RESEARCH ARTICLE

Effects of light and prey availability on Arctic freshwater protist communities examined by high-throughput DNA and RNA sequencing

Sophie Charvet[1,2,3], Warwick F. Vincent[1,3] & Connie Lovejoy[1,2]

[1]Département de Biologie, Université Laval, Québec, QC, Canada; [2]Québec-Océan and Institut de Biologie Intégrative et des Systèmes (IBIS), Québec, QC, Canada; and [3]Centre d'études nordiques (CEN), Québec, QC, Canada

Correspondence: Sophie Charvet, Département de Biologie, Université Laval, Québec, QC G1V 0A6, Canada.
Tel.: +1 418 656 2007;
fax: +1 418 656 2339;
e-mail: sophie.charvet.1@ulaval.ca

Received 22 July 2013; revised 22 January 2014; accepted 6 March 2014. Final version published online 7 April 2014.

DOI: 10.1111/1574-6941.12324

Editor: Riks Laanbroek

Keywords
Arctic; chrysophytes; climate change; dinoflagellates; polar lakes.

Abstract

Protists in high-latitude lakes are constrained by cold temperatures, low inorganic nutrient supply and low light availability for much of the year due to ice cover and polar darkness. The lengthening ice-free periods in these freshwater ecosystems due to a warming climate results in increased light availability, but the overall impacts on phytoplankton and other protists are unknown. We experimentally investigated protist community responses to changes in light and prey availability in a dilution series in Ward Hunt Lake (latitude 83°05′N), in the Canadian High Arctic. The communities at the end of the experiment were characterized using high-throughput pyrosequencing of the V4 region of the 18S rRNA gene as a measure of taxonomic presence, and of 18S rRNA (from RNA converted to cDNA) as a taxon-specific indicator of community response. At the end of the experiment under low irradiance, cDNA reads were dominated by photosynthetic dinoflagellate genera, except at the greatest dilution where *Cercozoa* were most abundant. In contrast, the cDNA reads in the high light treatments were dominated by chrysophytes. Given the known trophic differences among dinoflagellates, cercozoans and chrysophytes, this apparent environmental selection implies that the rise in underwater irradiance associated with increasing ice-free conditions may affect microbial food web structure and function in polar lakes.

Introduction

Arctic freshwater ecosystems are increasingly viewed as sentinels of global change (Schindler & Smol, 2006; Mueller *et al.*, 2009). The Arctic is experiencing accelerated warming at rates well above the global average (Anisimov *et al.*, 2007), and this rapid climate change has already resulted in visible perturbations, including ice-shelf breakup (Mueller *et al.*, 2003; Copland *et al.*, 2007) and irreversible loss of cryospheric habitats (Vincent, 2010). High Arctic perennially ice-covered lakes are presently crossing thresholds, with more frequent loss of their summer ice covers (Mueller *et al.*, 2009). The onset of summer open water in these once-perennially ice-covered lakes leads to an unusual exposure to wind-induced mixing, extreme increases in underwater irradiance and changes in the water inflow dynamics (Veillette *et al.*, 2011).

Chrysophytes often dominate the summer phytoplankton community of Arctic lakes (reviewed in Charvet *et al.*, 2012a) when 24-h sunlight is available for photosynthesis. However, the overall biological production in many polar lakes is low due to poor nutrient input from the catchment and little or no deep-water mixing over most of the year, resulting in strong nutrient limitation (Vincent *et al.*, 2008). Chrysophytes have several strategies for surviving in low-nutrient environments, including small cell size, which provides a higher surface to volume ratio favouring efficient dissolved inorganic nutrient uptake (Nicholls, 2009), and high-affinity transporters for certain nutrients (Raven, 2009). The capacity of many photosynthetic chrysophytes to graze on bacteria or other protists is another strategy for obtaining nutrients in oligotrophic waters (Bird & Kalff, 1986; Maranger *et al.*, 1998; Raven, 2009) and is consistent with chrysophytes

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

FEMS MICROBIOLOGY ECOLOGY

occurring under the high light and low-nutrient conditions during Arctic summers. In contrast to summer conditions, in spring and at high latitudes where lakes may be ice-covered throughout the year including over summer, the phytoplankton may be light-limited. Such conditions would favour species capable of obtaining both energy and nutrients from bacteria or protist prey. In the case of energy-limiting conditions, mixotrophic protists compete with strictly heterotrophic protists, but have the additional energetic cost of maintaining cellular machinery for phagocytosis and digestion, along with chloroplasts (Raven, 1997). A question remains as to whether, over short time spans, mixotrophs could successfully compete with specialist heterotrophs at low prey densities (Rothhaupt, 1996; Tittel *et al.*, 2003).

Although protist communities under the spring ice cover differ from those in late summer (Veillette *et al.*, 2011; Charvet *et al.*, 2012b), the direct influence of light on natural protist communities has not been extensively studied (Llames *et al.*, 2009). As the multiannual ice-cover melts more frequently, the course of seasonal succession and the taxonomic makeup of photosynthetic protist communities in these lakes could be altered, depending on the influence of overall irradiance in species selection.

In the present study, we investigated the response of protist communities from an Arctic lake to high and low irradiance levels over a gradient of potential prey concentrations. We hypothesized that irradiance and prey density would favour specific taxa over the short term (4 days) and that the resultant communities would be sorted by the stronger of these two environmental drivers. We evaluated this hypothesis by way of an *in situ* incubation experiment in a High Arctic lake. The experiment was designed to create a resource matrix of light and prey to investigate how protist species respond to a trophic continuum (Granéli & Carlsson, 1998). In the context of disappearing summer ice cover, we assessed the effect of underwater irradiance by simulating low light, ice-covered conditions and high light, ice-free conditions. We predicted that the low light regime would challenge the primarily phototrophic members in the initial communities. With the dilutions, the intention was to create low prey conditions that could affect phagotrophic protists. The changes in protist community structure were tracked using high-throughput sequencing of the taxonomically informative V4 region of 18S rRNA from DNA and from RNA converted to cDNA. The 18S rRNA gene reads provided information on the historic and current presence of taxa, while the cDNA reads provided a record of the taxa that were more likely to be active, meaning those with increased ribosomal activity and the potential for protein synthesis (Blazewicz *et al.*, 2013).

Materials and methods

Study site

Ward Hunt Lake is located on Ward Hunt Island, at latitude 83°05′N and longitude 74°10′W, off the northern coast of Ellesmere Island in Nunavut, Canada. The lake's maximum measured depth is 10 m with a total area of 0.37 km^2. Ward Hunt Lake is ultra-oligotrophic and typically completely covered by thick perennial ice throughout the summer; limnological, catchment and climate details are given in Vincent *et al.* (2011).

Nutrients

Aliquots of 120 mL of sampled water were stored in glass bottles with polypropylene caps. These samples were later analysed at the Institut National de Recherche Scientifique-Centre Eau-Terre-Environnement (INRS-ETE, Quebec, QC, Canada) after being transported in the dark at *c.* 4 °C. Concentrations of nitrate and nitrite (NO_X-N), total nitrogen (TN), ammonia (NH_3-N) and soluble reactive phosphorus (SRP) were determined using standard colorimetric techniques (Gibson *et al.*, 2002). Total phosphorus was determined from a separate 125 mL aliquot by the continuous flow analyser stannous chloride method. The detection limit for NO_X-N was 0.005 mg N L^{-1} and for SRP was 0.001 mg P L^{-1}.

Experimental set-up

Ward Hunt Lake was sampled on 4 July 2009, immediately below a 40-cm hole in the ice, which was 2 m away from the north-western shoreline. Water depth at the sampling site was 1 m, and at the time of sampling, there was a 20 to 30-cm ice cover. Approximately, 100 L were collected, of which 50 L was sequentially filtered through 3.0- and 0.8-μm pore-size 47-mm-diameter polycarbonate filters (PC) then through 0.2-μm Sterivex units (Millipore). Given the logistic and material constraints, 100 L was the maximum quantity of water that could be filtered over 12 h before starting the experiment. As the experimental design called for gradients of conditions, the water was used to increase the number of treatments across the gradients rather than replication. The filtrate (FW) was used to dilute the experimental series (Landry & Hassett, 1982) by mixing with the unfiltered lake water (LW) in 10-L cubitainers (polyethylene collapsible containers; Reliance Products, Winnipeg, MB, Canada), to obtain the following dilution series: dilution factor 1 (LW only), 0.5 (half LW, half FW), 0.2 (2 L of LW, 8 L of FW) and 0.1 (1 L of LW, 9 L of FW). For each dilution, one cubitainer was exposed to ambient surface irradiance (high light)

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

and the other was kept in two heavy weight black plastic bags (low light). The combination of dilutions and light treatments provided a matrix of resources, along which a continuum of potential feeding strategies might be expected to develop (Granéli & Carlsson, 1998).

