa OTUs were only abundant during winter and autumn, when they were found in the small fraction.

### 3.3. Seasonal variation in the biomass and export of small and large pelagic protists at IsA

The 3 sample groups (small and large suspended fractions and total exported protists) showed significantly different (ANOSIM: R = 0.202, p = 0.001) com-

munity compositions. A high percentage of the total OTUs were rare (89%), meaning their read abundances were <0.01% of the total dataset (data not shown). The amounts of shared OTUs between the small suspended fraction and total exported protists (30%) and the large suspended fraction and total exported protists (25%) were very similar (Fig. 4a).

During winter, the chl *a* biomass in the water column was relatively low with a dominance of small phototrophs (mean of 0.5 and 0.01 mg $m^{-3}$ for the small and large suspended fractions, respectively). In accordance, the exported chl *a* biomass in winter was low, but interestingly, here the large fraction prevailed (Table 1). Despite low chl *a* biomass, the winter OTU richness was high (suspended and exported samples in Fig. 4b and c, respectively). The highest small phototrophic biomass in the water column was measured at SI, when the maximum exported chl *a* flux was detected (<10 µm, between 20 and 60 m: 8.3–10.8 mg chl *a* $m^{-2}$ $d^{-1}$). The peak of chl *a* biomass for the large protist fraction was at SII, in concert with the highest chl *a* export (>10 µm, between 20 and 60 m: 13.8–15.5 mg chl *a* $m^{-2}$ $d^{-1}$; Table 1). Towards autumn, both the chl *a* biomass in the water column and the chl *a* export flux decreased (Table 1).

All suspended as well as the exported material differed between seasons (WI–III, SI–III and AI; ANOSIM: R = 0.753, p = 0.001). The exported material resembled the small fraction during winter and autumn, whereas it was more similar to the large fraction in spring (Fig. 3, Fig. S2 in the Supplement). However, the relative contribution of the 2 different size fractions seemed to vary between the sampling dates: whereas the WII trap samples clustered with the large fraction, the WI and WIII trap samples clustered with the small fraction (Fig. S2). During winter and autumn, many potential heterotrophic/mixotrophic Dinophyceae and parasitic MALV OTUs were abundant in both the water column and traps. A similar pattern was seen for MAST 1a, while the Picozoa OTUs were numerous in the traps only during winter (Table 2).

In late April and early May (SI and SII), the exported community was mainly made up of Bacillariophyceae (87–92% of total DNA reads) that were also highly abundant in the water column (large fraction) at this time. The pennate diatom *Stauroneis* was only present in the trap material (SI and SII), being absent (= not dominant) in the water column. In contrast, *Micromonas* Arctic (CCMP2099), which was very numerous during early spring (SI) and autumn, was never detected in the exported material. In late May (SIII), the community assembly changed again, with Haptophyta dominating in the water column (47–90% of reads in large fraction), and also contributing strongly to the vertically exported material (47–75% of total reads). *Phaeocystis* sp. was numerous during spring (small and large fractions) and autumn (small fraction), but was only exported in mid and late spring (SII and SIII, respectively). Ciliophora were only found in the exported material during spring (SIII, Table 2).

While most of the OTUs in the exported material were the same as those abundant in the water column at the same date, some OTUs seemed to be enriched in the traps (Table 2). For instance, Cercozoa clone_SA24H12 was only present in the traps (WII, WIII and SI). Parasitic organisms such as an unclassified MALV I (WI), MALV II clade 7 (AI), *Leptolegnia* (SII) and 2 OTUs representing the Dinophyceae *Chytriodinium* (SIII and AI) were all present in the exported material but not in the water column during the same period. In contrast, certain groups and OTUs did not seem to contribute to the exported material, i.e. Choanoflagellida, *Gymnodinium beii* and a few Dinophyceae sp. (strains: 27, 35, 60) as well as several Strombidiidae sp. ciliates (strains: 23, 37, 4).

### 3.4. Depth distribution and community shifts

The system was well mixed on all of our sampling dates, even during autumn, when the sampling co-occurred with an upwelling event (see above). No significant differences were found when the community composition of our entire dataset (suspended and exported) was tested according to depth (R = –0.047, p = 0.965), shallow versus deep (R = –0.01, p = 0.5) or light (R = –0.01, p = 0.6). In addition, the fractionated chl *a* biomass (suspended and exported) displayed only minor differences between depths, except in SI, when the largest suspended chl *a* biomass was measured at 5 m (1.4 mg $m^{-3}$, Table 1). The large fraction communities were very similar throughout the water column and also in the sediment traps during winter and autumn (Fig. 3). In spring, however, slight depth-dependent differences in community composition were observed for the large fraction (Fig. 3), mainly due to shifts in the abundances of a few dominant taxonomic groups.

