Marine microbial eukaryotes in Svalbard waters:
Seasonality, community composition and diversity

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A dissertation for the degree of Philosophiae Doctor – April 2016
Cover image: Adventfjorden in different seasons (Photography by 1,4: Stuart Thomson, 2: Miriam Marquardt, 3: David Wrangborg)
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Thesis submitted in partial fulfillment of the requirements for the degree of Philosophiae Doctor (Ph.D) in Natural Science

Longyearbyen, 78°North, April 2016

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Preface

In 2006/07 I was doing a student exchange year at the University of Tromsø (UiT). At the time when I had to choose my courses to study there, the UNIS course catalogue was still included in the one from UiT, but I was not aware of that. I was so exited to see all these amazing courses about Arctic ecology, Arctic fauna and flora…and basically chose just UNIS courses. The student administrator wrote disappointingly back to me that I actually had to go to Svalbard, not Tromsø, if I wanted to take these courses. So I did, one year later; first on a cruise in 2007, then for a course in 2008 and for fieldwork in 2010. At that point, I never would have imagined that I would soon move to Svalbard to do a 4-year-PhD study there…

People say once you have fallen for the Arctic - you always return. This is one of my return stories.

For this PhD thesis roughly 2,500,000 millilitres (or more…) of seawater were filtered
Acknowledgements

"Alone we can do so little, together we can do so much." (Helen Keller)

With this truthful sentence I would like to thank a number of persons, without whose help and contribution, this study would not have been possible.

First of all my three wonderful supervisors Tove M. Gabrielsen, Anna Vader and Marit Reigstad for their guidance and support. Tove and Anna, when I first took this position I was not sure if I really wanted to live in Longyearbyen for four years, but very soon after my arrival, after meeting you two and Archana and the rest of the Biology department, I felt this was my place to be. Tove - you are a wonderful mentor; well sometimes a little bit too busy and surrounded by too many master students, but no matter what; your door was always open for me (even when a note on your door said “Do not disturb until 11 am” 😎). I could always come to you with all my problems of any nature; whether molecular challenges, the search for equipment (on which I surely spent 50% of my time during my first year…) or daily (PhD) life problems - you always listened and tried your best to solve the problems – thank you so much. The same is true for Anna: Thank you for being my friend and supervisor in one person. It was very important to have you at my side, whether at UNIS, on fieldwork, or elsewhere in the world (Hola Granada!), especially in this last year while Tove was in Canada. Marit thank you so much for your support and supervision. Without you in my team, I probably would not have been finished before the end of the year 😎. I know I should have “used” you even more, but let me tell you that the talks and discussion we had always gave me a new light and motivated me again.

I would like to thank the UNIS logistics department, especially Lars F. Stangeland for his helping hands and all the enjoyable polar circle trips that we had together. Further, the captains and the crews of R/V Helmer Hanssen (UiT), K/V Svalbard, M/V FARM and M/V Stálbás for valuable help during all my field sampling in Svalbard waters and the sea ice. Huge thanks to my good friend, colleague, sampling and filtration buddy Stuart Thomson – the fieldwork would not have been as much fun as it was without you and Bob Dylan!

Additional thanks to Stuart and Magnus Aune for linguistic assistance. I would also like to say that Stuart is the king of the world. Furthermore, I have to thank many helping hands who supported me in the field- and/or laboratory: Vincent Carrier, Prasad Rao, Lilith Kuckero,
Thanks also to all my splendid co-authors Ingrid Wiedmann, Eike I. Stübner, Archana R. Meshram, Ragnheid Skogseth and Finlo Cottier, for their fruitful comments on manuscripts and figures and partly the making of figures. I also would like to acknowledge the collaboration with Anna M. Kubiszyn and Jozef Wiktor (IOPAS Poland), Ramon Massana and Irene Forn Hernan (CSIC Barcelona) and Connie Lovejoy (IBIS Université Laval).
Furthermore, huge thanks go to all my lovely (previous and current) colleagues and friends here in the Arctic Biology Department, for good discussions during ’goodcoffee’- coffee breaks in the Orange (Terracotta?) Lounge! Also thanks to all co-workers and friends in the other UNIS departments – I had a great time here at the northernmost University of the world! Acknowledgements also go to the ARCTOS PhD school for providing an excellent research network, as well as forums and seminars. Thanks also to all my colleagues and friends at UiT. Special thanks to Daniel Vogedes who proof-read my thesis on a weekend despite not having much knowledge about molecular research, and thanks to Malin Daase for her special drawing talents.
Thanks to all my lovely friends on this island and in the rest of the world (Karin, Berit, David, Anatoly, Calle, Alexa, Chantal, Pernilla, Eike, Milena, Mona, Alba…never ending list…) for keeping my social life busy with tours, dogsledding, diving, kayaking, knitting or dinners – you’re making life like it is supposed to be – just beautiful.
And last but not least I am very grateful for my parents, Gaby and Kalli. You two always supported me during my whole life; throughout kindergarten, school and university; whether I had good or crazy plans (moving to the Arctic was one of the crazy ones I believe…). You always encouraged me and believed in me and I owe you so much!

My research was internally financed by the University Centre in Svalbard. This study is part of the MicroFun project, funded by ConocoPhillips and Lundin Petroleum via their Northern Area Program. Fieldwork was supported by the Arctic Field Grant (RIS 5264 and 5688).

Miriam Marquardt, Longyearbyen, April 2016
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Abstract

Microbial eukaryotes are critically important for the functioning of marine ecosystems. In Arctic waters, where marine planktonic cyanobacteria are infrequent, microbial eukaryotes are the predominant primary producers. The ongoing changes in the Arctic reflected by sea-ice retreat, freshening of the ocean and increased stratification may favour smaller protists in the future and that will potentially alter production and downward flux. In spite of their importance, our knowledge of the diversity, seasonality and fate of pico- and nanosized eukaryotic plankton is still limited in the polar regions, especially during the polar night period. High-Arctic regions are characterized by extreme seasonality in light conditions, with 24 hours of sunlight in summer giving way to several months of complete darkness in winter.

Molecular tools are now available for identifying even the smallest protist species. To investigate microbial eukaryotes in Svalbard waters two sampling approaches were applied: (1) Spatial sampling at multiple locations around West Spitsbergen to study the distribution of two small key-phototrophs (*Micromonas pusilla* and *Phaeocystis pouchetii*) during the polar night. PCR screening with specific primers was used to overcome the difficulty of identifying these small flagellates in low-biomass winter samples. (2) High-resolution temporal sampling (December 2011 to December 2012) at the Isfjorden-Adventfjorden time series station (IsA, West Spitsbergen) was conducted to investigate the succession and diversity of small protists and to determine their contribution to the vertical carbon flux. The community composition of suspended microbial eukaryotes (two size fractions: 0.45 – 10 µm and > 10 µm) from four different depths (5, 15, 25 and 60 m) was determined using 454 sequencing of the 18S V4 region amplified from both DNA and RNA. Additionally, microbial eukaryotes (> 0.45 µm) were sampled from short-time sediment traps (20, 30, 40 and 60 m) to study their contribution to the vertical flux. Hydrographical profiles and *in situ* environmental conditions were recorded at all stations.

Strong seasonal shifts of the community composition, species richness and photosynthetic biomass were observed in Adventfjorden. The winter and early-spring
communities were more diverse than the spring and summer/autumn communities. Small *Gyrodinium* species were predominant in both DNA and RNA libraries of the suspended material throughout the year, and in the trap material. The Arctic *Micromonas* ecotype was most abundant in the early bloom and fall periods at IsA, but was widely distributed and active at almost all locations and depths around Svalbard. Also *Phaeocystis pouchetii* was widespread during the polar night, and blooming from mid to end of May at the ISA station. Heterotrophs such as Marine Stramenopiles (MASTs), Picozoa and the parasitic Marine Alveolates (MALVs) displayed higher relative abundances in winter than in other seasons in Adventfjorden. Strategies such as kleptoplasty and parasitism might have helped certain species (e.g. *Strombidium* sp. and MALVs, respectively) to cope with unfavourable conditions at certain times of the year. Smaller cells (< 10 µm) contributed more to the vertical flux during autumn and winter, possibly due to increased flocculation and ballasting. In contrast, larger and more typical spring bloom taxa (e.g. diatoms) dominated both the water column and the sedimented material in spring. In March an advective event, which replaced cold and less saline Local Water with warm and saline Transformed Atlantic Water was potentially responsible for a change in the IsA community composition.

The combined use of RNA and DNA data was of large benefit when opening the "black box" of the polar night, revealing that the Arctic winter protist communities are active and more diverse than expected. Molecular tools not only revealed novel taxa contributing to the vertical export, but also suggested new mechanisms for vertical export demonstrated by parasite-host induced transport. Together, these results emphasize the extreme seasonality of Arctic microbial communities driven by the environment (e.g. light regime, nutrient availability), but also point to the necessity of a thorough knowledge of hydrography to fully understand their succession, variability and fate.
List of papers

Paper I

Paper II

Paper III
1. Introduction

Microbial eukaryotes can be defined as any microscopic organism with a complex cell (or cells), in which the genetic material is organized into a membrane-bound nucleus (or nuclei) (Caron et al. 2012 and references within), and include microalgae as well as non-autotrophic protists (Poulin et al. 2011). These tiny organisms can be found anywhere on the planet (terrestrial, aquatic, atmospheric), inhabit niches of diverse forms and sizes (Fenchel 1987) and are often found in high cell numbers (e.g. 2.8 x 10^6 cells L^-1 of the haptophyte *Phaeocystis pouchetii* in the Fram Strait, Lasternas and Agusti 2010). Microbial eukaryotes are of critical importance for the functioning of the earth ecosystems. Focusing on the marine ecosystem, microbial eukaryotes have diverse and important roles: (1) As primary producers: global annual net production of approx. 50 petagrams carbon (Chavez et al. 2011); (2) As consumers/predators, e.g. heterotrophic dinoflagellates/ciliates controlling bloom dynamics (Sherr and Sherr 2007, Seuthe et al. 2011); (3) As maintainers of biogeochemical cycles (microbial loop – Azam et al. 1983): several protists are able to transform carbon-, nitrogen-, sulfur- and phosphate-containing compounds to make them available for biological production (Strom 2008, Richardson and Jackson 2007, Worden et al. 2015). Until recently, the biodiversity of marine microbial eukaryotes was mainly studied by microscopy and morphological identification techniques. These techniques largely limited our knowledge of the diversity and role of pico- (0.2–2 µm) and nano-sized (2–20 µm) cells. However, the last decades’ rapid developments in sequencing techniques and molecular genomics have finally given us the tools to charter the true and immense biodiversity of microbial life as well as discovering new taxonomic relationships (e.g. López-García et al. 2001, Moon-Van Der Staay et al. 2001, Moreira and López-García 2002, Dinsdale et al. 2008). Yet, seasonal studies of pico- and nanoflagellates are still limited in the world’s oceans (e.g. Romari and Vaulot 2004, Piwosz and Perenthaler 2009, Terrado et al. 2008 and 2009, Gilbert et al. 2009 and 2012, Sørensen et al. 2012), especially during the polar night period in the Arctic marine systems.