Phosphorus (K_2HPO_4) and nitrogen ($NaNO_3$) were added to each cubitainer at concentrations of 3.5 μg L^{-1} of P and 18 μg L^{-1} of NO_3^--N. This enrichment was at 10× and 0.78×, respectively, the normal ambient concentrations in the lake (see Results below), and was made to prevent senescence due to strong nutrient limitation at the beginning of the experiment. Differences in the protist communities exposed to the same nutrient conditions were then expected to be due to light and prey availability. The cubitainers were left for 4 days, anchored in place and allowed to float half-submerged at the surface in an ice-free region of the lake, to maintain *in situ* temperatures and exposure to natural irradiance levels.

Sampling

To determine cell abundance and growth rates, subsamples of 10 mL for prokaryotes and 40 mL for eukaryotes were collected at the start of experiment (T_0), after 2 days (T_2), and after 4 days (T_4) at the end of the experiment. These were fixed in 1% glutaraldehyde for 1 h, then 5 μg mL^{-1} of 4′-6-diamino-2-phenylindol (DAPI) was added for 5 min (Porter & Feig, 1980), and the samples filtered onto 25-mm-diameter black polycarbonate filters (Millipore); 0.2- and 0.8-μm pore-size filters were used for prokaryotes and eukaryotes, respectively. The filters were mounted onto microscope slides with a drop of nonfluorescing immersion oil, and stored in the dark, at −20 °C. Samples for Chl *a* were collected by filtering 1 L of water onto 25 mm GF/F filters (Fisher Scientific) and stored at −80 °C.

For eukaryote DNA, 2 L of water were sequentially filtered onto 3.0- and 0.8-μm pore-size 47-mm polycarbonate (PC) filters, which were placed into lysis buffer (50 mM Tris, 40 mM EDTA, 0.75 M sucrose) and stored at −80 °C. For eukaryote RNA, 2 L were filtered as for DNA, but filters were placed into a 1 : 100 mix of β-mercaptoethanol and RNAlater (Sigma-Life Sciences), then stored at −80 °C.

Microscopy and growth rate calculations

DAPI stained cells were enumerated at 1000× magnification under ultraviolet (UV) excitation, and to visualize chlorophyll *a* autofluorescence, the same fields were examined under blue (420 nm) excitation, with a Zeiss Axiovert inverted epifluorescence microscope. Cells for which the nucleus was apparent under UV and with pigments that autofluoresced red under blue excitation were classified as capable of photosynthesis, and cells that only appeared using DAPI were considered heterotrophs. Cells were counted across 40 fields, along a cross section of the filter, with a minimum count of 200 cells for each sample.

The cell concentrations in each cubitainer were estimated at the start of the experiment (N_0), after 2 days (N_2) and at the end of the experiment (N_4). Growth rates (K) were calculated as generations per day, for each time interval, as:

$$K = (\log N_t - \log N_0)/(\log 2 \times t),$$

where N_0 is the number of cells in the initial population, N_t is the number of cells at time t (Prescott *et al.*, 2002). The average of the growth rates of the first 2 days and the last 2 days was used as the mean growth rate for the experiment.

Pigment analyses

Chlorophyll *a* (Chl *a*) was extracted in 95% methanol following sonication, and concentrations were measured by high-performance liquid chromatography (ProStar HPLC; Varian, Palo Alto, CA), as in Bonilla *et al.* (2005).

Nucleic acid extractions

Community DNA was extracted using a salt (NaCl)-based method modified from Aljanabi & Martinez (1997) with lysozyme and proteinase K steps (Diez *et al.*, 2001) as detailed in Charvet *et al.* (2012a). The final ethanol-rinsed DNA pellets were dried and resuspended in 100 μL of 1× TE buffer (10 mM Tris–HCl, 1 mM EDTA) and stored at −80 °C. The community RNA was extracted using the Qiagen RNeasy Mini Kit (Qiagen, Toronto, ON, Canada) as per the manufacturer's indications and included a DNase (RNase-free DNase kit; Qiagen) step. A PCR, using eukaryote primers Euk336F and EukR (Sogin & Gunderson, 1987; Medlin *et al.*, 1988), was used to verify the DNase step. Directly after extraction, the total community RNA was reverse transcribed into the more stable form of complementary DNA (cDNA) using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Life Technologies). As most samples had very low concentration of cDNA, random amplification of all cDNA samples was conducted by multiple displacement amplification (MDA; Illustra GenomiPhi V2 DNA Amplification Kit; GE Healthcare, UK). Negative controls from the MDA were preserved and subjected to the same downstream tag PCRs as the samples, to verify that there was no contamination from the reagents.

© 2014 Federation of European Microbiological Societies. Published by John Wiley & Sons Ltd. All rights reserved

Tag-PCR amplifications

The hypervariable V4 region of the 18S rRNA gene was amplified using the primers from Comeau *et al.* (2011). The resulting *c.* 500 nt amplicons were quantified using a Nanodrop ND-1000 spectrometer. At this point, PCR products for each treatment from the 3.0 and 0.8 μm extracts were combined in equal proportions to ensure representation of the entire community. Sequencing was carried out on a 454 GS FLX-Titanium system (Roche), at the Plateforme de Séquençage de l'Institut de Biologie Intégrative et des Systèmes, Université Laval, Québec, Canada. The raw reads were deposited in the NCBI Sequence Read Archive with the BioSample accession number SAMN02258453.

Preprocessing, quality control and taxonomy analyses

The quality filters, resampling of sequences and alignment protocols used in this study are fully described in Comeau *et al.* (2011). The alignments were further checked for chimeras using the UCHIME chimera check function (Edgar *et al.*, 2011) against the Silva reference alignment in MOTHUR (Schloss *et al.*, 2009). The final aligned reads were clustered into operational taxonomic units (OTUs) at the $\geq$ 98% similarity level using MOTHUR (furthest-neighbour clustering). This first clustering step of the pooled sequences allowed identification of singletons (OTUs represented by a single sequence in the total data set), and these were removed as potential errors (Huse *et al.*, 2010; Kunin *et al.*, 2010).

Taxonomic assignation (using a 50% bootstrap cut-off) of the remaining sequences was made against our curated sequence database, which is based on NCBI taxonomy (Comeau *et al.*, 2011). This version of the database included sequences from 18S rRNA gene clone libraries of Arctic lakes published in Charvet *et al.* (2012a) and is available upon request. Taxa in this database were binned by likely trophic status: unambiguous phototrophs, mixotrophs and heterotrophs, based on literature searches. This reference sequence database was trimmed to the V4 region, as recommended by Werner *et al.* (2012). Common 'unclassified sequences' were further investigated using BLASTN against the GenBank nr database (NCBI).

OTUs-based analyses

Bray–Curtis clustering of communities from the experimental treatments was based on relative abundance of OTUs generated in MOTHUR. The phylogenetic diversity of cDNA OTUs between the different treatments was tested by computing net-relatedness (NRI) and nearest taxon (NTI) indexes (Webb *et al.*, 2002). Briefly, representative OTU reads were placed in an approximate maximum-likelihood phylogenetic tree using FASTTREE v2.1 (Price *et al.*, 2010) based on a multiple sequence alignment generated with PyNAST (Caporaso *et al.*, 2010b) as implemented in Quantitative Insights Into Microbial Ecology (QIIME v1.5; Caporaso *et al.*, 2010a). The phylogenetic tree was refined in the R package 'Picante' (Kembel *et al.*, 2010) to compute mean pairwise distance (MPD) and mean nearest taxon distance (MNTD) against a null model comprised of 999 randomized trees with taxa shuffling, leading to standardized metrics (SES_{MPD} and SES_{MNTD}, corresponding to $-1 \times$ NRI and $-1 \times$ NTI, respectively).

Statistical analyses

Significance of differences at the genus level along the resource continuum was tested with Metastats (White *et al.*, 2009). A canonical correspondence analysis (CCA) was used to visualize variability among communities and to analyse this variability according to the experimental treatments, based on the experimental variables: light, dilution factors, final bacterial cell concentrations, Chl *a*. The sequence abundance data were transformed to relative proportions of the major groups detected: alveolates, stramenopiles (which include chrysophytes), *Cercozoa*, *Cryptophyta*, *Chlorophyta*, *Haptophyta* and *Telonemia*.