The identified inflow of warmer and more saline water into Adventfjorden in February and March (Fig. 2) co-occurred with significant changes in the small community at 25 m depth (re-analysis of data from Marquardt et al. 2016; 14 December 2011 to 30 April 2012). MALVs (Table S2 in the Supplement:

wine-red), MAST 1a (Table S2: yellow) and Picozoa (Table S2: blue), which were dominant during the first LW period (LW1), disappeared during the period dominated by TAW in March to April. During the shift back to LW dominance in mid-April, ciliates and phototrophs such as diatoms, chlorophytes and *Phaeocystis* sp. increased in abundance. However, some Dinophyceae (e.g. *G. fusiforme* and *G. helvecticum*) were abundant throughout the whole period. It must be noted that although the community changes were significantly correlated with water mass (water mass: LW1, TAW, LW2; ANOSIM: R = 0.7962, p = 0.001, re-analyzed data from Marquardt et al. 2016), we cannot conclude whether the change in water mass was the causative agent.

## 4. DISCUSSION

The use of high-throughput sequencing proved highly useful in identifying potentially important and to date unrecognized contributors to the vertical flux of Arctic marine systems. Our results provide new insight into the relative contributions of different pelagic protist groups to the vertical flux in Adventfjorden, also highlighting the importance of small (<10 µm) cells to the vertical export.

The results obtained must, however, be interpreted with the methodological limitations in mind. Firstly, 454 sequencing does not give quantitative data on species abundances. The read numbers of an OTU relative to the other identified OTUs in the samples may give an estimate of its relative abundance, although it is known that rDNA copy numbers vary between taxa, i.e. 1 DNA read ≠ 1 individual (Zhu et al. 2005), and that there are methodological reasons as to why the read abundances of some OTUs may be over- or underestimations of taxon abundances (Huse et al. 2007). Secondly, we were only able to subsample the dataset to 3400 reads, which is a relatively low sequence volume and may have limited the findings on the present dataset. Thirdly, the correct assignment of an OTU to a known species relies on an appropriate amount of sequence variation within the target gene as well as a highly accurate and complete 18S rDNA sequence database, which presently does not exist.

### 4.1. Advection and layering at IsA

The concurrent hydrographical profiles from IsA and the Isfjorden proper showed that IsA represents the hydrographic changes in the upper 100 m in Isfjorden during the sampling period (Fig. S3 in the Supplement). The warm events at IsA in winter and early spring were accompanied by a topographically steered inflow of comparably warm and saline TAW, which entered Adventfjorden from Isfjorden after being separated from the WSC following the topography along the West Spitsbergen Shelf. This inflow of AW is most likely governed by regional-scaled atmospheric conditions on the shelf (Cottier et al. 2007, Nilsen et al. 2016, Tverberg et al. 2019). During strong southerly wind events, such as in winter 2011/2012, the AW inflow penetrated Isfjorden as TAW along the bottom and reached above 100 m depth and influenced the entire water column at the IsA station (Fig. 2). The regular inflow of AW is the reason why the high-Arctic Adventfjorden has been ice free in the recent winters.

We found a significant shift in the community composition when the water mass changed in the beginning of 2012. The biogeography and community structure (and diversity) of microbial eukaryotes can be strongly associated with the oceanographic conditions (Lovejoy et al. 2007, Hamilton et al. 2008, Li et al. 2009, Monier et al. 2015). It was primarily the rare biosphere (e.g. rare OTUs, read abundances <0.01%) that was exchanged during the advective event, while the abundant OTUs remained numerous in both LW and TAW.

The water column at IsA showed weak or no stratification during the field campaigns of the present study (winter 2011–autumn 2012; Fig. S1 in the Supplement), suggesting that strong mixing processes were taking place. This agrees with there being no significant differences in community composition with depth for either the suspended small (0.45–10 µm) and large (>10 µm) pelagic protists or in the sediment traps. At the same time, several species that were abundant in the water column were not detected in the traps, and vice versa (Table 2), suggesting that organisms were avoiding the traps either because they used their buoyancy and actively moved away or because the cells were too light to sink out. Furthermore, the non-stratified water column did not prevent the development of a spring bloom at the IsA station, which started in end of April in a nutrient-replenished water column (4.5 µM nitrate; Kubiszyn et al. 2017). Blooms in non-stratified water are not abnormal and similar situations have been observed previously in high-latitude regions (Townsend et al. 1992, Ellertsen 1993) and are explained by reduced mixing facilitating bloom development.

### 4.2. Seasonally influenced contribution of protists to the vertical export

Interestingly, small protists (<10 µm) contributed substantially to the downward flux during winter and autumn (Fig. S2 in the Supplement), supported by microscopic counts by Kubiszyn et al. (2017). This trend may increase in the future with limited seasonal sea ice in Adventfjorden and other Arctic fjords, and a concomitant cessation of the contribution of ice-algae to the pelagic–benthic coupling (Wiedmann et al. 2016). In winter, particulate organic carbon (POC) export (90–140 mg POC $m^{-2}$ $d^{-1}$; Wiedmann et al. 2016), chl *a* biomass (Table 1) and protist cell numbers (0.01–0.65 × $10^9$ cells $m^{-2}$ in Kubiszyn et al. 2017) were low and a high C:N ratio indicated the presence of partly degraded material in the water column (Wiedmann et al. 2016). Nevertheless, the total species richness and the number of unique (i.e. not shared between suspended and exported; Fig. 4) OTUs were highest at this time of the year, suggesting that winter water contained many different taxa but in low biomass, potentially in a dormant stage. RNA libraries in Marquardt et al. (2016) were also diverse, supporting a high winter richness of living cells. Apart from the living cells identified by the RNA method, the DNA method allowed us to detect dormant stages (i.e. resting cells, resting spores) as well as recently living cells, incorporated in fecal pellets and thus not identifiable in the microscope (Nejstgaard et al. 2003, Amacher et al. 2009, Troedsson et al. 2009).