The marine Arctic is a highly seasonally pronounced environment and known to be tremendously productive at certain times of the year (e.g. *in situ* primary production in
spring: 107.87 mg C m\(^{-3}\) d\(^{-1}\) and winter: 13.91 mg C m\(^{-3}\) d\(^{-1}\), in Arctic Ocean surface waters from 1954 – 2007, Matrai et al. 2013). Seasonal variability in e.g. solar radiation, temperature, sea-ice cover and nutrient availability (Walsh 2008, Thomas and Dieckmann 2010) as well as large spatial variations (e.g. different surface types such as open ocean, landmasses and sea ice – Walsh 2008) characterize the habitat and determine the life in this remote and extreme environment.

The ongoing rapid changes in the northern hemisphere, reflected by increasing air temperatures, stronger stratification and a decreasing sea ice cover, will strongly impact the Arctic marine ecosystems (Cottier et al. 2007, Moline et al. 2008, Hoegh-Guldberg and Bruno 2010, Stroeve et al. 2012) and the question arises of how this will affect the annual carbon budget and primary production in the future (Hill et al. 2013, Arrigo and van Dijken 2011). In the northern latitude regions, where marine planktonic cyanobacteria are infrequent, marine microbial eukaryotes are the predominant primary producers (Li 1998, Vincent 2000, Li et al. 2009). Recent studies suggest that changes in the vertical structure of the water column (i.e. stronger stratification) will lead to a shift in the microbial communities, favouring smaller picoplankton cells (Daufresne et al. 2009, Li et al. 2009, Tremblay et al. 2009, Worden et al. 2015) and potentially altering the carbon flux (Li et al. 2009).

Few seasonal studies exist from the Pacific sector / Canadian Arctic, describing the succession of microbial eukaryotes in a limited time period based on molecular analysis (e.g. Terrado et al. 2008: December to May, 2009: November to July, and 2011: March to May, Comeau et al. 2013: March to May, Hassett et al. 2016: January to August). On the Atlantic side, such studies are even rarer (Sørensen et al. 2012: January to June, Piquet et al. 2013: April to June, Metfies et al. 2016: June to August). As the Atlantic influenced Arctic differs from the Canadian Arctic with respect to freshwater supply, stratification gradients and surface nutrients (Bluhm et al. 2015), a different community and successional pattern may exist. This study represent a full years coverage of the microbial eukaryote succession in an Atlantic influenced, but high Arctic fjord, with additional focus on two specific species and on the potential contribution of these small sized organisms to the vertical export.
2. Objectives

The overall aim of this thesis was to investigate the community composition, diversity, seasonality and fate of marine microbial eukaryotes in Svalbard waters. To retrieve a high-resolution year-round dataset from a high-Arctic fjord system, the main focus was on the IsA station during the year 2011 – 2012. The main objectives were:

(I) To describe the seasonal variation in community composition and diversity of marine microbial eukaryotes (0.45 – 10 µm) in a high-latitude fjord system over the course of a year (Paper I, III).

(II) To identify the most abundant taxa (size: >10µm, <10µm), more specifically OTUs (Operational Taxonomic Units), at the IsA station and determine the relationship between the taxa/OTUs and their environment. Special focus was set on advection and sedimentation in the system (Paper I, III).

(III) To compare the presence (who is there?) and activity (are they viable?) of two arctic key-phototrophs, *Micromonas* sp. and *Phaeocystis* sp., in Svalbard waters, especially during the polar night (Paper I, II, III).
3. Background

3.1. Svalbard Arctic archipelago: seasons and processes

The Svalbard Arctic archipelago is located between 74° and 81° North and is characterized by its extreme light regime ranging from four months of darkness in winter (no sunrise from November to February) to a period of 24 hours of light during the midnight sun period (no sunset from April to August). The light regime gradually decreases northwards towards the pole; the polar night is not ‘just dark’ but in fact quite heterogeneous (Fig. 1). The polar night has generally been considered as a period of limited biologic activity. However, recent studies show that several species of plankton are active and even feeding during this period in Svalbard waters (Berge et al. 2009, Kraft et al. 2013, Berge et al. 2015).

Figure 1: The polar night light regime in the Arctic: Civil twilight between the 66°N (polar circle) and 72°N, civil polar night between 72°N and 78°N, and nautical polar night at latitudes above 78°N (illustration from Berge et al. 2015).
Sea ice is another important character of the Arctic, as it plays a crucial role for the ecosystem and the climate (e.g. Søreide et al. 2006, Walsh 2008, Arndt et al. 2009, Thomas and Dieckmann 2010). The extent and thickness of the sea ice has declined dramatically during the last decades (Hoegh-Guldberg and Bruno 2010, Stroeve et al. 2012, Dobricic et al. 2016). This is also affecting the Svalbard archipelago as several fjords on the west coast of Svalbard showed large fluctuations in their seasonal ice-cover (Onarheim et al. 2014, Muckenhuber et al. 2016, Nilsen et al. 2016).

Arctic species and ecosystems are, by nature, finely tuned to the timing of seasonal events and biomass production is characterized by large fluctuations over the year. In the Arctic, the pelagic spring bloom commonly takes place between April and June at latitudes 70 - 80° N (Leu et al. 2011). At this time the production can be several orders of magnitude higher than during low production periods (Sakshaug et al. 2009). The onset and timing of the Arctic spring bloom depends on several factors including light, turbidity, nutrients, trace metals, sea ice break-up and stratification (Smetacek and Nicol 2005, Sakshaug et al. 2009, Hodal et al. 2012). Of these especially light limits the growth of arctic phytoplankton (Sherr et al. 2003, Sakshaug et al. 2009, Hodal et al. 2012). It is proposed that the retreat of sea ice, with delayed freezing and earlier melting and change in snow cover, will have large impact on the timing of ice algal and pelagic blooms (Ardyna et al. 2014, Katlein et l. 2015, Arrigo and van Dijken 2015). Typically the Arctic spring bloom is dominated by diatom species such as Thalassiosira antarctica var. borealis, Chaetoceros socialis and Fragilariopsis cylindrus (von Quillfeldt 2000, Hodal et al. 2012) or the haptophyte Phaeocystis pouchetii (Wassmann et al. 1999, Olli et al. 2002, Sherr et al. 2003, Hodal et al. 2012). Outside bloom periods, during winter, summer (mid June to August) and autumn (September and October), ciliates, dinoflagellates and smaller nanoflagellates often dominate the water column in Svalbard and adjacent waters (Seuthe et al. 2011, Kubizyn et al. 2014).

The vertical particle flux plays a major role in the global carbon cycle by removing carbon from the upper ocean/atmosphere where it was originally sequestered by photosynthetic organisms (biological pump – Volk and Hoffert 1985). Globally more than 10 billion tons of carbon are removed per year by this process (Buesseler and Boyd,
2009). The vertical particle transport (or flux) increases and decreases according to the amount of organic matter produced in the euphotic zone, and also depends on other factors and processes in the water column such as grazing, community composition and depth (Wassmann et al. 2003, Turner 2015). In this context, the fate of the production very much depends on the secondary producer match/mismatch situation (Cushing 1990, Reigstad et al. 2000), since primary producers can either be grazed and recycled in the surface layers or contribute to sedimentation processes (vertical flux) (Wassmann 1998, Turner 2015). Both grazing and sinking are important but competing processes when it comes to the fate of particles and algal cells. The vertical particle flux is often very high at the time when production in the euphotic zone is largest, usually during spring (or ice-edge) bloom occasions (Dore et al. 2008, Thompson et al. 2008, Michels et al. 2008, Lalande et al. 2011, Martin et al. 2011, Rynearson et al. 2013). Diatoms are important contributors to the vertical flux and are frequently observed in sediment traps (Smetacek 1980, Riebesell et al. 1995, Olli et al. 2002, Reigstad et al. 2008). *Phaeocystis* is known for its massive blooms composed of gelatinous colonies. These can also sink out and contribute to the flux (e.g. as part of fecal pellets, Hamm et al. 2001), albeit their contribution to the deep water layers is usually low compared to diatoms (Beaulieu 2002, Reigstad and Wassman 2007). Very little is known regarding the contribution of smaller protist cells (< 10 µm) to the downward flux (Richardson and Jackson 2007). However, aggregates formed by flocculation of dissolved organic carbon, and marine snow (sticky matter of marine organisms) are important for vertical particle transport, and may increase the contribution of smaller plankton cells (Richardson and Jackson 2007, Worden et al. 2015).