Estimation of photosynthetically active radiation

The incident irradiance at Ward Hunt Lake during the experiment between June 4 and 8 was calculated based on data from the Ward Hunt Island meteorological station < 1 km from the lake (Centre d'Études Nordiques, 2013). The proportion of photosynthetically active radiation (PAR) transmitted through the black polyethylene bags was estimated using a QSL100/101 Quantum Scalar Irradiance meter (Biospherical Instruments Inc., San Diego, CA).

Results

At the start of the experiment, phosphate and nitrate concentrations in the LW were low and the addition of nutrients decreased the N : P ratio in all treatments (Table 1). The PAR values over the experimental run ranged from 572 μmol photon m^{-2} s^{-1} at midnight to 1052 μmol photon m^{-2} s^{-1} at noon, with a mean ($\pm$ SD) daily incident PAR in the high light treatment of 769 ($\pm$ 19) μmol photon m^{-2} s^{-1}. Approximately, 0.1% of the incident PAR was transmitted through the low

© 2014 Federation of European Microbiological Societies. Published by John Wiley & Sons Ltd. All rights reserved

Table 1. Nutrients (µg N or P L^{-1}) and N : P ratios at the beginning of the experiment (T_0), and Chlorophyll *a* (Chl *a*, in µg L^{-1}) and bacterial and protist abundances (10^4 cells mL^{-1}) at T_0 and the end of the experiment

		Low light				High light			
	LW	0.1	0.2	0.5	1	1	0.5	0.2	0.1
NOx-N	23	20.3*	20.8*	30.0*	41.0*	41.0*	30.0*	20.8*	20.3*
SRP	0.35	3.53*	3.54*	3.67*	3.85*	3.85*	3.67*	3.54*	3.53*
N : P	66 : 1	6 : 1*	6 : 1*	8 : 1*	10 : 1*	10 : 1*	8 : 1*	6 : 1*	6 : 1*
Time of sampling	T_0	T_4				T_4			
Chl *a*	0.39	0.09	0.22	0.25	0.32	0.13	0.12	0.05	0.07
Bacteria	7.21	ND	2.63	5.56	7.00	8.17	5.84	4.33	4.94
Heterotrophs	0.51	0.19	0.37	0.47	0.53	0.31	0.30	0.13	0.07
Phototrophs	2.44	0.36	0.53	0.72	0.85	1.08	0.96	0.66	0.35

LW, lake water (corresponds to initial conditions at time of sampling for the experimental set-up); ND, no data; SRP, soluble reactive phosphorus.
*Estimates of concentrations at the start of the experiment are calculated from the dilution of the T_0 concentrations and the subsequently added nutrients.

light polyethylene bags, corresponding to a mean daily PAR of 0.77 (± 0.02) µmol photon m^{-2} s^{-1}.

The final bacterial concentrations from microscopy were lower at high dilutions than at low dilutions (Table 1). In contrast, the bacterial growth rates were greater at high dilutions (Fig. 1a). Microbial eukaryotic abundance was also greatest at low dilutions (Table 1), but growth rates varied among functional groups (Fig. 1b). For the heterotrophic eukaryotes, the growth rates were greater in the low light treatments at highest dilutions. Under high light, the growth rate trend of heterotrophs was similar to that of phototrophs (Fig. 1b), although growth rates of phototrophs were less in the lowest dilutions (dilutions 1 and 0.5).

The community profiles from DNA and cDNA templates differed, with a maximum of 22% of OTUs shared between the two templates for any one treatment. The Bray–Curtis community analysis showed that DNA communities clustered together, apart from the cDNA communities (Fig. 2). There was no clear clustering in the DNA-derived communities with respect to either light or dilution (Fig. 2). Among the cDNA-derived communities, the treatments were clustered into high light and low light communities (Fig. 2), with the exception of the low light dilution 0.1, which branched apart. The NRI and NTI were positive for most communities, but values were mostly greater for cDNA communities compared with DNA (Fig. 3). Both phylogenetic metrics obtained from DNA communities were similar among the different treatments, while the cDNA communities showed greater variability (Fig. 3).

Initial 18S rRNA gene reads consisted of 61% alveolates, 20% stramenopiles and 19% *Cercozoa*, with very few chlorophytes or cryptophytes (Fig. 4a). At the end of the incubations, the DNA-derived reads from all of the treatments were still mostly alveolates with 50–80% of the total (Fig. 4b); the majority of these were dinoflagellates and ciliates (Table 2). The only exception was in dilution 0.2 under high light, where stramenopiles accounted for 60% of the total reads, mostly corresponding to chrysophytes (Table 3). In contrast, reads obtained from the cDNA template showed more variability among the different light and dilution treatments (Fig. 4c). Reads corresponding to *Cercozoa* related to *Protaspis* dominated the highest dilution (dilution 0.1), with some oomycete and *Synurophyceae* reads (Table 3), while the other low light treatments were dominated by dinoflagellate reads (Table 2). In the high light treatments, chrysophytes dominated the cDNA reads (Table 3). In terms of functional classification, genera were grouped into three trophic categories: strict heterotrophs, strict autotrophs and mixotrophs, based on literature documentation (Fig. 5). This analysis suggests that mixotrophy was the dominant trophic strategy among the protist communities, along the resource continuum of the experiment.

To compare the results from the two nucleotide templates used for sequencing, the number of reads from DNA was plotted against the number of reads from cDNA for each different taxonomic entity reported above (Fig. 6). The *Cercozoa* had highest cDNA levels compared with DNA in dilution 0.1 at low light, but in the rest of the incubations, had similar low numbers of reads for both DNA and cDNA. The oomycetes and *Synurophyceae* were barely represented in terms of cDNA reads at high light, but reached relatively high numbers of cDNA at low light, although DNA read numbers remained low. Dinoflagellate and chrysophyte DNA to cDNA ratios were relatively constant (no significant difference) between the different treatments (Fig. 6). However, the total number of both DNA and cDNA reads for dinoflagellates was

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

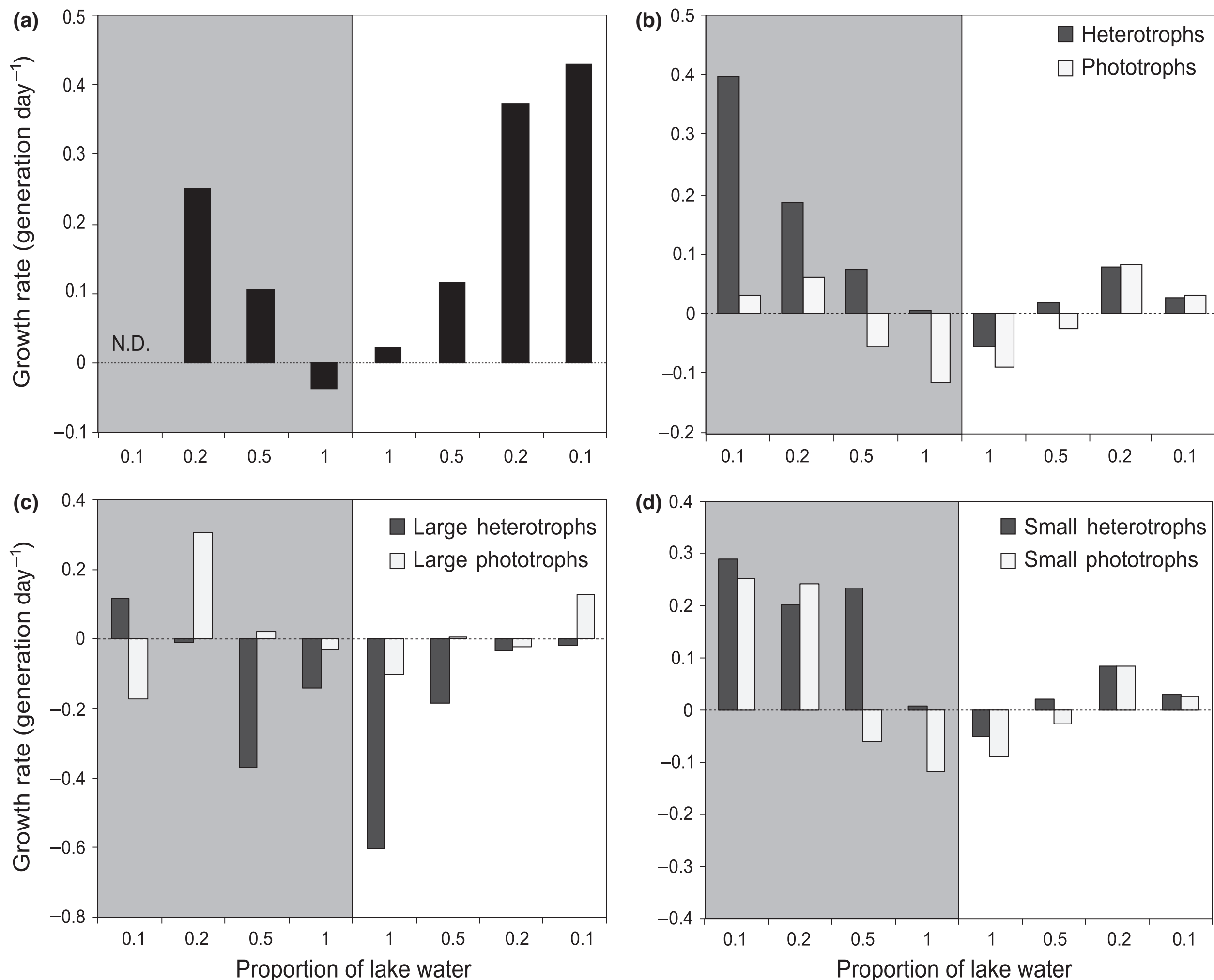

Fig. 1. Growth rates of prokaryotes (a) and eukaryotic heterotrophs and phototrophs (b) along the resource continuum. (c, d) Growth rates of the large (> 10 μm) and small (< 10 μm) eukaryotes, respectively. The grey zone corresponds to the low light treatment, and the white zone corresponds to the high light treatment.