The winter to spring transition was characterized by a substantial increase in chl *a* biomass and POC concentration (Wiedmann et al. 2016), a decrease in OTU richness and a large change in the community composition (Fig. 3). Only a few OTUs were shared between the winter and spring communities, suggesting a strong succession of species at the initiation of the spring bloom. SII was characterized by a high vertical POC flux (>1000 mg POC $m^{-2}$ $d^{-1}$ in Wiedmann et al. 2016), and in terms of DNA reads, those assigned to diatoms dominated in the water column (large fraction) and the exported material (20–60 m), in line with findings that diatoms are the major contributors to the POC flux in the Arctic (Fortier et al. 2002, Reigstad et al. 2008, Wexels Riser et al. 2008, Dünweber et al. 2010). In the present study, diatoms were quantitatively the dominant primary producers; this is supported by cell counting of Kubiszyn et al. (2017). The haptophyte *Phaeocystis pouchetii* that was also numerous in early spring (Kubiszyn et al. 2017) was not identified among the exported material at that time (Table 2). This was an expected finding, as diatoms were highly abundant in the water column (Kubiszyn et al. 2017) and their heavily silicified cells and resting stages were sinking out. Such diatom mass sinking events have been observed in different high-latitude regions (e.g. Rynearson et al. 2013).

During late spring (SIII), more than 50% of the DNA reads in the deployed sediment traps belonged to Haptophyta (mainly *Phaeocystis* sp.), and the vertical POC flux was lower than in early spring (SI) (Wiedmann et al. 2016). In our previous study (Wiedmann et al. 2016), we concluded that this POC decline was due to a change from fast-sinking diatoms (Passow 1991) to slow-sinking detritus, including *Phaeocystis* cells. It is possible that the detected *Phaeocystis* DNA came from cells that were already dead or fading during late spring; this is supported by low relative read abundances of *Phaeocystis* sp. 18S rRNA at this time (Marquardt et al. 2016). Nevertheless, it is also possible that *Phaeocystis* DNA was sinking out, either repackaged into fecal pellets (Hamm et al. 2001), or when ballasted by inorganic or organic substances (Wollenburg et al. 2018). We conclude that *Phaeocystis* should be considered a genus of limited sinking capacity rather than a non-sinking genus as suggested by Wolf et al. (2016).

The highest vertical POC flux at IsA was measured in autumn (770–1530 mg POC $m^{-2}$ $d^{-1}$; Wiedmann et al. 2016). At this time, the pelagic chl *a* biomass was low (Table 1) and the community composition had shifted into an assemblage similar to that found during the previous winter (Fig. 3, Fig. S2 in the Supplement), with small eukaryotes predominant in the traps as well as in the pelagic ecosystem. The OTU richness increased again and it looked as if the community was returning to its starting point in winter, indicating the existence of an annual cyclic pattern (Marquardt et al. 2016, Lambert et al. 2019). Wiedmann et al. (2016) reported that the vertical flux during autumn was made up of large to very large (>0.5 mm) fast sinking particles, hypothesizing that these particles may have been formed by flocculation and ballasting by lithogenic material entrained with the meltwater run-off. These meltwater sediments may contribute to physical flocculation, a process in which aggregates with high sinking velocity are formed (Kranck 1973, Sutherland et al. 2015). We suggest that the presence of small cells (<10 µm) in the autumn sediment traps is due to their inclusion in these large, fast sinking particles.

Heterotrophs such as MAST and Picozoa were abundant in the water column during winter and contributed to the vertical carbon flux. The higher

relative abundances of heterotrophs in the water column in winter were in agreement with the findings of Kubiszyn et al. (2017), who found that heterotrophs and potentially heterotrophic dinoflagellates predominated the suspended winter communities, although at low cell numbers (e.g. *Gymnodinium* sp. 81 cells $l^{-1}$ at WII in Kubiszyn et al. 2017). Detritus was proposed as the main vehicle of the vertical flux in winter at IsA (Wiedmann et al. 2016), and we suggest that the small heterotrophs were sinking in the form of aggregates (Waite et al. 2000, Richardson & Jackson 2007).

### 4.3. Pelagic protists in the vertical export

Our results emphasize that small cells can be important contributors to the vertical flux. This is in line with recent research that questions the traditional idea of a negligible contribution of small cells to the vertical flux (Richardson & Jackson 2007, Amacher et al. 2009, 2013, Worden et al. 2015). The 2 Dinophyceae OTUs assigned to *Gyrodinium helveticum* and *G. fusiforme* that were ubiquitous in all samples (suspended and exported) have an approximate cell size of 10 µm (A. M. Kubiszyn pers. comm.), and seem to be sampled by both filter fractions (small and large). They are frequently observed in Arctic studies (Barents Sea, Ratkova & Wassmann 2002; Canadian Arctic, Comeau et al. 2011; Greenland Sea, Richardson et al. 2005; Fram Strait, Kilias et al. 2013), and their relatively high abundances at IsA throughout all seasons, despite changes in hydrography and environmental conditions, suggest they are generalists (Thaler & Lovejoy 2015, Marquardt et al. 2016).