3.2. Marine microbial eukaryotes in the Arctic – an overview of major groups

Phylogenetics (the study of the evolutionary relationship among groups of organisms) and the methodical identification and classification of organisms (i.e. taxonomy) are research fields of perpetual change and open gaps (Adl et al. 2012, Burki 2014). The eukaryotic tree of life is continuously growing (Figure 2) and during the last decades 18S rRNA gene surveys (18S rDNA, see 4.2) have unveiled many new lineages and relationships (Adl et al. 2012, Terrado et al. 2009, Monier et al. 2013, Burki 2014). In the
following paragraph some members of the eukaryotic tree with high relevance to the Arctic marine environment are briefly described. The taxonomy used in this study follows Adl et al. (2005 and 2012) with some deviations indicated by references.

**Figure 2:** Eukaryotic tree of life based on phylogenetics (illustration taken from Burki 2014). Dotted lines: uncertain relationships.

Stramenopiles, Alveolata and Rhizaria are combined under the supergroup ‘SAR’ (Burki et al. 2007), which is strictly phylogenetically defined (Burki 2014). Stramenopiles (earlier Heterokontophyta, Baldauf 2000) are distinguished by their “heterokont” (two different shaped) flagella. They include a large variety of multi- (e.g. Phaeophyceae – brown algae) and unicellular algae with a phototrophic lifestyle (e.g. class Bacillariophyceae – diatoms). The class Bacillariophyceae is quite well studied and has a
crucial rule in the arctic spring bloom production (von Quillfeldt 2000, Sakshaug et al. 2009, Hodal et al. 2012) as well as the sea-ice habitat (Horner 1980, Gosselin et al. 1997, Werner et al. 2007, Arrigo et al. 2010). Several (potential) mixotrophic taxa are also found among Stramenopiles and have been reported from Arctic environments: Chrysophyceae (Lovejoy et al. 2002, Rózanska et al. 2008), Dictyophyceae, Pelagophyceae and Raphidophyceae (Poulin et al. 2008). Mixotrophy, the ability to use both phototrophy and heterotrophy to gain energy and nutrients, can be an advantageous strategy during the long polar night or in times of unfavourable conditions (Nygaard and Tobiesen 1993, Jones 1994, Moorthi et al 2009, Bachy et al. 2011, Flynn et al. 2013). A very common and phylogenetically diverse group of purely heterotrophic flagellates within the Stramenopiles is the novel eukaryotic lineages of Marine Stramenopiles (MASTs, Massana et al. 2004 and 2006). MASTs are important picoplankton grazers (Massana et al. 2004 and 2006). Several MAST clades have been identified (Massana et al. 2004 and 2006, Massana and Pedros-Alio 2008) with clades 1, 2, 3, 7 and 8 being frequently found in arctic water and sea ice (Lovejoy et al. 2006, Comeau et al. 2011 and 2013, Thaler and Lovejoy 2014).

Alveolata contain a range of morphologically diverse heterotroph or mixotroph groups such as Dinoflagellata (including Dinophyceae and Marine Alveolates - MALV), Ciliophora and Ampicomplexa. An Alveolata-specific characteristic is the presence of cortical alveoli; a system of vesicles supporting the membrane (Cavalier-Smith 1991). Alveolata play important roles as grazers and parasites in the marine system (Levinsen and Nielsen 2002, Seuthe et al. 2011, Chambouvet et al. 2008, Skovgaard et al. 2009, Gómez et al. 2009), but also have many phototrophic representatives. Alveolata have high rDNA copy numbers compared to other superphyla, and therefore often dominate 18S gene surveys (Zhu et al. 2005, Medinger et al. 2010, Gong et al. 2013). Large Arctic Dinophyceae have been thoroughly investigated by microscope studies (e.g. Okolodkov and Dodge 1996, Levinsen and Nielsen 2002, Seuthe et al. 2011) however small naked genera such as Gymnodinium and Gyrodinium are often difficult to identify to species level, while they are very frequently detected and identified in 18S rRNA studies (Comeau et al. 2011). Uncultivated groups of Marine Alveolates (MALVs, López-García
et al. 2001, Guillou et al. 2008) play an important role as parasites in the sea (Skovgaard et al. 2009, Chambouvet et al. 2008, Noguchi et al. 2013). In the Arctic mostly the MALV Group I and II clades are recovered in genes surveys (Lovejoy et al. 2006, Terrado et al. 2009). Ciliophora are of relevance in the marine food web as both grazers and prey (Levinsen and Nielsen 2002, Levinsen et al. 2000, Turner et al. 2001, Sherr and Sherr 2007, Seuthe et al. 2011). They can vary greatly in size (from 10 µm to 4 mm, Denis 2008) and are diverse in both water (Sherr et al. 1997, Levinsen and Nielsen 2002, Seuthe et al. 2001) and sea ice (Agatha et al. 1993, Bachy et al.2011). Strombidiidae-type ciliates have been reported to dominate 18S surveys in the Arctic (Lovejoy and Potvin 2011). Some Ciliophora possess the ability of kleptoplasty (see below), as found in for instance Strombidium spp. (Stoecker and Silver 1990), Mesodinium rubrum and Loboea stobila (reported in Lovejoy et al. 2002 and 2006, Comeau et al. 2011). Kleptoplasty (Rumpho et al. 2006) enables the protist to use ingested chloroplasts to perform photosynthesis, allowing the host cell to switch between different trophic modes (i.e. mixotrophy) and thus retain activity under both light and dark conditions. Kleptoplasty is also known from several Dinophyceae species (e.g. Dinophysis spp. – Minnhagen et al. 2008, and Gymnodinium spp. – Skovgaard 1998).

Rhizaria primarily contain heterotrophic groups (Burki 2014) and are considered to be important Arctic predators of phytoplankton (Lovejoy et al. 2014). Rhizaria includes three main groups; Cercozoa, Foraminifera and Radiolaria (Moreira et al. 2007); which are based on molecular characteristics (e.g., Keeling 2001, Archibald et al. 2003, Nikolaev et al. 2004, Bass et al. 2005, Burki and Pawlowski 2006, Burki et al. 2010, Brown et al. 2012, Sierra et al. 2013). Rhizaria vary in form but a large part of them are naked and testate amoeboid with filose or reticulose pseudopods (Burki 2014). Radiolaria, such as the classes Polycystinea and Acantharia as well as phylum Cercozoa, are often found in Arctic 18S libraries (Lovejoy et al. 2014). Cryothecomonads from the Cercozoa group are frequently found in Arctic waters (Thaler and Lovejoy 2012 and 2014). Recent discoveries indicate that they may play an essential ecological role in the sea ice habitat (Comeau et al. 2013).

Opisthokonta are mainly heterotrophs characterized by a uniflagellated (zoo)spore phase.
The kingdoms of fungi and animals belong to this group as do the bacterivorous choanoflagellates. Parasitic/saprophytic Chytridiomycota have been found to dominate marine fungal communities in the Arctic (Terrado et al. 2008, Hassett et al. 2016) and have recently been discovered to also infect sea ice algae (Hassett et al. 2016). Knowledge on choanoflagellates in the Arctic is still limited, but they have been suggested to be diverse on a pan-arctic scale (Lovejoy 2014).

Archaeplastida include red and green algae, as well as land plants, and the unicellular group of Glauco phytes. The class Chlorophyceae includes the globally distributed small (ca. 2 µm) green algae *Micromonas pusilla* with several strains identified (Slapeta et al. 2006). One strain (strain CCMP2099, *Micromonas Arctic*) has only been isolated from Arctic waters (Lovejoy et al. 2007) and is capable of phagotrophy (McKie-Krisberg and Sanders 2014).

The phyla Crypto- and Haptophyta together form the potentially monophyletic group Hacrobia (Sakaguchi et al. 2009, Okamoto et al. 2009), with cells typically having two unequal flagella. Both phyla include mainly plastid-bearing phototrophs, but also some hetero- and mixo-trophic species. Several algal species belonging to Haptophyta have ecological importance as they can form large (toxic) blooms and have significant impact on the biogeochemical cycles (e.g. Wassmann et al. 1990, Verity and Smetacek 1996, Reigstad and Wassmann 2007, Schoemann et al. 2005, and refs. therein). The single-cell or colony-living *Phaeocystis pouchetii*, for instance, can dominate microbial eukaryote communities in the Arctic (Olli et al. 2002, Sherr et al. 2003, Olli et al. 2007, Lovejoy et al. 2007).

Hacrobia also include heterotrophic flagellates belonging to Telonemia, Picozoa (previously named picobiliphytes, Not et al. 2007 and Seenivasan et al. 2013) and Katablepharidae, although their true phylogenetic relationship is so far poorly understood (Burki et al. 2009, Zhao et al. 2012). These flagellates are found in low to high abundance in arctic marine 18S surveys (Monier et al. 2013, Thaler and Lovejoy 2015). The phylum Telonemia has a cosmopolitan distribution, is often found in surface waters in the Arctic (Shalchian-Tabrizi et al. 2006 and 2007, Monier et al. 2013) and has also
previously been found in Svalbard waters (Shalchian-Tabrizi et al. 2007, Bråte et al. 2010). It has been suggested that Telonemia, due to its frequent occurrence in both marine and brackish water, may play an important ecological role (Vørs 1992, Lee and Patterson 1998, Shalchian-Tabrizi et al. 2007). Picoplankton-sized (< 3 µm) Picozoa have been measured in high abundance in certain oceanic regions (up to 30% in tropical eddy-influenced surface waters; Seenivasan et al 2013). Picozoa comprise (at least) three subclades with Arctic representatives (Lovejoy 2014). It is hypothesized that Picozoa, based on their ultra-structure (Seenivasan et al. 2013), may feed on TEP (transparent exopolymers; Riedel et al. 2006) in the Arctic, making them potentially important in sedimentation processes.
4. Approach

4.1. Study area and sampling program

Study area

The Svalbard Arctic archipelago is surrounded by the Arctic Ocean to the north, the Fram Strait to the west and the Barents Sea to the east and south. Spitsbergen, the largest island of the Archipelago, is a unique area to study the Arctic marine ecosystem, as it is easily accessible thanks to the infrastructure in Longyearbyen, the islands main town. The archipelago is influenced by different watermasses transported by the West Spitsbergen Current (WSC) and the East Spitsbergen Current (ESC) (Ingvaldsen and Loeng 2009, Fig. 3). The WSC is an extension of the Gulf Stream system that transports warm and saline Atlantic water (AW) on the western coast of Spitsbergen, while the eastern coast is influenced by colder and less saline water (Arctic Water - ArW) from the Arctic Ocean which is transported with the ESC (Ingvaldsen and Loeng 2009). Due to the warm WSC, the climate on Spitsbergen is relatively mild compared to similar latitudes in other regions of the Arctic (Cottier et al. 2005).