greater in the low light incubations, while chrysophyte cDNA reads were significantly higher ($P < 0.05$) at high light.

Dinoflagellate cDNA reads were mostly *Scrippsiella* and *Woloszynskia* with a lower contribution of *Gymnodinium* (Fig. 7a). The *Woloszynskia* reads were more abundant at low light than under high light, in which *Scrippsiella* reads were more frequent. The chrysophytes showed a greater diversity at low light, with best matches to the cultivated chrysophyte CCMP2296, a Beaufort Sea clone ST320-ES069_E8, *Ochromonas* and *Chrysolepidomonas* (Fig. 7b). In contrast, under the high light treatments, the chrysophyte reads were largely composed of the Beaufort Sea clone ST320-ES069_E8 (80% of the chrysophytes and 40–70% of total reads) and some *Ochromonas* (15–20% of the chrysophyte reads).

A CCA based on reads of major phyla from cDNA showed a separation of the communities according to the light treatment along Axis 1 (Fig. 8). The low light communities were separated following higher Chl *a* concentrations and were characterized by the presence of alveolates, except for dilution 0.1, where *Cercozoa* were a major factor. The high light communities were influenced by light and bacterial concentrations and were characterized by the stramenopiles. The Metastats analyses (Table 4) showed that the significant differences between the high and low light treatments were attributable to the dinoflagellate *Woloszynskia*, which was significantly (P-value = 0.005) more abundant at low light, and the chrysophytes *Ochromonas* and clone St320_ES069_E8, which were significantly (P-values < 0.005) more abundant at high light.

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

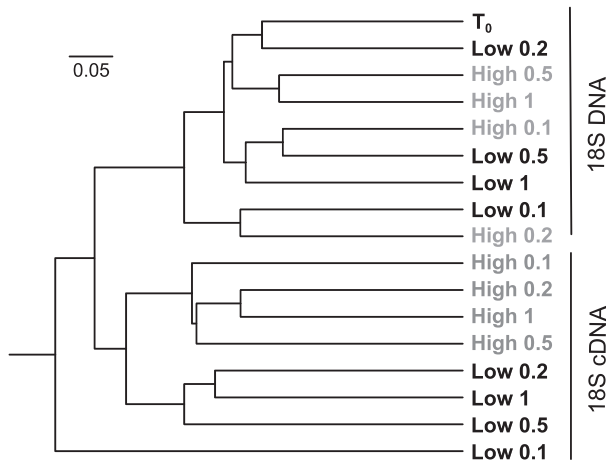

Fig. 2. Bray–Curtis dendrogram based on OTU (98% similarity) abundance data.

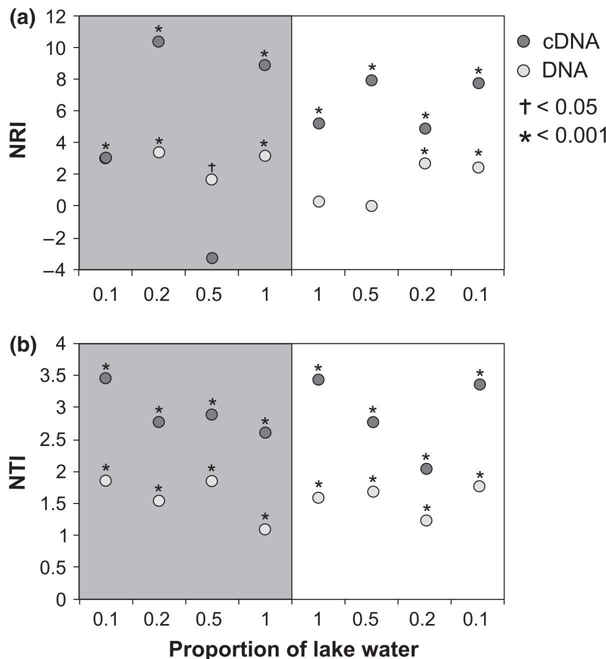

Fig. 3. NRI (a) and NTI (b) of each treatment combination, based on cDNA (dark circles) and DNA (light circles) along the resource continuum. The grey zone corresponds to the low light treatment, and the white zone corresponds to the high light treatment. Note that for the NRI, the values overlap for DNA and cDNA at dilution 0.1 in the low light treatment.

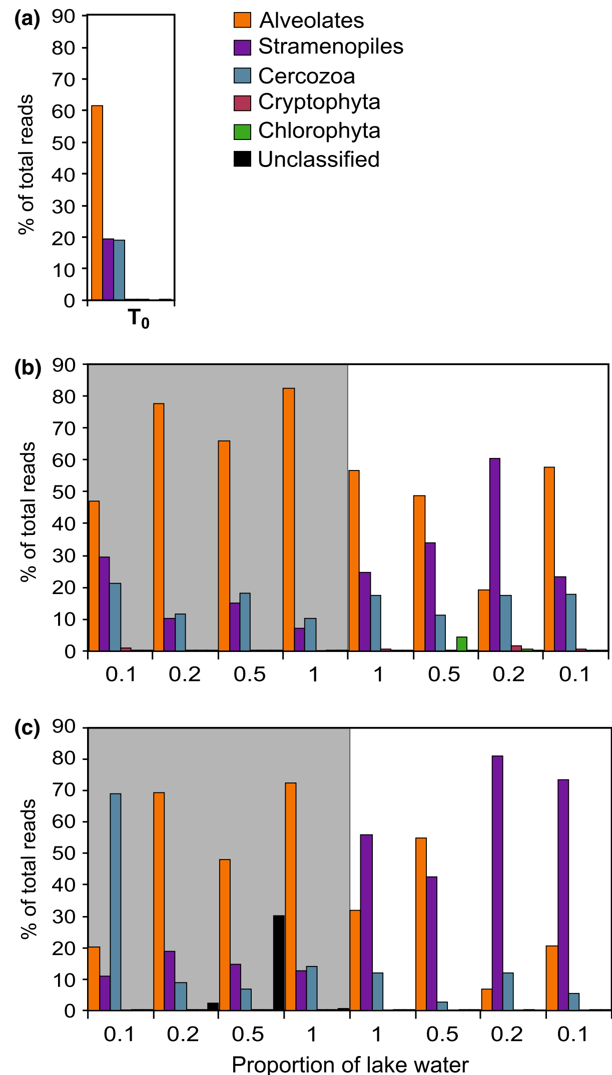

Fig. 4. Phylum composition of the initial lake community DNA (a) and along the resource continuum at the end of the experiment, obtained with DNA (b) and cDNA (c) templates. The grey zone corresponds to the low light treatment, and the white zone corresponds to the high light treatment.