In contrast, small pico- and nanoflagellates such as MAST-1a (ca. 7.7 µm; Massana et al. 2006) and Picozoa (e.g. 2.5–3.8 µm for *Picomonas judraskeda* sp. nov, Seenivasan et al. 2013) were only found in the small fractions and in the trap samples only during winter. *Micromonas* Arctic (1.5–2 µm, Lovejoy et al. 2007), on the other hand, was present in the water column at certain times but was not identified among the contributors to the downward flux. Our study therefore hypothesizes a species-specific fate for small cells, potentially linked to their life strategy. While some stay rather suspended in the water column, others are preferentially vertically exported.

While larger protists in general are thought to contribute to the flux (Michaels & Silver 1988, Boyd & Newton 1999, Sarthou et al. 2005), we found several OTUs that were abundant in the water column, but rare in the exported material (e.g. Dinophyceae sp. strains 27, 35 and 60, *Gymnodinium beii*, several Strombidiidae sp. strains 23, 37 and 4). Possible explanations for this include grazing, remineralization or advection (Gismervik 2006, Hamilton et al. 2008, Amacher et al. 2009, Dünweber et al. 2010, Turner 2015 and references within), but as these ciliates and dinoflagellates are mobile to a certain degree, they could also be actively staying suspended in the water column.

Several species were only detected in the traps. Some of these are known to be parasites and were potentially transported into the traps along with their hosts. Among these parasites were a MALV I, the oomycete *Leptolegnia*, the dinoflagellate *Chytriodinium* and potentially some of the Cercozoa OTUs (Drebes et al. 1996). Marine alveolates (Guillou et al. 2008) commonly parasitize marine organisms such as crustaceans, dinoflagellates, fish and bivalves (Stentiford & Shields 2005, Chambouvet et al. 2008, Skovgaard et al. 2009, Miller et al. 2012, Noguchi et al. 2013). Several dinoflagellate species were common in winter, and could be putative host species for the MALV I. Oomycetes are important parasites on marine algae (Wei et al. 2010) and have been reported to infect invertebrates, especially crustaceans (Hubschman & Schmitt 1969). The dinoflagellate *Chytriodinium* sp., which is known to infect copepod eggs (Gómez et al. 2009), was found in the traps in late spring and autumn, at the same time when *Calanus* sp. was abundant in both the water and trap samples (data not shown). We potentially identified a new mechanism for vertical export exemplified by parasite-host-induced transport, increasing attention onto parasitism when studying carbon cycles and vertical flux.

*Acknowledgements*. The authors thank the UNIS logistical staff, especially Lars Frode Stangeland, and the crew of RV 'Helmer Hanssen' (UiT) and MS Farm. Thanks to S. Thomson and E. I. Stübner for great support in the field. A helping hand was highly appreciated in the lab (C. D. Nadeau, S. Øygaarden), during the Carbon Hydrogen Nitrogen analyses (S. Øygaarden) and the Next Generation Sequencing analyses (M. L. Davey). Special thanks to C. Lovejoy (l'Université Laval, Québec) for providing the protist database and feedback about the study design of this project. Further, thanks to the 2 anonymous reviewers for valuable input that significantly improved the manuscript. This project is part of the MicroFun project and funded by ConocoPhillips/Lundin Petrolium and UNIS. Fieldwork was supported by the Svalbard Science Forum grant (Arctic Field Grant RIS 5264 and 5688), UNIS and the CONFLUX project (Tromsø Forskningsstiftelse). Funding for R. Skogseth merits project Grønn-Bille (Research Council of Norway project no. 227067) and I. Wiedmann was supported by project ARCEx (Research Council of Norway project no. 228107).

LITERATURE CITED


Alldredge AL, Gottschalk CC (1989) Direct observations of the mass flocculation of diatom blooms: characteristics, settling velocities and formation of diatom aggregates. Deep Sea Res A 36:159–171

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Amacher J, Neuer S, Anderson I, Massana R (2009) Molecular approach to determine contributions of the protist community to particle flux. Deep-Sea Res I 56:2206–2215

Amacher J, Neuer S, Lomas M (2013) DNA-based molecular fingerprinting of eukaryotic protists and cyanobacteria contributing to sinking particle flux at the Bermuda Atlantic time-series study. Deep-Sea Res II 93:71–83

Assmy P, Fernández-Méndez M, Duarte P, Meyer A and others (2017) Leads in Arctic pack ice enable early phytoplankton blooms below snow-covered sea ice. Sci Rep 7: 40850 EP

Beaulieu SE (2002) Accumulation and fate of phytodetritus on the sea floor. Oceanogr Mar Biol Annu Rev 40:171–232