The west coast of Spitsbergen is characterized by several fjord systems which are potentially good indicators for environmental changes due to the alternating inflow of different watermasses and changing conditions (i.e. Atlantic, Arctic, brine- and freshwater inputs) (Nilsen et al. 2008). Isfjorden, the largest fjord system on the west coast, is directly influenced by warm WSC water as it is open to the shelf and its mouth does not have a sill (Nilsen et al. 2008). Isfjorden is a very shallow system with more than half of the area having a depth less than 100 m (Nilsen et al. 2008). The Isfjorden system includes several smaller fjords and fjord systems including Billefjorden in the north east and Tempelfjorden and Adventfjorden on the southern side. Earlier efforts to study the biological processes in the Isfjord system have mostly been concentrated on the sill fjord Billefjorden (e.g. Arnekvæn et al. 2005, Sørensen et al. 2012, Grigor et al. 2014). However, Adventfjorden is much more easily accessible from Longyearbyen (Fig. 3) and this proximity makes high resolution temporal sampling possible.

Adventfjorden is a ~7 km long and ~5 km wide fjord with depths of < 100 m, and does
not have a sill. ArW formed locally or advected with the coastal current originating from the ESC normally occupies Adventfjorden, but seasonal (late summer and fall) and occasional (winter) inflow of warm and saline AW from the WSC to Isfjorden influences the area (Nilsen et al. 2008, Cottier et al. 2007). Adventfjorden has mostly been free of sea ice since 2007 (www.met.no; ice-free: 2006 – 2007, 2010, 2012 – 2014). During summer and autumn glacial run-off (Advent River, Longyear River, Fig. 3) affect the fjord by substantial input of sediment-loaded freshwater (Węsławski et al., 1999).

Sampling program

The temporal sampling for Paper I and III was centered around the Adventfjorden time series station (IsA) established during autumn 2011 in Isfjorden, at the mouth of Adventfjorden close to Longyearbyen (N 78°15.6, E 15°31.8), while spatial sampling for Paper II took place at multiple locations around Svalbard (Fig. 3). A moored observatory located at IsA, which included two CTDs, light sensors, temperature loggers and an ADCP, provided us with a unique opportunity to continuously monitor the marine environment. The IsA station was sampled bi-/weekly from December 2011 to June 2012, and biweekly to monthly from July to December 2012 (Paper I, III). Spatial sampling (fjords and open deep water) took place between December 2008 and January 2013 (Paper II).
Figure 3: Sampling area. Left: The Svalbard Archipelago with the location of the sampling stations for Paper II. The West Spitsbergen Current (WSC) and the East Spitsbergen Current (ESC) are indicated. R3: Rjpfjorden; BAB: Billefjorden Adolfbukta. Right: Detailed map of Adventfjorden showing the position of the IsA time series station. Glacial run-off into the fjord from Longyear and Advent Rivers indicated with lines. The bathymetric data are from the International Bathymetric Chart of the Arctic Ocean (IBCAO version 3, Jacobsen et al. 2012).

The Adventfjorden sampling and analyses were done in collaboration with other marine scientists from UNIS, UiT – The Arctic University of Norway, University of Bergen (UiB) and Institute of Oceanology Polish Academy of Science (IOPAS, Poland), providing a better understanding of the system as a whole. The sampling campaign was part of the UNIS MicroFun project (http://www.mare-incognitum.no/index.php/microfun).
At each sampling date additional environmental data from a vertical CTD profiler was obtained and light measurements were performed. Seawater was collected with a Niskin bottle from four standard depths (5 m, 15 m, 25 m, 60 m) at IsA, and a combination of other depths at the other stations (5, 15, 35, 60, 75, 150, 500 and 2290 m; see Paper II). The standard depths for IsA were decided based on the placement of the mooring CTDs (25 and 65 m). Additionally short-term sediment traps were deployed (20, 30, 40, 60 m) at seven time-points during 2011 – 2012 (Paper III).

Collected seawater was used to analyze the community composition, amplified from DNA, and the active community, amplified from RNA, of microbial eukaryotes (0.45 – 10 µm, > 10 µm). Additional water samples were used to determine particulate carbon and nitrogen (POC/PON), nutrients (N, P, Si) and fractionated Chl a biomass (> 0.7 µm or > 10 µm) according to standard protocols. Short-term sediment traps were used to investigate the potential contribution of the microbial eukaryote community to the vertical flux, and to evaluate their fate. Water collected from the sediment trap was likewise analyzed for DNA, fractionated Chl a, POC/PON and particle sizes.

4.2. Molecular analysis, data processing and statistics

The 18S ribosomal RNA (rRNA) is part of the small subunit (40S) of the ribosome, a complex which is responsible for protein biosynthesis in all living cells. The genes that encode the rRNA are called ribosomal DNA (rDNA). 18S rRNA is a basic component of all eukaryotic cells and investigations of the 18S gene have revolutionized the field of phylogenetics. The 18S rRNA gene is easy to amplify because it exits in multiple copy in each cell, and contains conserved and variable (V) regions enabling the study of evolutionary relationships through time (Lovejoy et al. 2007, Amaral-Zettler et al. 2009, Burki 2014). Usually primers are designed to bind to the conserved regions in order to amplify the variable regions.

In the study I and III (Papers I, III) Roche 454-pyrosequencing (see BOX 2) was performed using universal eukaryotic primers (designed by Comeau et al. 2011) targeting the V4 hypervariable region of the 18S rDNA. An end-point PCR assay with species-
specific primers was used to identify the key phototrophs (*Micromonas* and *Phaeocystis*) in Paper II. The PCR products of both species were verified with Sanger sequencing (Sanger and Coulson, 1975). While PCR screening with species-specific primers is a quick tool to identify target species in environmental samples, 454-pyrosequencing with universal primers allow for chartering the total diversity in the communities. Because 454-pyrosequencing is prone to a higher error rate compared to traditional Sanger sequencing (Huse et al. 2007, Balzer et al. 2011) the sequence reads should be clustered at a level of sequence similarity relevant for the target gene and species. After clustering the 454-sequence reads at different similarity levels (97%, 98% and 99%), clustering into operational taxonomic units (OTUs) was done using the less strict similarity level of 97% to avoid inflating the OTU richness of the samples. OTU clustering is the most frequent used diversity unit to characterize microbial taxa (Schmidt et al. 2014). To simplify the text, the word species is used instead of ‘OTU’ in the results and discussion chapters of this thesis.

Diversity estimations, statistical tests and multivariate analyses were performed after normalization of the samples to an even sequencing depth in R (R version 3.2.1, R Core Team 2015). The use of frequently applied diversity indices (species richness, Shannon-Wiener and Pielou’s Evenness, described in BOX 1) allowed comparison to available literature. Statistical tests and multivariate analyses were always performed with three types of data to ensure the reliability of the results: raw data (total sequence reads), unweighted data (presence/absence of individual OTUs in each sample) and log10(n+1)-transformed data. After finding that there were no large differences between the three outcomes, transformed data were presented to down-weight the influence of highly abundant OTUs in Paper I and III.
BOX 1: BIODIVERSITY

In the Convention on Biological Diversity (UN Conference on Environment and Development, Rio de Janeiro, 1992, Convention on Biological Diversity, Article 2), biodiversity is defined as follows:

“ ‘Biological diversity’ means the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems. ”

Different concepts have been assessed to describe diversity in more detail, as for instance in Norse et al. (1986) where they describe three levels of diversity: genetic (genetic variability within species), species (species numbers) and ecological (community) diversity. Species diversity is also often considered part of ecological diversity, in which species richness, the number of species in a community, is assessed. Biodiversity varies greatly across the Earth, and between its different regions and habitats and there are a variety of diversity indices to estimate that. A few selected very commonly used diversity indices will be shortly presented here (Clarke and Warwick 2001):

Species richness (S) is a count of the total number of species in a sample. It depends on the sample size and thus large sample sizes tend to have higher species richness compared to small sample sizes.

The Chao 1 ($S_{\text{chao1}}$) diversity index (Chao, 1984) is very frequently used, and is based on species richness but takes into account the number of rare classes (i.e. OTUs) found in a sample:

$$S_{\text{chao1}} = S_{\text{obs}} + n_1(n_1-1)/2(n_2+1)$$

with the observed number of species ($S_{\text{obs}}$), and $n_1$ as the number of OTUs with only one sequence (i.e. singletons) and $n_2$ the number of OTUs with only two sequences (i.e. doubletons).

Also very frequently used is the Shannon-Wiener ($H'$) diversity index:

$$H' = -\sum p_i \log(p_i)$$

where $p_i$ is the proportion of the total count (or biomass) estimated by the $i$th number of species. Since $H'$ can be sensitive to sampling effort, it should be used only to compare samples with equivalent sampling designs.