Discussion

The dilution treatments resulted in changes in net growth rates for the prokaryotes and Eukarya. Prokaryote growth rates increased with increasing dilution, consistent with lowered encounter rates and reduced predation pressure at higher dilutions (Landry & Hassett, 1982). Chloroplast-containing eukaryotes also increased with increasing dilutions, as reported in other systems (Landry & Hassett, 1982; Dolan *et al.*, 2000). Heterotrophic (colourless) eukaryotes showed the same trend, which differed from previous reports (Dolan *et al.*, 2000). There was a high proportion of taxa nominally classified as nanoflagellates among the heterotrophic eukaryotes, and the results imply that this size class was subject to the same predation pressure as the small autotrophs. Negative growth rates were recorded for the pigmented protists, consistent with net losses of phototrophs and potential mixotrophs,

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

Table 2. Proportions of ciliates and dinoflagellates for each light and dilution treatment, obtained with DNA or cDNA templates (expressed as percentage total reads), at the beginning (T_0) and end of the experiment (T_4)

			Low light T_4				High light T_4			
Taxonomic group	Template	T_0	0.1	0.2	0.5	1	1	0.5	0.2	0.1
Dinoflagellates	cDNA	ND	20.17	65.83	44.82	63.19	29.61	54.64	6.64	20.20
	DNA	47.37	38.89	45.51	37.73	31.06	21.80	10.29	12.90	40.70
Ciliates	cDNA	ND	0.03	3.20	3.03	9.14	2.37	0.15	0.03	0.36
	DNA	13.91	7.74	32.01	28.09	51.44	34.83	38.03	5.93	16.88
Total	cDNA	ND	20.20	69.03	47.85	72.32	31.98	54.79	6.67	20.56
	DNA	61.29	46.63	77.51	65.83	82.50	56.63	48.32	18.84	57.58

ND, no data.
The dilution treatments ranged from 10% (labelled 0.1) to 100% (1) unfiltered lake water.

Table 3. Proportions of DNA or cDNA reads of stramenopile groups (as percentage of total reads) along the resource continuum at the beginning (T_0) and end of the experiment (T_4)

			Low light T_4				High light T_4			
Taxonomic group	Template	T_0	0.1	0.2	0.5	1	1	0.5	0.2	0.1
Chrysophyceae	cDNA	ND	2.22	8.72	9.34	8.72	55.09	41.83	81.07	72.83
	DNA	22.37	18.81	27.68	9.70	14.09	6.29	23.82	31.92	59.36
Diatoms	cDNA	ND	0.03	0.12	0.00	0.62	0.03	0.03	0.00	0.00
	DNA	0.00	0.00	0.03	0.12	0.00	0.00	0.09	0.24	0.03
Oomycetes	cDNA	ND	8.42	3.98	1.51	0.39	0.03	0.03	0.15	0.06
	DNA	0.06	0.12	0.30	0.09	0.06	0.00	0.00	0.00	0.00
Pelagophyceae	cDNA	ND	0.12	0.56	0.65	0.36	0.83	0.33	0.03	0.47
	DNA	0.21	0.36	0.71	0.30	0.59	0.71	0.21	0.56	0.15
Synurophyceae	cDNA	ND	0.00	5.28	1.42	2.20	0.03	0.27	0.00	0.00
	DNA	0.30	0.09	0.24	0.06	0.27	0.03	0.21	1.10	0.50
Unclass. Stram	cDNA	ND	0.00	0.21	1.60	0.15	0.00	0.00	0.00	0.09
	DNA	0.00	0.00	0.06	0.00	0.00	0.00	0.03	0.00	0.03
Total	cDNA	ND	10.80	18.87	14.54	12.43	56.01	42.48	81.25	73.45
	DNA	22.93	19.37	29.01	10.26	15.01	7.03	24.35	33.82	60.07

ND, no data; Unclass. Stram., unclassified stramenopiles.

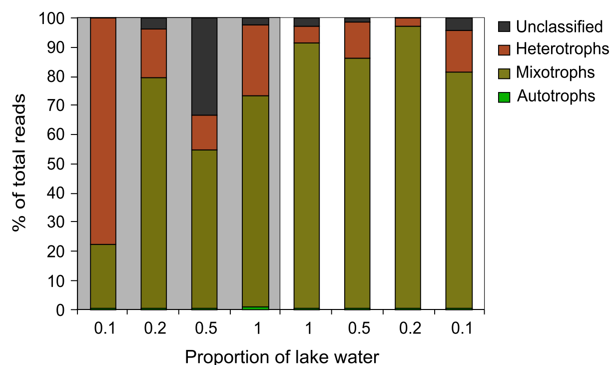

Fig. 5. Community composition of functional groups along the resource continuum at the end of the experiment, based on cDNA reads. The grey zone corresponds to the low light treatment, and the white zone corresponds to the high light treatment.

either due to inorganic nutrient depletion over time or as a result of higher predation pressure by other protists in these treatments.

The taxonomic composition of the initial protist community, collected from under the ice, differed from that reported in August 2008 from the same lake (Charvet *et al*., 2012a), where chrysophytes dominated the Ward Hunt Lake clone libraries. In the present study, dinoflagellates represented a greater proportion of the community. Interestingly, the ratio of dinoflagellates to chrysophytes was similar to that of Lake A in May 2008 when the ice cover was intact (Charvet *et al*., 2012b).

The initial DNA sequences of the protist community clustered with those at the end of the experiment, suggesting persistence of the major taxonomic groups and little response to either experimental treatments or containment (Kim *et al*., 2011). However, the cDNA template reads showed evidence of change, with the high and low light treatments clustering together (Fig. 2). The NRI and NTI can be used to infer the ecological processes involved in shaping phylogenetic community structure. Positive values for both phylogenetic metrics indicated significant clustering in communities from most

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

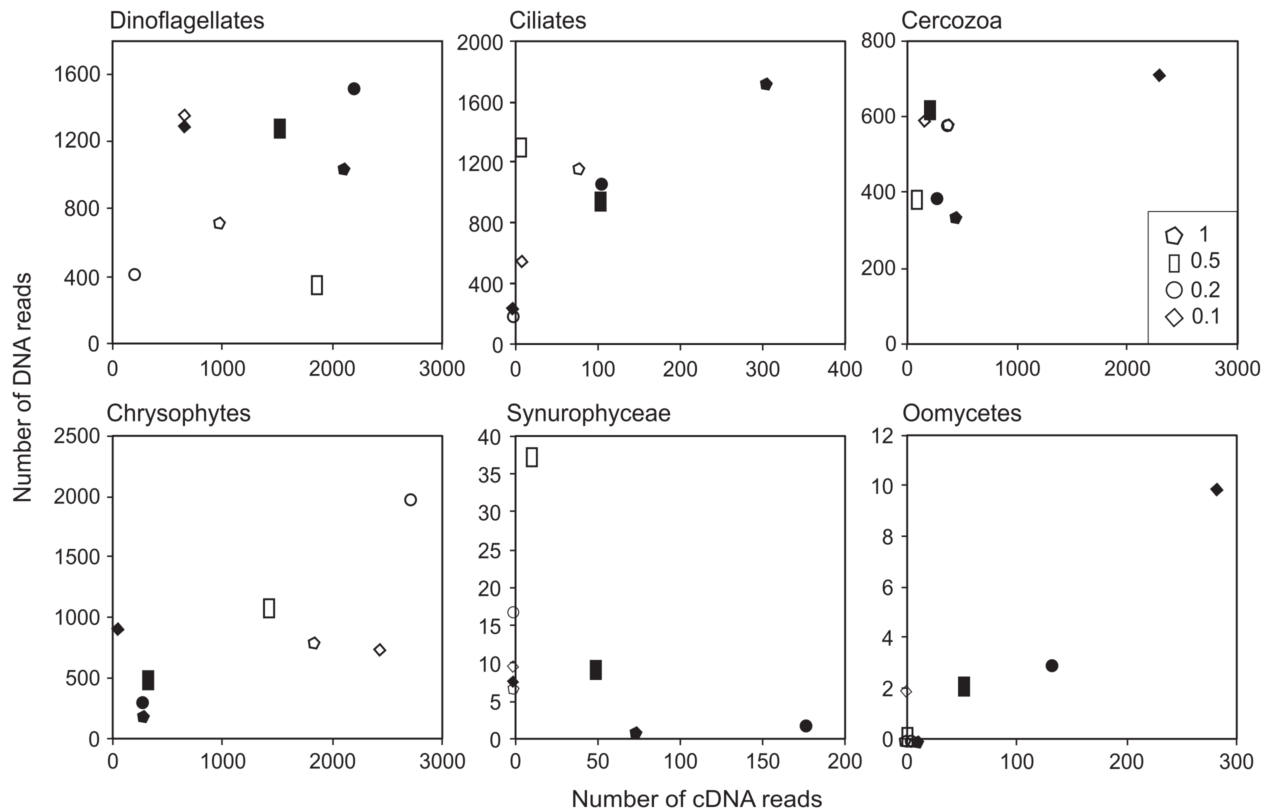

Fig. 6. Number of DNA reads plotted against number of cDNA reads, for dinoflagellates, ciliates, and *Cercozoa* (top panel) and chrysophytes, *Synurophyceae* and oomycetes (lower panel). Open symbols indicate the high light treatments, and closed symbols indicate low light. Dilution is indicated by symbols given in the insert in the *Cercozoa* panel. Note the different scales for each taxonomic class.

treatments, suggesting a strong influence of habitat selection on community structures (Webb *et al.*, 2002). Recently disturbed systems have been observed to be relatively more 'clustered' than undisturbed communities (Ding *et al.*, 2012). The higher NRI and NTI values found for cDNA communities compared with the DNA suggest that the experimental treatments had a greater clustering effect on the cDNA OTUs. This was also reflected at the taxonomic level below phyla and classes, with taxa changing markedly among treatments, which was in contrast to the communities inferred from DNA.