Billett DSM, Lampitt RS, Rice AL, Mantoura RFC (1983) Seasonal sedimentation of phytoplankton to the deep-sea benthos. Nature 302:520–522

Boyd PW, Newton P (1999) Does planktonic community structure determine downward particulate organic carbon flux in different oceanic provinces. Deep-Sea Res I 46:63–91

Brandner MM, Stübner E, Reed AJ, Gabrielsen TM, Thatje S (2017) Seasonality of bivalve larvae within a high Arctic fjord. Polar Biol 40:263–276

Brussaard CPD, Noordeloos AAM, Witte H, Collenteur MCJ, Schulz K, Ludwig A, Riebesell U (2013) Arctic microbial community dynamics influenced by elevated $CO_2$ levels. Biogeosciences 10:719–731

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K and others (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336

Chambouvet A, Morin P, Marie D, Guillou L (2008) Control of toxic marine dinoflagellate blooms by serial parasitic killers. Science 322:1254–1257

Chevenet F, Brun C, Bañuls AL, Jacq B, Christen R (2006) TreeDyn: towards dynamic graphics and annotations for analyses of trees. BMC Bioinformatics 7:439

Chevenet F, Croce O, Hebrard M, Christen R, Berry V (2010) ScripTree: scripting phylogenetic graphics. Bioinformatics 26:1125–1126

Clarke KR (1993) Non-parametric multivariate analysis of changes in community structure. Austral Ecol 18:117–143

Comeau AM, Li WKW, Tremblay JE, Carmack EC, Lovejoy C (2011) Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. PLOS ONE 6:e27492.

Cottier F, Tverberg V, Inhal ME, Svendsen H, Nilsen F, Griffiths C (2005) Water mass modification in an arctic fjord through cross-self exchange: the seasonal hydrography of Kongsfjord, Svalbard. J Geophys Res Oceans 110: C12005

Cottier F, Nilsen F, Inall M, Gerland S, Tverberg V, Svendsen H (2007) Wintertime warming of an Arctic shelf in response to large-scale atmospheric circulation. Geophys Res Lett 34:L10607

Degerlund M, Ellertsen HC (2010) Main species characteristics of phytoplankton spring blooms in NE Atlantic and Arctic waters (68–80° N). Estuaries Coasts 33:242–269

Drebes G, Kühn SF, Gmelch A, Schnepf E (1996) *Cryothecomonas aestivalis* sp. nov., a colourless nanoflagellate feeding on the marine centric diatom *Guinardia delicatula* (Cleve) Hasle. Helgol Meeresunters 50:497–515

Dünweber M, Swalethorp R, Kjellerup S, Nielsen TG and others (2010) Succession and fate of the spring diatom bloom in Disko Bay, western Greenland. Mar Ecol Prog Ser 419:11–29

Durkin CA, Estapa ML, Buesseler KO (2015) Observations of carbon export by small sinking particles in the upper mesopelagic. Mar Chem 175:72–81

Ellertsen HC (1993) Spring blooms and stratification. Nature 363:24

Foissner W, Hawksworth DL (eds) (2009) Protist diversity and geographical distribution. In: Topics in biodiversity and conservation. Dordrecht, Springer, p 1–42

Fortier M, Fortier L, Michel C, Legendre L (2002) Climatic and biological forcing of the vertical flux of biogenic particles under seasonal Arctic Sea ice. Mar Ecol Prog Ser 225:1–16

Gismervik I (2006) Top-down impact by copepods on ciliate numbers and persistence depends on copepod and ciliate species distribution. J Plankton Res 28:499–507

Gómez F, Moreira D, López-García P (2009) Life cycle and molecular phylogeny of the dinoflagellates *Chytriodinium* and *Dissodinium*, ectoparasites of copepod eggs. Eur J Protistol 45:260–270

Greene CH, Pershing AJ (2007) Climate drives sea change. Science 315:1084–1085

Guillou L, Viprey M, Chambouvet A, Welsh RM and others (2008) Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). Environ Microbiol 10:3349–3365

Guillou L, Bachar D, Audic S, Bass D and others (2013) The Protist Ribosomal Reference database ($PR^2$): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res 41:D597–D604

Hamilton AK, Lovejoy C, Galand PE, Ingram RG (2008) Water masses and biogeography of picoeukaryote assemblages in a cold hydrographically complex system. Limnol Oceanogr 53:922–935

Hamm C, Reigstad M, Wexels Riser C, Mühlebach A, Wassmann P (2001) On the trophic fate of *Phaeocystis pouchetii*. VII. Sterols and fatty acids reveal sedimentation of *P. pouchetii*-derived organic matter via krill fecal strings. Mar Ecol Prog Ser 209:55–69

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics software package for education and data analysis. Palaeontol Electronica 4:1–9

Hegseth EN, Tverberg V (2013) Effect of Atlantic water inflow on timing of the phytoplankton spring bloom in a high Arctic fjord (Kongsfjorden, Svalbard). J Mar Syst 113-114:94–105