Pielou’s evenness ($J'$) is a commonly used index for the equitability or evenness in a sample. It measures how evenly distributed the individuals are among different species:

$$J' = H'/H'_{\text{max}} = H'/\log S$$

$H'_{\text{max}}$ is the maximum possible value of the Shannon-Wiener index meaning that which would be achieved if all species were equally abundant (i.e. $\log S$).
4.3. High-throughput sequencing – a useful challenge

The recent developments in sequencing technologies have revolutionized the field of molecular genomics. From the traditional Sanger approach where single amplicons are sequenced one at a time (one tube one reaction), the current high-throughput sequencing (HTS) approaches perform parallel runs of millions of sequencing reactions at once, thus allowing for the simultaneous sequencing of amplicons from all species in e.g. environmental samples. Different HTS technologies have been developed during the last two decades, and due to the increased read length compared to other technologies (e.g. Illumina, Illumina Inc.), we chose to use Roche 454 pyrosequencing technology (BOX 2) for this project.

Along with the wealth of sequencing information obtained by HTS come limitations and potential errors that are important to be aware of. From starting in the field (e.g. varying field protocols for DNA and RNA sampling and filtration), and continuing in the molecular laboratory (e.g. contaminations, RNA instability, primer choice and bias, sequencing errors, introduction of chimeric sequences), errors can be introduced to the data and these needs to be dealt with (Huse et al. 2007, Balzer et al. 2011). HTS itself can produce errors in the sequence reads that potentially generate incorrect phylotypes with the consequence of inflated OTU richness (Huse et al. 2010, Behnke et al. 2011). Another challenge is to correctly assign the different OTUs to the correct species; in many cases this is not possible, as most of the available databases are still incomplete compared to the tremendous marine microbial diversity. Further, since the OTU clustering can be run with different methods and programs (e.g. hierarchical clustering algorithms in mothur, Schloss et al. 2009) and is strictly defined by the users constraints (e.g. similarity level used), results between different studies can be inequivalent (Schmidt et al. 2014). The effects of these combined errors and challenges can be reduced by the use of bioinformatic pipelines to trim the dataset and improve assignments (e.g. QIIME - Carporaso et al. 2010, mother - Schloss et al. 2009, UCHIME - Edgar et al. 2011), as reviewed by several authors (e.g. Huse et al. 2010, Quince et al. 2009 and 2011, Nilsson et al. 2011, Schloss et al. 2011, Nguyen et al. 2014, Tedersoo et al. 2015). The comparison of libraries prepared from extraction of DNA versus RNA (by sequencing
cDNA reverse-transcribed from rRNA) are expected to differ in the identified OTU richness and relative read abundances of different OTUs because the libraries prepared from RNA extracts are expected to give higher relative read abundances of active taxa. In contrast, libraries prepared from DNA extracts may amplify DNA also from dead and dying cells as well as extracellular DNA (Egge et al. 2014, Logares et al. 2014). The combined use of both molecules thus give valuable insights into the species present in an environment compared to the ones that are active at the time of sampling (Logares et al. 2014). Environmental sequencing of rRNA/cDNA can also give an impression of the activity and growth rates of protists (Dortsch et al. 1983, Dittami and Edvardsen 2012, Logares et al. 2014). A potential source of error using the 18S rRNA or its gene product (rDNA) lies in the different gene copy numbers (Zhu et al. 2005, Not et al. 2009, Medinger et al. 2010, Gong et al. 2013) and different rRNA content per cell among taxa (Dittami and Edvardsen 2012). Thus, the relative abundances of sequence reads has to be interpreted with these caveats in mind.

With potential methodological caveats in mind, HTS has revealed an amazing diversity of marine microbes, with the identification of to date unknown phylogenetic diversity (Massana et al. 2002, Moreira and López-Garcia 2002, del Campo et al. 2016). So, how can we compare the HTS data with species identification based on traditional tools? We know that one DNA read ≠ one individual, so how do results from HTS studies compare to for instance similar surveys based on light microscopy? A freshwater study by Xiao et al. (2014) compared the use of light microscopy with 454 sequencing to investigate the phytoplankton composition. The results of the two methods were not always in agreement. While HTS identified more of the rare species and especially pico-sized organisms (i.e. revealing higher diversity), light microscopy was better at identifying to a higher taxonomic level (e.g. species level) (Xiao et al. 2014). All in all, the benefit of HTS lies rather in in the large amount of provided information of protist communities in large samples and the short time and low cost of processing the data (Lindahl et al. 2013).
BOX 2: 454 PYROSEQUENCING

Pyrosequencing is one of several high-throughput sequencing technologies (and the main technique used in Paper I and III). It is based on the “sequencing by synthesis” principle by detection of a pyrophosphate release after nucleotide incorporation (Ronaghi et al. 1998), and distinguishing the procedure from Sanger sequencing where the order of nucleotides is detected by dideoxynucleotides terminating the chain reaction (Sanger and Coulson 1975).

In 454 pyrosequencing DNA is amplified inside water droplets floating in an oil solution (i.e. emulsion PCR). Each of these water droplets contain a single DNA template that is immobile and surfaced to a single primer-coated bead that then forms a clonal colony (i.e. genetically identical replicates). Beads are then loaded onto a picotiter plate for sequencing with only one bead per well. The plates are flushed with one deoxynucleotide, i.e. A, C, G or T nucleotide at a time, and when the DNA polymerase incorporates the complementary dNTP onto the template, pyrophosphate is released, converted to ATP, and acts as a substrate for the luciferase enzyme which generates a visible light signal when oxidising luciferin. The light signal of each dNTP incorporation is proportional to the number of dNTPs that were incorporated, and thus the identity and number of nucleotides added to the growing strain are detected. Sequencing errors are more prone to occur across homopolymers (i.e. sequences of identical bases: AAAA) with erroneous interpretation of the strength of the light signal, and as the homopolymer length increases, it becomes more difficult to accurately quantify the flash of light. This potential error source is mostly dealt with bioinformatically.
5. Summary of results

5.1. Paper I: Seasonal variation in community composition and diversity of microbial eukaryotes at the high-arctic IsA station

Sampling was conducted over the course of one year (26 sampling days) at the IsA time series station to assess the community composition and diversity of microbial eukaryotes (size: 0.45 to 10 µm) from a depth of 25 m by 454 pyrosequencing. The results showed that community composition as well as diversity of microbial eukaryotes was strongly influenced by season. Two large shifts in the composition were observed, during early spring and in summer/autumn, with a potential assemblage resetting the following winter. Multivariate analyses indicated that the changes in community composition throughout the year corresponded to seasonal events (e.g. increased light regime) rather than hydrography. The winter and early-spring communities were more diverse than the spring and summer/autumn communities. Dinophyceae, especially *Gyrodinium helveticum* and *G. fusiforme*, were predominant in both the DNA and RNA libraries throughout the year. *Micromonas Arctic* was abundant mostly in the early-bloom and autumn periods, whereas heterotrophs such as marine stramenopiles (MASTs) and Picozoa, and the parasitoid marine alveolates (MALVs), displayed higher relative abundances in the winter than in other seasons.

5.2. Paper II: Distribution of *Micromonas pusilla* and *Phaeocystis puchetii* during the polar night in Svalbard waters

The presence and depth distribution of the two phototrophs *Phaeocystis puchetii* (Haptophyceae) and *Micromonas pusilla* (Mamiellophyceae) was investigated during the civil polar night. Samples from around Svalbard were used, representing many locations with different characteristics (open water – fjord, ice-covered – ice-free, shallow – deep, Atlantic – Arctic Water). PCR screening with species-specific primers was applied to overcome the difficulties of identifying small flagellates in low-biomass winter samples. It was possible to detect phototrophic biomass (Chl *a*) even during the polar winter, but concentrations were very low. The investigations indicated that both *Phaeocystis*
*pouchetii* and *Micromonas pusilla* were widespread during the polar night in Svalbard waters as they were detected in nearly all locations at different depths (e.g. Rijpfjorden at 240 m). RNA samples from some of the stations suggested that most of the cells were actually viable.

5.3. Paper III: Hydrography, sedimentation and the fate of pelagic protists

The fate of marine pelagic protists and their contribution to the vertical flux was investigated at the IsA station by 454 pyrosequencing. Samples were collected during seven sampling events in winter, spring and autumn from December 2011 to September 2012. Protists (> 0.45 µm) were sampled from short-time sediment traps (depths: 20, 30, 40, 60 m) as well as from the water column (0.45 – 10 µm and >10 µm, depths: 5, 15, 25, 60 m). Hydrographic profiles, which were obtained regularly during the whole period, indicated that the shallow Adventfjorden was vertically homogeneous, except during the stratified summer. An advective event during winter, replacing cold and less saline Local Water (LW) with warm and saline Transformed Atlantic Water (TAW), co-occurred with a shift in the community composition of small protists in March. The community composition and the diversity within the suspended and exported material showed strong seasonal changes during 2011-2012. Smaller protists (< 10µm) dominated the exported material during autumn and winter with small taxa such as Marine Alveolates (MALVs) and Dinophyceae predominating. In contrast, larger and more typical spring bloom taxa (e.g. diatoms) were dominant in the water column and sunk into the traps during spring. Species showed very contrasting patterns: *Gyrodinium fusiforme* and *G. helvetica* were dominant in the water column and in the traps throughout the year, while several Choanoflagellida and Dinophyceae strains were not abundant in the exported material at all. Interestingly, some parasitic species, such as MALV 1a and *Chytriodinium* were abundant in the trap but not found in the water column. Small-sized metazoans (e.g. copepods and benthic larval stages) were also abundant in the exported material. Whether they were actually contributing to the passive downward flux or actively migrating into the traps remained unclear.
6. Discussion

This study is, to my knowledge, the first whole year seasonal 18S-gene study of arctic microbial eukaryotes (< 10 µm) performed in a high-resolution perspective at 78°N. The IsA station showed strong seasonal shifts (2011 – 2012) in the community composition and diversity of microbial eukaryotes as well as downward flux contribution reflecting the extreme seasonality of the Arctic (Fig. 4).