Nuclear DNA includes the 18S rRNA gene, which is present in both active and dormant cells, and can also persist as extracellular DNA (Nielsen *et al.*, 2007; Borin *et al.*, 2008; Charvet *et al.*, 2012b). 18S rRNA reads recovered from the cDNA template indicate the presence of ribosomes from specific protists, and generally, more ribosomes in a cell indicate more active protein synthesis (Poulsen *et al.*, 1993; Not *et al.*, 2009). The phylogenetic metrics based on cDNA suggested a strong influence of habitat selection on the structure of the active community. The proportion of DNA reads vs. cDNA reads provided an indication of which taxa were represented in the active portion of the community. Although the variability of 18S rRNA gene copy numbers among eukaryote taxa makes it difficult to estimate actual abundances, the graphical relation between DNA and cDNA number of reads in Fig. 6 was an indication of relative activity levels for a given taxon in the different treatments. The persistence of the starting community as inferred from the DNA template over the 4-day incubation would suggest insufficient time for degradation of DNA from dead or lysed cells (Danovaro *et al.*, 2005; Stoeck *et al.*, 2007; Terrado *et al.*, 2011; Charvet *et al.*, 2012b). Furthermore, given the slow generation times of one doubling over the 4 days, the experiment may have been too short to detect differences. As exposure to new environmental conditions can cause mortality or trigger a metabolic shutdown with low protein production and less need for ribosomes (Stoeck *et al.*, 2007), cDNA is a more sensitive tool to detect changes in active communities, compared with DNA.

Many taxa identified using high-throughput sequencing of the 18S rRNA genes and cDNA belonged to genera in which mixotrophy has been reported (Sanders, 2011). However, even within the same genus, species may have markedly different trophic strategies (Jones, 1997;

© 2014 Federation of European Microbiological Societies. Published by John Wiley & Sons Ltd. All rights reserved

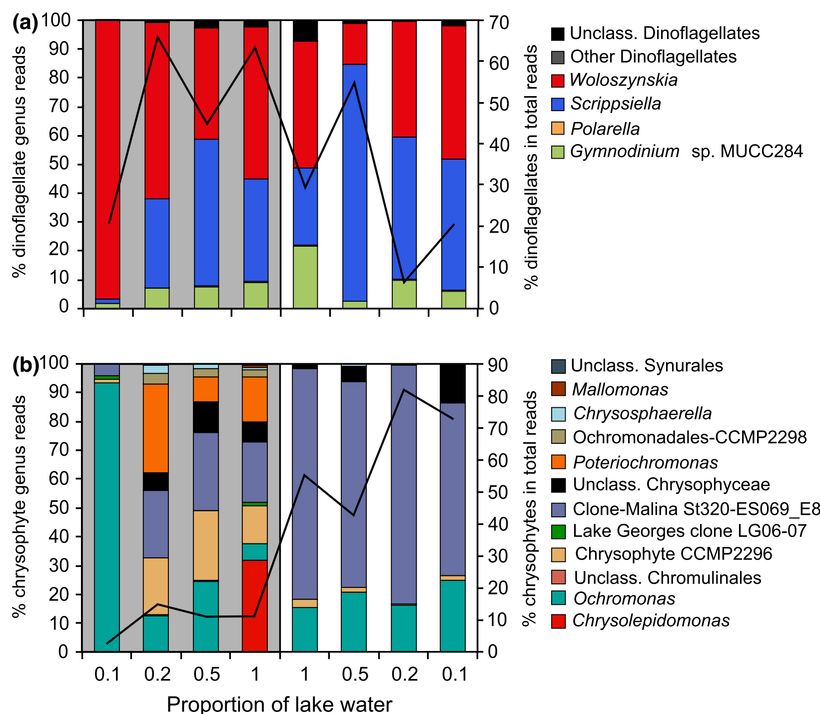

Fig. 7. Details of the proportions of dinoflagellate (a) and chrysophyte (b) genera along the resource continuum based on cDNA sequences. The left *y*-axes indicate the reads for each genus as a percentage of the total reads for dinoflagellates (a) or chrysophytes (b), represented as bar graphs. The right *y*-axes correspond to the phylum reads as a percentage of the total protist reads, represented by the black line in the graph. The grey zone corresponds to the low light treatment, and the white zone corresponds to the high light treatment.

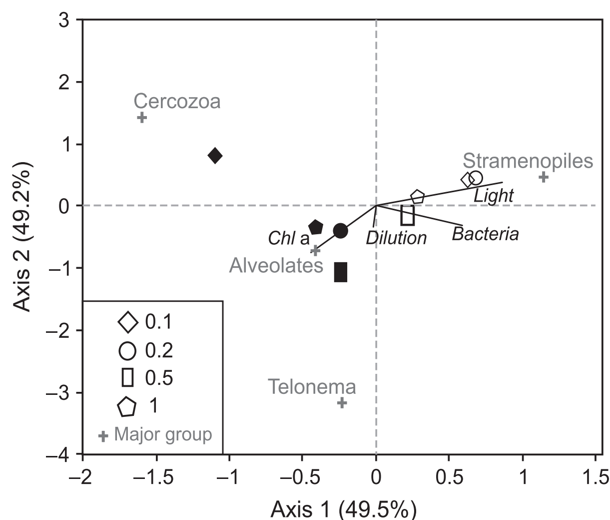

Fig. 8. CCA of cDNA community structure from each treatment combination. Open symbols indicate the high light treatments, and closed symbols indicate the low light treatments. Dilution levels are indicated by the symbols in the insert.

Stoecker, 1998) and may react differently to changed environmental conditions. Protists can switch strategies depending on ambient conditions (Marshall & Laybourn-Parry, 2002; Flynn *et al.*, 2013), and rates or results from feeding experiments may be particular to the experimental conditions. Furthermore, most phytoflagellates are not in cultivation and have not been tested for mixotrophic potential. As we did not directly measure taxon-specific particle uptake, the mixotrophic nature of the genera identified here is speculative and will require further evaluation by direct field and laboratory experiments. By focusing on the taxa identified from cDNA and on the ratios between DNA and cDNA reads, the outcome of each experimental treatment was interpreted in terms of community response to those conditions. These responses indicate the likely community shifts over the course of a summer season as the ice cover recedes, and they are consistent with changes previously reported in a High Arctic lake during an unusual period of ice cover loss (Charvet *et al.*, 2012b).

Impact of light on protist communities

We found that two chloroplast-containing dinoflagellate genera, *Scrippsiella* and *Woloszynskia*, dominated the cDNA reads under low light conditions, indicating active communities. The *Scrippsiella* cDNA reads showed close similarity (99% in GenBank) to the cold ecotype *Scrippsiella* aff. *hangoeii*, reported to coexist with *Gymnodinium* and *Polarella glacialis* in saline lakes in the Vestfold Hills of Antarctica (Rengefors *et al.*, 2008). The *Woloszynskia* cDNA reads were 99% similar to the freshwater species *Woloszynskia pascheri* (Logares *et al.*, 2007), and in a maximum-likelihood tree grouped close to *Woloszynskia* reads from meromictic Lake A near Ward Hunt Lake (Charvet *et al.*, 2012b), suggesting a polar ecotype of the

© 2014 Federation of European Microbiological Societies. Published by John Wiley & Sons Ltd. All rights reserved

Table 4. Results from the Metastats analysis, based on a genus data matrix

Taxon	High light	Low light	*P*-value
Unclassified *Intramacronucleata*	−	+	0.047
Woloszynskia	−	+	0.005
Unclassified *Cercozoa*	−	+	0.008
Unclassified *Trebouxiophyceae*	−	+	0.014
Ochromonas	+	−	0.002
Chrysophyte_clone_ MALINA_St320_ES069_E8	+	−	0.001
Poteriochromonas	−	+	0.047
Unclassified *Ochromonadales*	−	+	0.018

+: significantly more reads; −: significantly less reads.
Values indicate significant differences between high and low light treatments. All dilutions for each treatment were pooled for replication (White *et al.*, 2009).

genus. The mixotrophic capacity of species of *Scrippsiella* and *Woloszynskia* to feed on small eukaryote cells (Jeong *et al.*, 2005; Kang *et al.*, 2011) may explain the strong persistence of these pigmented dinoflagellates at low light. The relatively lower proportion of *Scrippsiella* reads detected by cDNA in the low light, compared with high light, was consistent with reports of encystment at low irradiances (Rintala *et al.*, 2007; Lundgren & Granéli, 2011). In contrast, *Woloszynskia* reads were significantly (Metastats P-value < 0.05) more represented in the low light bottles. These profiles suggest that different strategies were used by the dinoflagellates, with *Woloszynskia* having a lower light threshold than *Scrippsiella*. The presence of reads from both genera at various dilutions suggests that coexistence of the different strategists can occur, although specific light conditions tend to favour one dinoflagellate over another.