Hodal H, Falk-Petersen S, Hop H, Kristiansen S, Reigstad M (2012) Spring bloom dynamics in Kongsfjorden, Svalbard: nutrients, phytoplankton, protozoans and primary production. Polar Biol 35:191–203

Holm-Hansen O, Riemann B (1978) Chlorophyll *a* determination: improvements in methodology. Oikos 30: 438–447

Hubschman JH, Schmitt JA (1969) Primary mycosis in shrimp larvae. J Invertebr Pathol 13:351–357

Huse SM, Huber JA, Morrison HG, Sogin ML, Welch DM (2007) Accuracy and quality of massively parallel DNA pyrosequencing. Genome Biol 8:R143

Iversen MH, Robert ML (2015) Ballasting effects of smectite on aggregate formation and export from a natural plankton community. Mar Chem 175:18–27

Jacobsen M, Mayer L, Coakley B, Dowdeswell JA and others (2012) The International Bathymetric Chart of the Arctic Ocean (IBCAO) Version 3.0. Geophys Res Lett 39: L12609

Kilias E, Kattner D, Wolf C, Nöthig EM, Peeken I, Metfies K (2013) Protist distribution in the western Fram Strait in summer 2010 based on 454-pyrosequnecing of 18S rDNA. J Phycol 49:996–1010

Kiørboe T, Hansen JLS (1993) Phytoplankton aggregate formation: observations of patterns and mechanisms of cell sticking and the significance of exopolymeric material. J Plankton Res 15:993–1018

Kranck K (1973) Flocculation of suspended sediment in the sea. Nature 246:348–350

Kubiszyn AM, Piwosz K, Wiktor JM (2014) The effect of inter-annual Atlantic water inflow variability on the planktonic protist community structure in the West Spitsbergen waters during the summer. J Plankton Res 36: 1190–1203

Kubiszyn AM, Wiktor JM, Wiktor JMJ, Griffiths C, Kristiansen S, Gabrielsen TM (2017) The annual planktonic protist community structure in an ice-free high Arctic fjord (Adventfjorden, West Spitsbergen). J Mar Syst 169: 61–72

Kuwata A, Hama T, Takahashi M (1993) Ecophysiological characterization of two life forms, resting spores and resting cells, of a marine planktonic diatom, *Chaetoceros pseudocurvisetus*, formed under nutrient depletion. Mar Ecol Prog Ser 102:245–255

Lalande C, Nöthig EM, Somavilla R, Bauerfeind E, Shevchenko V, Okolodkov Y (2014) Variability in under-ice export fluxes of biogenic matter in the Arctic Ocean. Global Biogeochem Cycles 28:571–583

Lambert S, Tragin M, Lozano JC, Ghiglione JF, Vaulot D, Bouget FY, Galand PE (2019) Rhythmicity of coastal marine picoeukaryotes, bacteria and archaea despite irregular environmental perturbations. ISME J 13:388–401

Li WKW, McLaughlin F, Lovejoy C, Carmack EC (2009) Smallest algae thrive as the Arctic Ocean freshens. Science 326:539

Lovejoy C (2014) Changing views of arctic protists (marine microbial eukaryotes) in a changing Arctic. Acta Protozool 53:91–100

Lovejoy C, Vincent WF, Bonilla S, Roy S and others (2007) Distribution, phylogeny, and growth of cold-adapted picoprasinophytes in Arctic seas. J Phycol 43:78–89

Marquardt M, Vader A, Stübner EI, Reigstad M, Gabrielsen TM (2016) Strong seasonality of marine microbial eukaryotes in a high-Arctic fjord (Isfjorden, in West Spitsbergen, Norway). Appl Environ Microbiol 82:1868–1880

Massana R, Terrado R, Forn I, Lovejoy C, Pedrós-Alió C (2006) Distribution and abundance of uncultured heterotrophic flagellates in the world oceans. Environ Microbiol 8:1515–1522

Michaels AF, Silver MW (1988) Primary production, sinking fluxes and the microbial food web. Deep-Sea Res A 35: 473–490

Miller JJ, Delwiche CF, Coats DW (2012) Ultrastructure of *Amoebophrya* sp. and its changes during the course of infection. Protist 163:720–745

Monier A, Comte J, Babin M, Forest A, Matsuoka A, Lovejoy C (2015) Oceanographic structure drives the assembly processes of microbial eukaryotic communities. ISME J 9:990–1002

Muckenhuber S, Nilsen F, Korosov A, Sandven S (2016) Sea ice cover in Isfjorden and Hornsund, Svalbard (2000–2014) from remote sensing data. Cryosphere 10: 149–158

Nejstgaard JC, Frischer ME, Raule CL, Gruebel R, Kohlberg KE, Verity PG (2003) Molecular detection of algal prey in copepod guts and faecal pellets. Limnol Oceanogr Methods 1:29–38

Nilsen F, Cottier F, Skoseth R, Mattson S (2008) Fjord-shelf exchanges controlled by ice and brine production: the interannual variation of Atlantic water in Isfjorden, Svalbard. Cont Shelf Res 28:1838–1853

Nilsen F, Skogseth R, Vaadal-Lunde J, Inall M (2016) A simple shelf circulation model — intrusion of Atlantic Water on the West Spitsbergen Shelf. J Phys Oceanogr 46: 1209–1230