6.1. Marine microbial eukaryotes in the Svalbard Arctic display strong seasonality

Winter

The unique discovery of the high winter diversity (see species richness; Fig. 4, Paper I, III) in this high-Arctic fjord system (cf. sea-ice protists in the Canadian Arctic - Niemi et al. 2011), contradicts with the classic Arctic paradigm that implies only negligible biological activity during the polar night (Paper I – III, cf. Berge et al. 2009, Kraft et al. 2013, Blachowiak-Samołyk et al. 2014, Berge et al. 2015). The relatively high read abundance of heterotrophs such as MAST, Picoza, Choanoflagellida and the parasitic MALV (both group I and II) during fall and winter compared to spring is probably a response to the Arctic winter where the absence of light restricts the phototrophic organisms (Paper I, III). Thus, these results give new insights into the assemblages of the smallest protists in the Arctic, where studies based on microscopy only are able to identify “unidentified nanoflagellates” (e.g. Sommer et al. 2005, Werner et al. 2007, Iversen and Seuthe 2011, Kubiszyn et al. in revision). Interestingly these small protists seemed to play an important role for the vertical particle flux during winter (and autumn) (Fig. 4; Paper III), possibly due to increased flocculation and ballasting processes (Olli and Heiskanen 1999, Richardson and Jackson 2007, Wiedmann et al. 2016).

The phototrophic biomass was also detectable during the winter months and was dominated by cells < 10 µm (Paper I – III), as has been observed in Kongsfjorden (Iversen and Seuthe 2011). The Svalbard winter communities also included key phototrophs (e.g. Micromonas and Phaeocystis, Paper II), and the RNA libraries showed that they were active most of the time. There are two known overwintering strategies for
phototrophs: (1) Keeping viable persisting stocks of cells throughout the winter, which may be important when the conditions change to more favorable ones (Paper II) and consequently for seeding the next spring bloom (Lewis et al. 1999, Niemi et al. 2011, Błachowiak-Samolyk et al. 2014). (2) To produce resting spores which then settle into the sediments and are brought up with vertical mixing in the water column in spring (Eilertsen et al. 1995, Hegseth et al. 1995, Brown et al. 2013). Kubiszyn et al. (in revision) could not confirm the presence of any vegetative forms of typical bloom-seeding phototrophs during winter at IsA. However the DNA and RNA libraries in this study (Paper I) do not agree with that, as for example Skeletonema sp. and Thalassiosira spp., possibly as resting spores (cf. Eilertsen et al. 1995, Błachowiak-Samolyk et al. 2014), and Chlorophyta besides M. pusilla, were found in low read abundances (i.e. they were present and alive) even during the dark winter months. These taxa increased in relative read abundances in early spring (March), in a similar fashion to Phaeocystis sp. and M. pusilla. These findings are pushing the need for more polar night studies to potentially uncover new species and life strategies of those night survivors.

Winter-spring transition

The return of the sun above the horizon correlated with an increase in the estimated Chl a biomass, increased contribution of phototrophs such as Bacillariophyceae, Chlorophyta and Haptophyta and a reduction in the relative proportion of heterotrophs (Fig. 4; Paper I, III), a common finding in Arctic areas (Terrado et al. 2009, Tremblay et al. 2008, Terrado et al. 2011, Iversen and Seuthe 2011). At IsA, the community changes seen in the early spring (early March) were potentially a combination of ongoing advective processes (Paper III) as well as a seasonal response to changes in the environment (among others: increased solar radiation and nutrient availability, Paper I). Also in Adventfjorden Micromonas constituted a higher importance in the pre-bloom phase as reported previously from Arctic waters (Lovejoy et al. 2007, Terrado et al. 2008, Sørensen et al. 2012, Nordgård 2014) and that in turn agrees with the theory that the viable winter cells were “just waiting” for better conditions to seed (Paper I, II). Due to its small cell size (i.e. high surface:volume ratio; Key et al. 2010) Micromonas is
potentially more efficient in nutrient uptake than large protists (> 20 µm) and has lower requirements.

Spring
The IsA spring communities were less diverse and more uneven compared to winter communities (Fig. 4), and included common arctic spring bloom species (agreeing with Kubiszyn et al. in revision) that were abundant in both the small and large size-fractions of the suspended samples (Paper I, III). Interestingly, the seasonal diversity changes (i.e. high in winter, low in spring) of small eukaryotes identified with 454-sequencing did not agree with the results of Kubiszyn et al. (in revision). Kubiszyn et al. (in revision) encountered high species richness during the spring months at IsA and rather less diverse winter communities. There are two potential technical explanations for this disagreement; for one, winter samples are very dilute and a 200 ml water sample (as analysed by Kubiszyn et al. in revision) is possibly not enough to cover the full diversity of winter protists; and secondly, the taxonomic knowledge of Arctic spring bloom species is much better compared to that of winter species - which often include high numbers of those “unidentified nanoflagellites”.

Two phototrophic key groups, Bacillariophyta and Haptophyta, dominated the spring bloom at IsA (April to May, Paper I, III, cf. Kubiszyn et al. in revision) and they were also the main contributors to the vertical flux during that time (Paper III), showing a change of the vertical flux from small to large cells. The succession occurred in a fairly well-mixed water column which persisted most of the year apart from the more stratified summer months (Paper I, III) contradicting the Sverdrup paradigm regarding a need of stratified conditions (Smetacek and Passow 1990, Eilertsen 1993) for the spring bloom to occur. Blooming events in the presence of an unstratified or disrupted water column are not abnormal and have been reported earlier from temperate and cold waters fjord systems (e.g. Norwegian fjords – Eilertsen 1993, Kongsfjorden - Seuthe et al. 2011 and Hodal et al. 2012). Heterotrophic/mixotrophic taxa and notably sequences with best matches to the dinoflagellate Gyrodinium spp. and the ciliate Strombidium (especially in the RNA libraries) were common in spring in Adventfjorden (Paper I) and Kongfjorden
(Seuthe et al. 2011) as well as in the Canadian Arctic (Terrado et al 2011, Comeau et al. 2011).

Summer/Autumn
The reestablishment of the microbial community at the end of the year 2012, in terms of diversity estimates and community composition (Paper I) hints at a re-occurring seasonal pattern, although more data is needed to validate that (Fig. 4). Interestingly, in autumn, an intensive sedimentation event was observed at IsA (770-1530 mg POC m$^{-2}$ d$^{-1}$, Wiedmann et al., 2016) that co-occurred with sediment-loaded glacial run-off (Wiedmann et al. 2016). Possibly due to this event and concurrent increased flocculation and ballasting processes (Wiedmann et al. 2016), the contribution of smaller eukaryotes increased again in the trap material in September (Paper III). Besides the increased flocculation and ballasting processes during summer and autumn, river meltwater potentially brung putative freshwater species into Adventfjorden, which is evidenced by increased reads of different Telonemia species (June – August; cf. Kongsfjorden - Bråte et al. 2010).

This is the first study that has revealed seasonal patterns of different taxa according to a series of snapshots, even during the dark polar night, by use of combined DNA and RNA libraries in the Atlantic influenced Arctic. The uniqueness and importance of time-series data is unquestioned, as these are needed in order to further evaluate the stability of seasonal successions (cf. Gilbert et al. 2012) as well as their main biotic and abiotic drivers (cf. Kim et al 2014).
Figure 4: Illustration of the seasonal succession of marine microbial eukaryotes at the IsA station during 2011 – 2012 (based on Paper I and III). a: *Micromonas pusilla*, b: heterotrophic flagellates (e.g. MASTs, MALVs, Picozoa), c: Dinophyceae spp., d: Ciliophora spp., e: single cells and colonies of *Phaeocystis pouchetii*, f: Bacillariophyceae spp., g: aggregates.
6.2. Dominant Svalbard protists in a pan-Arctic perspective

The number of 18S surveys of smaller eukaryotes (< 20 μm) has increased tremendously in the last years (e.g. Massana et al. 2004, Kilias et al. 2014, Thaler and Lovejoy 2015), making it easier to assign taxonomy to OTUs and maybe to identify potential key-species. I defined a “key-species” in this study, as a species that is abundant in a period of time and which hence may play an important ecological role in the system.

*Micromonas and Phaeocystis: true arctic survivors - present all-year around*

This study reported the presence of viable life stages of the two key phototrophs, *Phaeocystis pouchetii* and *Micromonas pusilla* during the polar night in Svalbard waters (Paper I – III), and hence supports the significant role of pico- and nanoplankton as Arctic key-phototroph substituting for cyanobacteria, which are scarce in the northern regions (Li 1998, Vincent 2000, Li et al. 2009). While these two flagellates are truly widespread in Arctic waters (Svalbard – Paper II, Eilertsen et al. 1989, Barents Sea – Wassmann et al. 1990, Thronsden and Kristiansen 1991, Central Arctic Ocean - Sherr et al. 2003, Metfies et al. 2016, Fram Strait – Kilias et al. 2013 and 2014, Canadian Arctic – Lovejoy et al. 2007, Terrado et al. 2008 and 2011), winter reports of these two key species are still rare (Northern Norway - Thronsden and Heimdal 1976, Arctic - Sherr et al. 2003, Iversen and Seuthe 2011, Bachy et al. 2011), often due to logistical constraints. The Svalbard polar night DNA/RNA libraries revealed that these two phototrophs are able to survive unfavorable conditions without daylight (Paper II) and may overwinter in the pelagic, and go on to seed the spring bloom as discussed in 6.1 (Paper I, II; cf. Sørensen et al. 2012). However, resting stages (for *Phaeocystis*: Hegseth and Tverberg 2013, Gaebler-Schwarz et al. 2010) or alternative trophic abilities, e.g. phagotrophy as recently reported for *Micromonas pusilla*, (Gonzales et al. 1993, Sanders and Gast 2011, McKie-Krisberg and Sanders 2014) are alternative ways of surviving. Independently of the sampling location and watermass characteristics, most of the *Micromonas* sequences in this study were assigned to the arctic subclade *Micromonas CCMP2099* (Paper I – III: *Micromonas* Arctic; Lovejoy et al. 2007). This was in contrast with Metfies et al. (2016) who found the Arctic subclade abundant only in polar waters, while *M. pusilla*
Clade C was predominant in Atlantic water. The predominance of *Micromonas* Arctic in the strongly Atlantic water influenced Svalbard archipelago possibly indicates that the arctic subclade is able to cope with changes in hydrography.