High light treatments could be expected to be favourable for photosynthetic protists, but nutrients in ultra-oligotrophic Ward Hunt Lake are generally limiting (Bonilla *et al.*, 2005). It is likely that despite additions of nitrate and phosphate at the start of the experiment, inorganic nutrients were rapidly depleted and insufficient to maintain growth of both bacteria and autotrophs over the 4-day incubation. Strict autotroph lineages were largely absent from the initial community, and the community was dominated by reads attributable to putative mixotrophic phototrophs. The final communities suggest that the addition of nutrients at the start of the experiment did not have a major effect. The proportion of chrysophyte cDNA reads, compared with DNA, was higher in the high light treatments, and the relatively similar distribution of taxa at all dilutions suggests that their activity was influenced more by light than cell concentrations. Numerous chrysophytes have been identified as bacterivores (Kimura & Ishida, 1985; Bird & Kalff, 1986; Caron *et al.*, 1993; Jones & Rees, 1994), while others are considered phytoplankton grazers (Tittel *et al.*, 2003). Most chrysophyte reads at high light were related to the uncultured Beaufort Sea clone St320-ES069_E8 (Balzano *et al.*, 2012) or *Ochromonas*. The Beaufort Sea clone groups with a *Dinobryon* clade in a GenBank distance tree, while the *Ochromonas* reads had BLAST results close to 98–99% similarity to an *Ochromonas* (CCMP1899) isolated from sea-ice. Given the experimental conditions under which they dominated, these two dominant taxa were likely primarily photosynthetic mixotrophs, capable of using phagotrophy to supplement photosynthesis as a source of organic nutrients when dissolved inorganic nutrients are limiting (Stoecker, 1998).

In the low light dilution series, the genus *Poterioochromonas* of the *Synurophyceae* had relatively high proportions of cDNA reads compared with DNA, in contrast to the high light treatments. *Poterioochromonas malhamensis* is primarily heterotrophic, but can rely on photosynthesis when prey concentrations fall below a threshold (Caron *et al.*, 1990). In addition to bacterivory, *P. malhamensis* can also feed on larger phytoplankton cells such as chlorophytes (Zhang & Watanabe, 2001; Tittel *et al.*, 2003). Among chrysophytes, the cDNA reads at low light, in dilutions 0.2, 0.5 and 1, included strain CCMP 2296, isolated originally from sea-ice melt water in Baffin Bay. Its activity exclusively under low light suggests that it is also likely to be a phagotrophic phototroph. While the genera mentioned above dominated stramenopile reads, overall, they represented a small proportion of the total protist community (< 12%). The more abundant reads of other predators such as the strict heterotroph *Protaspis* or the larger dinoflagellates *Woloszynskia* and *Scrippsiella* suggest that the chrysophytes were outcompeted and perhaps under predation pressure from these latter competitors.

Impact of cell concentrations on protist communities

Maximum growth rates for the heterotrophs were observed at the highest dilution (0.1) in the low light treatment, which were dominated by sequences of *Cercozoa*, a specialist heterotroph clade. The main cercozoan genus among cDNA reads was *Protaspis*, a large *Cryomonadida* that uses pseudopodia for prey capture and ingestion (Hoppenrath & Leander, 2006; Adl *et al.*, 2012). *Cercozoa* were not strongly represented by either DNA or cDNA reads in the rest of the experimental communities, and *Protaspis*-like organisms seemed to take advantage of

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

the very specific conditions where both light and prey resources were low. Similarly, among the heterotrophic stramenopiles, oomycetes increased in the low light treatment when prey were scarce. The oomycete reads detected by cDNA were related to *Aplanopsis* and *Leptolegnia*, both belonging to the saprotrophic water moulds, *Saprolegniales* (Spencer *et al.*, 2002; Beakes & Sekimoto, 2009). With prey concentrations below the minimum requirement for most phagotrophs and insufficient light for photosynthesis to make up the energy shortfall, it is possible that dying phytoplankton were a substrate for the saprophytic oomycetes.

In the high light treatment, pigmented protists remained more abundant than nonpigmented cells (not shown) irrespective of cell concentrations. However, the dilutions influenced the community composition of active populations (Fig. 6). *Scrippsiella* was restricted to the relatively low dilutions (1 and 0.5) with the higher prey concentrations, while the chrysophytes produced 18S ribosomal RNA as long as light was favourable and independently of cell density. At the higher dilutions, the chrysophyte Beaufort Sea Malina clone ST320-ES069_E8 and *Ochromonas* represented a greater proportion of the cDNA reads. This would be consistent with a lower requirement for nutrients among chrysophytes compared with the energetic requirements of dinoflagellates, resulting in the stronger dependence of the latter on phagotrophy. Overall, generally we found that chrysophytes were favoured relative to dinoflagellates at high light, especially at higher dilutions, while under low light dinoflagellates were more advantaged.

Impacts of changing ice cover on microbial communities

During the Arctic winter, polar darkness lasts for 4 months and likely causes the collapse of phytoplankton communities, which may be outcompeted and preyed upon by strict heterotrophs such as *Protaspis*. During spring, summer and autumn, the lack of inorganic nutrients may be the factor limiting a strictly phototrophic mode of growth. In many Arctic lakes, picocyanobacteria contribute to the primary production and most likely provide a food source for chrysophytes, as postulated for nearby Lake A (Charvet *et al.*, 2012a). In Ward Hunt Lake, where picocyanobacteria have not been reported, summer primary production is likely carried out almost exclusively by potentially mixotrophic phytoplankton that may rely on heterotrophic bacteria as a nutrient resource. As implied by the results of the present study, the presence or absence of ice cover may influence which protist lineages dominate, with consequences for community succession within the lake.

When High Arctic lakes are ice-free during the summer, selection of chrysophytes might result in increased overall primary production. In oligotrophic Ward Hunt Lake, primary production and phytoplankton biomass are restricted by competition with bacteria for the low nutrients (Bonilla *et al.*, 2005). Bacterivorous chrysophytes could sustain food webs by carrying out primary production independently of inorganic nutrient supply. Their capacity to feed on their bacterial competitors for nutrients would allow them to be more numerous and active than strict phototrophs in these conditions. Furthermore, by transforming heterotrophic bacterial biomass into phytoplankton biomass, the C : N ratio of the seston in mixotrophic flagellate dominated systems would likely be greater than those in which heterotrophic flagellates are the main grazers (Ptacnik *et al.*, 2004). This would allow for more biomass build-up per limiting nutrient, thereby increasing energy transfer efficiency in planktonic food webs (Ptacnik *et al.*, 2004).

In conclusion, this study implies that protist community structure may change in response to light regime and thereby to changes in the frequency and extent of ice cover loss. Specifically, light-limiting conditions appeared to be advantageous to dinoflagellates, while the direct exposure to full sunlight favoured chrysophytes. The generality of these responses requires further investigation; however, they are consistent with a study of nearby Lake A, where an unusual ice-out event in 2008 was accompanied by an increased abundance of chrysophytes (Charvet *et al.*, 2012b). Such changes suggest that ongoing warming of the Arctic may induce a shift in protist communities and may modify the pathway of carbon flow to higher trophic levels.

Acknowledgements

We are grateful to S. Bourget, J. Veillette and D. Sarrazin for assistance in the field and to A. Comeau and A. Monier for bioinformatics support. We thank our colleagues at the IBIS/Université Laval Plateforme d'Analyses Génomiques for the 454 pyrosequencing; the IBIS Centre de Bio-informatique et de Biologie Computationnelle, Compute-Canada and the CLUMEQ Supercomputing Facility for bioinformatics support. This study was made possible by the Natural Sciences and Engineering Research Council of Canada, the Network of Centres of Excellence ArcticNet, the Canada Research Chair programme and the support from Fonds Québécois de la Recherche sur la Nature et les Technologies to Québec Océan and to Centre d'Études Nordiques (CEN). We thank Polar Continental Shelf Project for logistical support and Parks Canada for the use of their facilities in Quttinirpaaq National Park.