Noguchi F, Kawato M, Yoshida T, Fujiwara Y, Fujikura K, Takishita K (2013) A novel alveolate in bivalves with chemosynthetic bacteria inhabiting deep-sea methane seeps. J Eukaryot Microbiol 60:158–165

Oksanen J, Guillaume Blanchet F, Kindt R, Legendre P and others (2013) vegan: Community Ecology Package. R Package version 20-10 1. http://cran.r-project.org/package=vegan

Olli K, Heiskanen AS (1999) Seasonal stages of phytoplankton community structure and sinking loss in the Gulf of Riga. J Mar Syst 23:165–184

Olli K, Rieser CW, Wassmann P, Ratkova T, Arashkevich E, Pasternak A (2002) Seasonal variation in vertical flux of biogenic matter in the marginal ice zone and the central Barents Sea. J Mar Syst 38:189–204

Onarheim IH, Smedsrud LH, Ingvaldsen RB, Nilsen F (2014) Loss of sea ice during winter north of Svalbard. Tellus 66: 23933

Passow U (1991) Species-specific sedimentation and sinking velocities of diatoms. Mar Biol 108:449–455

Passow U, Alldredge A (1995) A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). Limnol Oceanogr 40:1326–1335

Pasternak A, Arashkevich E, Reigstad M, Wassmann P, Falk-Petersen S (2008) Dividing mesozooplankton into upper and lower size groups: applications to the grazing impact in the Marginal Ice Zone of the Barents Sea. Deep-Sea Res II 55:2245–2256

Pavlov AK, Tverberg V, Ivanov BV, Nilsen F, Falk-Petersen S, Granskog MA (2013) Warming of Atlantic Water in two west Spitsbergen fjords over the last century (1912–2009). Polar Res 32:11206

Piquet AMT, van de Poll WH, Visser RJW, Wiencke C, Bolhuis H, Buma AGJ (2014) Springtime phytoplankton dynamics in Arctic Krossfjorden and Kongsfjorden (Spitsbergen) as a function of glacier proximity. Biogeosciences 11:2263–2279

Ratkova TN, Wassmann P (2002) Seasonal variation and spatial distribution of phyto- and protozooplankton in the central Barents Sea. J Mar Syst 38:47–75

Reigstad M, Wassmann P (2007) Does *Phaeocystis* spp. contribute significantly to vertical export of organic carbon? Biogeochemistry 83:217–234

Reigstad M, Riser CW, Wassmann P, Ratkova T (2008) Vertical export of particulate organic carbon: attenuation, composition and loss rates in the northern Barents Sea. Deep-Sea Res II 55:2308–2319

Reigstad M, Carroll J, Slagstad D, Ellingsen I, Wassmann P (2011) Intra-regional comparison of productivity, carbon flux and ecosystem composition within the northern Barents Sea. Prog Oceanogr 90:33–46

Renaud P, Morata N, Carroll ML, Denisenko SG, Reigstad M (2008) Pelagic–benthic coupling in the western Barents Sea: processes and time-scales. Deep-Sea Res II 55: 2372–2380

Richardson K, Markager S, Buch E, Lassen MF, Kristensen AS (2005) Seasonal distribution of primary production, phytoplankton biomass and size distribution in the Greenland Sea. Deep-Sea Res I 52:979–999

Richardson TL, Jackson G (2007) Small phytoplankton and carbon export from the surface ocean. Science 315: 838–840

Rynearson TA, Richardson K, Lampitt RS, Sieracki ME, Poulton AJ, Lyngsgaard MM, Perry MJ (2013) Major contribution of diatom resting spores to vertical flux in the sub-polar North Atlantic. Deep-Sea Res I 82:60–71

Sarthou G, Timmermanns KR, Blain S, Tréguer P (2005) Growth physiology and fate of diatoms in the ocean: a review. J Sea Res 53:25–42

Schoemann V, Becquevort S, Stefels J, Rousseau V, Lancelot C (2005) *Phaeocystis* blooms in the global ocean and their controlling mechanisms: a review. J Sea Res 53: 43–66

Seenivasan R, Sausen N, Medlin LK, Melkonian M (2013) *Picomonas judraskeda* gen. et sp. nov.: the first identified member of the Picozoa phylum nov., a widespread group of picoeukaryotes, formerly known as 'picobiliphytes'. PLOS ONE 8:e59565

Seuthe L, Iversen KR, Narcy F (2011) Microbial processes in a high-latitude fjord (Kongsfjorden, Svalbard). II. Ciliates and dinoflagellates. Polar Biol 34:751–766

Sicko-Goad L, Stoermer EF, Kociolek JP (1989) Diatom resting cell rejuvenation and formation: time course, species records and distribution. J Plankton Res 11:375–389

Siegel DA, Buesseler KO, Behrenfeld MJ, Benitez-Nelson CR and others (2016) Prediction of the export and fate of global ocean net primary production: The EXPORTS Science Plan. Front Mar Sci 3:22