**Picozoa – contributing to the vertical export**

Picozoa (ca. 1-3 μm, previously called Picobiliphyta, Seenivasan et al. 2013) were frequently observed in Adventfjorden, especially in the winter samples, and seem to have a broad distribution in the Arctic (Thaler and Lovejoy 2015 and references within). One species in particular, Picobiliphyta-strain5 (*Paper I, III*), was identified as abundant over the course of a year in the pelagic as well as in the sediment traps (*Paper I, III*). The same species was also frequently found in the Canadian Arctic and to a much lesser degree in the Chukchi Sea (Thaler and Lovejoy 2015). Thaler and Lovejoy (2015) discussed possible diets of Picozoa and suggested that these flagellates have broader diets compared other heterotrophic nanoflagellates thus enabling Picozoa to inhabit different regions and depths competitive advantage. This enables these species to inhabit different regions and depths (i.e. a broad distribution, Thaler and Lovejoy 2015). Hence, this may explains their predominance during the polar night at IsA when there is strong food limitation.

**Alveolata – the kings of Adventfjorden**

Alveolata were the most abundantly represented group at IsA during the study year (*Paper I, III*) and are often dominant in 18S studies (Terrado et al. 2011, Comeau et al. 2011, Lovejoy and Potvin 2011). We excluded the fact that the dominance in this study (*Paper I*) was due to only high rDNA gene copy numbers that often prevail in Alveolata (Zhu et al. 2005, Medinger et al. 2010, Gong et al. 2013), as DNA and RNA libraries presented chiefly the same patterns.

The two Dinoflagellata species assigned to *Gyrodinium fusiforme* and *G. helvecccium* were ubiquitous in the IsA samples (small and large size fractions, and exported samples; *Paper I, III*) and in several locations around the Svalbard archipelago (Carrier 2016, Seuthe et al. 2011). Whereas *G. helvecccium* is originally described from freshwater, it
forms a single clade with the marine *G. rubrum* (Takano and Horiguchi 2004, Lovejoy et al. 2006), and assignments to both these taxa are commonly found in analyses of Arctic marine samples. *Gyrodninum* spp. have a widespread distribution in the Arctic (Barents Sea – Rat’kova and Wassmann 2002, Canadian Arctic – Comeau et al. 2011, Greenland Sea – Richardson et al. 2005, Central Arctic – Bachy et al. 2011, Kilias et al. 2014). An important *Gymnodinium* species at IsA was *Gymnodinium* sp. strain 7 which was observed during the polar night in the Central Arctic under the sea ice (Bachy et al. 2011) but in contrast, also in Atlantic water in the Scotian Shelf (Dasilva et al. 2014), thus the Atlantic influenced Svalbard archipelago seems to be a suitable habitat for this species. Many of the *Gyrodninum* and *Gymnodinium* species have a heterotrophic (or mixotrophic) lifestyle (Levinsen and Nielsen 2002) with a range of potential prey items (Sherr et al. 1989, Johansson et al. 2004, Aberle et al. 2007, Sherr and Sherr 2007, Jeong et al. 2010), but in turn dinoflagellates are also important prey for higher trophic levels (Rysgaard et al. 1999, Seuthe et al. 2011, Jeong et al. 2010). These potential generalist species may play an important biological role in the future Arctic Ocean as they were abundant regardless of any changes in the environment in Adventfjorden (e.g. changes in light, temperature, watermass - Paper I, III; and even changes in predator composition – Stübner et al. 2016) and hence may be able to cope easily in a fast-changing habitat. According to my data (Paper I, III), and in agreement with microscopic analyses from Kongsfjorden winter samples (Callesen 2015), it appears that there is a higher abundance of small dinoflagellates (< 10 µm) than reported earlier, which in the future should be more focused on.

Potential specialist groups such as marine alveolates (e.g. MALV I clade-1 and MALV II clade-7, Paper I, III) seemed to be strongly influenced by different hydrographic conditions (cf. Thaler and Lovejoy 2015, Kubiszyn et al 2014) and thus their presence fluctuated in Adventfjorden throughout the year-long study (Paper I, III). Both MALV I and MALV II are frequently reported from Arctic waters (Lovejoy et al. 2006, Canadian Arctic – Terrado et al. 2009, Central Arctic - Bachy et al. 2011 and Kilias et al. 2014, Fram Strait – Kilias et al. 2013, Svalbard - Sørensen et al. 2012). The decreased relative read abundance of MALVs in the winter-spring transition at IsA was supported by qPCR
cell counts investigated by Thomson (2014) during the same time period. Thomson (2014) measured MALV II (order Syndiniales) 18S copy numbers between 40 – 50 x 10^4 cells L^-1 in February, with max. copy numbers in April (90 x 10^4 cells L^-1), and determined a sudden decrease after the 19th April from which the Syndiniales did not recover. Thomson and I concluded that MALV II were transported out of the system with re-occurring cold water (advection event mentioned earlier; **Paper III**). However, since MALVs have a parasitoid lifestyle (Guillou et al. 2008) and are known to inhabit marine organisms such as crustaceans, dinoflagellates, fish and bivalves (Stentiford and Shields 2005, Chambouvet et al. 2008, Skovgaard et al. 2009, Noguchi et al. 2013, Miller et al. 2012), their appearance/disappearance possibly could relate to host availability (**Paper III**).

At the IsA station, ciliates had an increased relative abundance during spring (April and May, **Paper I**), potentially feeding on larger phytoplankton available (Kubiszyn et al. in revision) at that time. This successional pattern is common in the Arctic (Central Arctic - Sherr et al. 2003; Kongsfjorden - Seuthe et al. 2011) as ciliates are important grazers on other protists but also an essential diet for many higher trophic levels (Levinsen and Nielsen 2002, Turner et al. 2001, Sherr and Sherr 2007, Seuthe et al. 2011). Two important grazers in Adventfjorden were the ciliates Strombidiidae sp. (strain 37) and Choreotrichia-1 sp. (**Paper I, III**). They both belong to the subphyla Intramacronucleata, which is broadly distributed over the Arctic Ocean (Beaufort Sea – Terrado et al. 2009 and Comeau et al. 2011, Barents Sea – Fernandez-Leborans et al. 2006, Svalbard – Seuthe et al. 2011 and Sorensen et al. 2012). Members of the family Strombidiidae are known to have the ability of kleptoplasty (Rumpho et al. 2006) that certainly makes them well-fitted to living in the Arctic as they may remain active under limited food availability by converting light energy to organic compounds during summer and autumn.

**Stramenopiles predominate in winter**

Heterotrophic uncultured marine stramenopiles (MASTs, Massana et al. 2004 and 2006) were also commonly found in Adventfjorden and this is the first study that investigated their seasonal succession in the Arctic, little of which is known about to date.
Three subclades of MAST 1 (MAST 1a, 1b and 1c) are found in the Arctic and it is suggested that they are associated with certain environments; MAST 1a and 1b were associated with ice-covered and open waters (Thaler and Lovejoy 2014). This contradicted our data as MAST 1a especially was identified in all seasons (Paper I, III), at different depths (Paper III) and in the exported material (Paper III) in Adventfjorden, even though we investigated an enclosed ice-free fjordsystem. However, as MAST 1a was only dominant during winter (Paper I, III), possibly it was transported out of the fjord when the watermass shifted as suggested for the MALVs, however it did reappear at the end of the year. MAST 7 was also found at IsA, albeit in very low relative abundance, in contrasts to the findings by Thaler and Lovejoy (2015) who found MAST 7 to be ubiquitous and abundant in Arctic samples 18S clone libraries.

6.3. The fate of marine microbial eukaryotes and its potential drivers

Seasonal succession implies a shift in dominance of species and thus their fate. The fate of passively drifting small-sized plankton can have several pathways and drivers. Firstly, seasonally driven changes in community composition, in which species favour various conditions and hence grow successfully at different times of the year, are discussed in 6.1. Secondly, hydrographically driven changes – plankton species can be passively distributed by lateral advection in the water column (Hamilton et al. 2008). Thirdly, they can sink out and contribute to the vertical carbon flux. That may happen due to unfavourable growth conditions, i.e. limited spore development and increased sinking rate, but also due to enhanced flocculation and ballasting events (Kranck 1973, Sutherland et al. 2015). A fourth pathway would be by grazers who can boost the vertical C flux by fecal pellet production and protists cells may sink integrating in such aggregated fecal pellets (Wexels Riser et al., 2007, Lalande et al. 2011, Turner 2015 and ref. within).
Variations in water mass composition were important for the structure of the microbial eukaryote community at IsA. An advective event during early spring (March), which replaced Local Water with Transformed Atlantic Water, was probably the trigger for a change in the composition of small protists (0.45 – 10 μm, Paper III). Similar events have been reported from another West Spitsbergen fjord, Kongsfjorden (Hegseth and Tverberg 2013, Piquet et al. 2014, Kubiszyn et al. 2014) where Atlantic water intrusions can also strongly influence the system (Cottier et al. 2005) in combination with freshwater input from the adjacent glaciers (Svendsen et al. 2002, Cottier et al. 2005). The Kongsfjorden protist community composition was strongly affected by the changing hydrography in different seasons (spring and summer), i.e. different water mass origins and the strength of inflows (Hegseth and Tverberg 2013, Piquet et al. 2014, Kubiszyn 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 relatively highly affected by climate-driven changes (Foissner and Hawksworth 2009).

The current climate warming in the Arctic with freshening of the seawater and an increased stratification is predicted to favour picoplankton, in contrast to the current situation (Daufresne et al. 2009, Li et al. 2009, Sommer et al. 2016). The IsA station did not resemble such a situation in 2011 – 2012. While there was no seasonal sea ice cover in the fjord, the IsA station was fairly well-mixed and non-stratified most of the year, with a strong spring bloom of larger protists occurring in end of April – May (Paper I and III). The ISA station generally reflected the hydrographic conditions of the upper 100 m of the large Isfjorden system one week delayed indicated by the CTD data (Paper III). While a freshening of the water in the Svalbard fjords is a rather seasonal impact varying with fluctuations in sea ice and meltwater run-off from glaciers, this process may not necessarily affect the stratification (Carmack et al. 2015) as seen at IsA. Thus, the stratification-associated changes in the Arctic marine environment are potentially more prominent in the central Arctic Ocean and the Canadian Arctic rather than in the western
Svalbard fjords.

Nevertheless, the fluctuations and loss of seasonal sea ice in Svalbard fjords may result in earlier pelagic spring blooms and the cease of ice-algae blooms which may in return greatly affect the arctic food web and the contribution to the vertical flux (Grebmeier et al. 2006, Arrigo et al. 2012, Post et al. 2013, Lovejoy 2014, Wiedmann et al. 2016). Hence, this present annual study of the ice-free Adventfjorden system represents a possible scenario of future ice-free Svalbard fjords (Paper I, III).

**Predators, sinking and vertical flux**

The contribution of different taxa and species to the vertical flux at IsA largely differed between the seasons (Paper III) possibly due to the large variation in protist lifestyles and their adaptive strategies (generalist vs. specialist). Different patterns were observed, many species that were abundant in the water column were also present in the traps (e.g. *Gyrodinium fusiforme*, *G. helveticum* and others), whilst others were only abundant in the water column and absent from the traps (e.g. Choanoflagellida, several Dinophyceae strains). Yet others were only abundant in the trap and not in the pelagic (e.g. *Chytriodinium*, Cercozoa). The carbon cycle and sequestration is dependant on carbon oxidation rates as well as the process of photosynthesis (Worden et al. 2015). This interaction relies heavily on grazing rates, i.e. on heterotrophic species and their diverse feeding strategies (e.g. predation and parasitism; Worden et al. 2015). While grazing rates were not evaluated in this study, the presence of predators (e.g. zooplankton, hetero- and mixotrophic protist) was discussed since molecular traces from many predators were found in the trap material (Paper III). However, some of these predators are possibly intruders in the sediment traps as most of them are able to swim and potentially follow their prey, or conduct vertical migrations (Lee et al. 1988, Michaels et al. 1990, Amacher et al. 2009). Also parasitism is a way to access organic material, but it is challenging to determine this trophic mode in carbon cycling models (Worden et al. 2015), and interestingly several of the species only abundant in the trap are known to have an infective lifestyle (e.g. MALV 1a, *Chytridiomium*, Cercozoa). It is even hypothesized that parasitic feeding modes may be more important from an ecological perspective than
grazers controlling bloom dynamics (Montagnes et al. 2008, Chambouvet et al. 2008, Sherr and Sherr 2009). Thus parasitism needs to be paid more attention when studying carbon cycles and the vertical flux.

A large part of carbon flux literature states that size matters, because larger and heavier cells (e.g. diatoms, coccolithophores, spores) may sink faster and in turn contribute more to the downward flux (Michaels and Silver 1988, Boyd and Newton, 1999, Sarthou et al. 2005, Richardson and Jackson 2007, Ziveri et al. 2007).

That may not necessarily be true as we found small microbial eukaryotes (< 10 řm) to be dominant in the trap material at IsA (Paper III), and this finding is supported by other recent studies (Worden et al. 2004, Richardson and Jackson 2007, Amacher et al., 2009 and 2013, Cuvelier et al. 2010, Worden et al., 2015). The contribution of smaller cells to the vertical flux seemed to be strongly seasonally dependant, as there was a higher relative abundance of small-sized protists found in the traps during autumn and winter at IsA compared to spring (when diatoms were dominant in the traps). This was possibly due to a higher relative abundance of pico- and nanosized cells during winter and autumn (read abundance – Paper I, III, cell counts – Kubiszyn et al. in revision), and the ongoing mixing processes that transported cells down to the deeper water (Siegel et al. 2016), in combination with increased floculation and ballasting processes (Jackson and Richardson 2007, Worden et al. 2015) especially in autumn (Wiedmann et al. 2016).
7. Conclusions

Marine microbial eukaryotes were investigated in Svalbard waters with special focus on the high-Arctic and ice-free Adventfjorden from a seasonal perspective. The community composition and the diversity of small protists showed strong fluctuations during the course of a year and reflected the strong seasonality of the Arctic (Fig. 4). While the temporal differences in the community were potentially driven mostly by seasonal events and factors such as returning of the light and nutrient availability, the similar community composition at the four sampling depths was rather a result of the non-stratified water column that was apparent most of the year in 2011 – 2012.

Microbial eukaryote seasonal succession in Adventfjorden
The methods used in this study revealed the predominance of small heterotrophic nanoflagellate (HNF) taxa such as MASTs, MALVs and Picozoa during winter in the high-Arctic Adventfjorden. The succession of protists developed from a predominance of heterotrophic taxa in winter to a more typical Arctic spring bloom assemblages during spring (Fig. 4; cf. Terrado et al. 2009, Hodal et al. 2012). The diversity estimation in combination with the RNA libraries demonstrated that the protist world is rather species rich, with potentially many unknown species and activities even during the polar night. Future studies should focus more on this important time of the year in the Arctic, which still is a “black box” concerning biological knowledge.

Abundant species = key species?
Species and groups such as Phaeocystis, Micromonas, MALV I, Gyrodinium and Gymnodinium which were relatively abundant in Svalbard waters have been previously reported from Arctic regions. In this study I defined key species as those present in relatively high abundance (based on sequence reads) in the system over time or on a particular sampling date. Micromonas pusilla and Phaeocystis pouchetii have been reported to be key phototrophs (Ratkova and Wassmann 2002, Schoemann et al. 2005, Lovejoy et al. 2007, Kilias et al. 2013) and this study also reports their broad distribution around Svalbard, mostly in an active state (RNA) even during the dark winter months. Other abundant species like Gyrodinium helveeticum and G. fusiforme may have a rather
generalist lifestyle in the system. They seem to cope in many different environmental conditions, which may be an advantage in the ongoing climate changes in the Arctic. In contrast, there are specialists such as MALV I which can only cope in certain environmental conditions (cf. Thaler and Lovejoy 2015) and were advected out of the IsA system in spring when the watermass changed back from TAW to LW.

The fate of microbial eukaryotes

The life and death of marine microbial eukaryotes may depend on several factors (e.g. seasonal events, hydrography, grazing, unfavorable conditions, etc.). While certain phototrophs (e.g. Phaeocystis and Micromonas) may keep a small winter standing stock, possibly in a spore phase, ready to spawn when the environmental conditions improve, others stay relatively abundant in the pelagic all year around (Gyrodinium spp.). Strategies such as kleptoplasty (e.g. Strombidium sp.) and parasitism (MALVs, Chytriodinium sp., Leptolegnia sp.) may also help to cope with unfavorable conditions in the harsh Arctic.

While microscopy may be unable to identify small cells in detritus from trap material, the molecular approach used in this study was able to identify a contribution of small eukaryotes (< 10 µm) to the vertical flux (e.g. similar community composition of trap and small suspended eukaryotes in winter and autumn at IsA), contradicting the theory that predominantly large cells contribute to the downward flux. The reason behind this may be a general predominance of small HNFs in the water column during winter and autumn. Additionally in autumn, small eukaryotes got highly enriched in aggregates, induced by increased flocculation/ballasting from the river/glacial meltwater, which were then sinking out. Thus, especially in arctic and subarctic fjord systems, which are affected by glacial runoff in the melt season and where HNF are predominant during winter and autumn may the contribution of small eukaryotes to the vertical flux may be of greater importance compared to other systems (e.g. sea ice, open water).
8. Outlook

Although this study has contributed many new insights into the hidden world of marine microbial eukaryotes in Svalbard waters, there are still knowledge gaps to fill. First of all, we need to increase our knowledge of the biological activity during the polar night period. In the addition to the question ‘who is there?’ (quality) we should also address ‘how many are actually there?’ (quantity). So far, sequencing techniques leave us with only semi-quantitative results, and the combination of a HTS technique with quantitative approaches such as qPCR, flow cytometry, DAPI stained cell counts or Fluorescence In Situ Hybridization (FISH) would be an advantage.

Of further interest is also the annual pattern of marine microbial eukaryotes communities, which seemed to reset towards autumn to the composition from the start of the year. The IsA time series data is a unique dataset with a high resolution perspective, although clearly one year of data is not enough to clarify whether this event was random and maybe dependent on different watermass advections into the fjord, or a re-occurring pattern which is seasonally influenced. Analyses of samples that were continuously taken at the IsA station between the years 2012 – 2016 will give further insights into this question (Vader et al. in preparation).

The combined use of DNA and RNA libraries was of a large benefit for this study, although due to logistical reasons it was not possible to sample more frequently for RNA. I suggest increasing the combined use of DNA and RNA in future studies to reveal a more thorough picture of the community (‘Who is there and who is actually active?’). Especially for the polar night studies and the trap material this would be of advantage. Additionally it would be interesting to utilize comparative metatranscriptomics to gain more deeply insights into the ongoing activities and functions of the different species in the water column (‘What are they actually doing?’) as well as in the sediment traps (‘Are they grazing? Are they parasitic?’).

Lastly, the sequence databases still contain many gaps, especially for samples that come from remote areas like the Arctic, which makes it difficult to identify cryptic species.
Phylogenetics is a useful tool that should be included to identify these species and to possibly aid in the discovery of new species. In that sense, the comparison with other Arctic areas is of great importance to this rather local study, to identify endemic and widespread species in a pan-arctic perspective as well as to identify the OTUs phylogenetically (Gabrielsen et al. in preparation).
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Phylogenomics

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