Skovgaard A, Meneses I, Angélico MM (2009) Identifying the lethal fish egg parasite *Ichthyodinium chabelardi* as member of Marine Alveolate Group I. Environ Microbiol 11:2030–2041

Stentiford GD, Shields JD (2005) A review of the parasitic dinoflagellates *Hematodinium* species and *Hematodinium*-like infections in marine crustaceans. Dis Aquat Org 66:47–70

Stübner EI, Søreide JE, Reigstad M, Marquardt M, Blachowiak-Samolyk K (2016) Year-round meroplankton dynamics in high-Arctic Svalbard. J Plankton Res 38:522–536

Sutherland BR, Barrett KJ, Gingras MK (2015) Clay settling in fresh and salt water. Environ Fluid Mech 15:147–160

Svendsen H, Beszczynska-Møller A, Hagen JO, Lefauconnier B and others (2002) The physical environment of the Kongsfjorden-Krossfjorden, an arctic fjord system in Svalbard. Polar Res 21:133–166

Terrado R, Vincent WF, Lovejoy C (2009) Mesopelagic protists: diversity and succession in a coastal Arctic ecosystem. Aquat Microb Ecol 56:25–39

Thaler M, Lovejoy C (2015) Biogeography of heterotrophic flagellate populations indicates the presence of generalist and specialist taxa in the Arctic Ocean. Appl Environ Microbiol 81:2137–2148

Townsend DW, Keller MD, Sieracki ME, Ackleson SG (1992) Spring phytoplankton blooms in the absence of vertical water column stratification. Nature 360:59–62

Troedsson C, Simonelli P, Nägele V, Nejstgaard J, Frischer M (2009) Quantification of copepod gut content by differential length amplification quantitative PCR (dla-qPCR). Mar Biol 156:253–259

Turner JT (2015) Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. Prog Oceanogr 130:205–248

Turner JT, Levinsen H, Nielsen TG, Hansen BW (2001) Zooplankton feeding ecology: grazing on phytoplankton and predation on protozoans by copepod and barnacle nauplii in Disko Bay, West Greenland. Mar Ecol Prog Ser 221:209–219

Tverberg V, Skogset R, Cottier F, Sundfjord F and others (2019) The Kongsfjorden Transect: seasonal and interannual variability in hydrography. In: Hop H, Weinecke C (eds) The ecosystem of Kongsfjorden, Svalbard. Advances in Polar Ecology. Springer Verlag, Cham, p 49–104

Vader A, Marquardt M, Meshram AR, Gabrielsen TM (2015) Key Arctic phototrophs are widespread in the polar night. Polar Biol 38:13–21

Vargas CA, Manriquez PH, Navarrete SA (2006) Feeding by larvae of intertidal invertebrates: assessing their position in pelagic food webs. Ecology 87:444–457

Waite AM, Safi KA, Hall JA, Nodder SC (2000) Mass sedimentation of picoplankton embedded in organic aggregates. Limnol Oceanogr 45:87–97

Wassmann P, Reigstad M (2011) Future Arctic Ocean seasonal ice zones and implications for pelagic–benthic coupling. Oceanography 24:220–231

Wassmann P, Ratkova TN, Andreassen I, Vernet M, Pedersen G, Rey F (1999) Spring bloom development in the marginal ice zone and the central Barents Sea. PSZNI: Mar Ecol 20:321–346

Wei L, Tianyu Z, Xuexi T, Bingyao W (2010) Oomycetes and fungi: important parasites on marine algae. Acta Oceanol Sin 29:74–81

Węsławski JM, Szymelfenig M, Zajączkowski M, Keck A (1999) Influence of salinity and suspended matter of benthos of an Arctic tidal flat. ICES J Mar Sci 56(Supplement):194–202

Wexels Riser C, Wassmann P, Reigstad M, Seuthe L (2008) Vertical flux regulation by zooplankton in the northern Barents Sea during Arctic spring. Deep-Sea Res II 55: 2320–2329

Wiedmann I, Reigstad M, Marquardt M, Vader A, Gabrielsen TM (2016) Seasonality of vertical flux and sinking particle characteristics in an ice-free high Arctic fjord — different from subarctic fjords? J Mar Sys 154(B):192–205.

Wolf C, Iversen M, Klaas C, Metfies K (2016) Limited sinking of *Phaeocystis* sp. during a 12 days sediment trap study. Mol Ecol 25:3428–3435

Wollenburg JE, Katlein C, Nehrke G, Nöthig EM and others (2018) Ballasting by cryogenic gypsum enhances carbon export in a *Phaeocystis* under-ice bloom. Sci Rep 8:7703

Worden AZ, Follows MJ, Giovannoni SJ, Wilken S, Zimmermann AE, Keeling PJ (2015) Rethinking the marine carbon cycle: factoring in the multifarious lifestyles of microbes. Science 347:1257594

Zhu F, Massana R, Not F, Marie D, Vaulot D (2005) Mapping of picoeucaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene. FEMS Microbiol Ecol 52: 79–92

*Editorial responsibility: Klaus Jürgens, Rostock, Germany*

*Submitted: July 4, 2018; Accepted: March 14, 2019*
*Proofs received from author(s): May 28, 2019*