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

References

Adl SM, Simpson AGB, Lane CE *et al.* (2012) The revised classification of eukaryotes. *J Eukaryot Microbiol* **59**: 429–514.

Aljanabi S & Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res* **25**: 4692–4693.

Anisimov OA, Vaughan DG, Callaghan T, Furgal C, Marchant H, Prowse TD, Vilhjálmsson H & Walsh JE (2007) Polar regions (Arctic and Antarctic). *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (Parry ML, Canziani OF, Palutikof JP, van der Linden PJ & Hanson CE, eds), pp. 653–685. Cambridge University Press, Cambridge.

Balzano S, Marie D, Gourvil P & Vaulot D (2012) Composition of the summer photosynthetic pico and nanoplankton communities in the Beaufort Sea assessed by T-RFLP and sequences of the 18S rRNA gene from flow cytometry sorted samples. *ISME J* **6**: 1480–1498.

Beakes GW & Sekimoto S (2009) The evolutionary phylogeny of Oomycetes- insights gained from studies of holocarpic parasites of algae and invertebrates. *Oomycete Genetics and Genomics: Diversity, Interactions, and Research Tools* (Lamour K & Kamoun S, eds), pp. 1–24. John Wiley & Sons Inc., Hoboken, NJ.

Bird DF & Kalff J (1986) Bacterial grazing by planktonic lake algae. *Science* **231**: 493–495.

Blazewicz SJ, Barnard RL, Daly RA & Firestone MK (2013) Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. *ISME J* **7**: 2061–2068.

Bonilla S, Villeneuve V & Vincent WF (2005) Benthic and planktonic algal communities in a High Arctic lake: pigment structure and contrasting responses to nutrient enrichment. *J Phycol* **41**: 1120–1130.

Borin S, Crotti E, Mapelli F, Tamagnini I, Corselli C & Daffonchio D (2008) DNA is preserved and maintains transforming potential after contact with brines of the deep anoxic hypersaline lakes of the Eastern Mediterranean Sea. *Saline Syst* **4**: 10.

Caporaso JG, Kuczynski J & Stombaugh J (2010a) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**: 335–336.

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL & Knight R (2010b) PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26**: 266–267.

Caron DA, Porter KG & Sanders RW (1990) Carbon, nitrogen, and phosphorus budgets for the mixotrophic phytoflagellate *Poterioochromonas malhamensis* (Chrysophyceae) during bacterial ingestion. *Limnol Oceanogr* **35**: 433–443.

Caron DA, Sanders RW, Lim EL, Marrasé C, Amaral LA, Whitney S, Aoki RB & Porter KG (1993) Light-dependent phagotrophy in the freshwater mixotrophic chrysophyte *Dinobryon cylindricum*. *Microb Ecol* **25**: 93–111.

Centre d'Études Nordiques (2013) Environmental data from Northern Ellesmere Island in Nunavut, Canada, v.1.0 (2002–2012). Nordicana DI, DOI: 10.5885/44985SL-8F203FD3A CCD4138.

Charvet S, Vincent WF & Lovejoy C (2012a) Chrysophytes and other protists in high Arctic lakes: molecular gene surveys, pigment signatures and microscopy. *Polar Biol* **35**: 733–748.

Charvet S, Vincent WF, Comeau A & Lovejoy C (2012b) Pyrosequencing analysis of the protist communities in a high Arctic meromictic lake: DNA preservation and change. *Front Microbiol* **3**: 422.

Comeau AM, Li WKW, Tremblay J-É, Carmack EC & Lovejoy C (2011) Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. *PLoS ONE* **11**: e27492.

Copland L, Mueller DR & Weir L (2007) Rapid loss of the Ayles Ice Shelf, Ellesmere Island, Canada. *Geophys Res Lett* **34**: L21501.

Danovaro R, Corinaldesi C, Anno AD, Fabiano M & Corselli C (2005) Viruses, prokaryotes and DNA in the sediments of a deep-hypersaline anoxic basin (DHAB) of the Mediterranean Sea. *Environ Microbiol* **7**: 586–592.

Diez B, Pedròs-Aliò C & Massana R (2001) Study of genetic diversity of eukaryotic picoplankton in different oceanic regions by small-subunit rRNA gene cloning and sequencing. *Appl Environ Microbiol* **67**: 2932–2941.

Ding Y, Zang R, Letcher SG, Liu S & He F (2012) Disturbance regime changes the trait distribution, phylogenetic structure and community assembly of tropical rain forests. *Oikos* **121**: 1263–1270.

Dolan J, Gallegos C & Moigis A (2000) Dilution effects on microzooplankton in dilution grazing experiments. *Mar Ecol Prog Ser* **200**: 127–139.

Edgar RC, Haas BJ, Clemente JC, Quince C & Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–2200.

Flynn KJ, Stoecker DK, Mitra A, Raven JA, Glibert PM, Hansen PJ, Granéli E & Burkholder JM (2013) Misuse of the phytoplankton-zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. *J Plankton Res* **35**: 3–11.

Gibson J, Vincent WF, Van Hove P, Belzile C, Wang X & Muir D (2002) Geochemistry of ice-covered, meromictic Lake A in the Canadian high Arctic. *Aquat Geochem* **8**: 97–119.

Granéli E & Carlsson P (1998) The ecological significance of phagotrophy in photosynthetic flagellates. *NATO ASI Ser G Ecol Sci* **41**: 539–558.

Hoppenrath M & Leander BS (2006) Dinoflagellate, Euglenid, or Cercomonad? The ultrastructure and molecular phylogenetic position of *Protaspis grandis* n. sp. *J Eukaryot Microbiol* **53**: 327–342.

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

Huse SM, Welch DM, Morrison HG & Sogin ML (2010) Ironing out the wrinkles in the rare biosphere through improved OTU clustering. *Environ Microbiol* **12**: 1889–1898.

Jeong HJ, Du Yoo Y, Park JY, Song JY, Kim ST, Lee SH, Kim KY & Yih WH (2005) Feeding by phototrophic red-tide dinoflagellates: five species newly revealed and six species previously known to be mixotrophic. *Aquat Microb Ecol* **40**: 133–150.

Jones HJ (1997) A classification of mixotrophic protists based on their behaviour. *Freshw Biol* **37**: 35–43.

Jones RI & Rees S (1994) Influence of temperature and light on particle ingestion by the freshwater phytoflagellate *Dinobryon*. *Arch Hydrobiol* **132**: 203–211.

Kang N, Jeong HJ, Du Yoo Y, Yoon EY, Lee KH, Lee K & Kim G (2011) Mixotrophy in the newly described phototrophic dinoflagellate *Woloszynskia cincta* from western Korean waters: feeding mechanism, prey species and effect of prey concentration. *J Eukaryot Microbiol* **58**: 152–170.

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP & Webb CO (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**: 1463–1464.

Kim DY, Countway PD, Gast RJ & Caron DA (2011) Rapid shifts in the structure and composition of a protistan assemblage during bottle incubations affect estimates of total protistan species richness. *Microb Ecol* **62**: 383–398.

Kimura B & Ishida Y (1985) Photophagotrophy in *Uroglena americana*, Chrysophyceae. *Jpn J Limnol* **46**: 315–318.

Kunin V, Engelbrektson A, Ochman H & Hugenholtz P (2010) Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. *Environ Microbiol* **12**: 118–123.

Landry MR & Hassett RP (1982) Estimating the grazing impact of marine micro-zooplankton. *Mar Biol* **67**: 283–288.

Llames ME, Lagomarsino L, Diovisalvi N *et al.* (2009) The effects of light availability in shallow, turbid waters: a mesocosm study. *J Plankton Res* **31**: 1517–1529.

Logares R, Shalchian-Tabrizi K, Boltovskoy A & Rengefors K (2007) Extensive dinoflagellate phylogenies indicate infrequent marine-freshwater transitions. *Mol Phylogenet Evol* **45**: 887–903.

Lundgren V & Granéli E (2011) Influence of altered light conditions and grazers on *Scrippsiella trochoidea* (Dinophyceae) cyst formation. *Aquat Microb Ecol* **63**: 231–243.

Maranger R, Bird DF & Price NM (1998) Iron acquisition by photosynthetic marine phytoplankton from ingested bacteria. *Nature* **396**: 248–251.

Marshall W & Laybourn-Parry J (2002) The balance between photosynthesis and grazing in Antarctic mixotrophic cryptophytes during summer. *Freshw Biol* **47**: 2060–2070.

Medlin L, Elwood HJ, Stickel S & Sogin ML (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* **71**: 491–499.

Mueller DR, Vincent WF & Jeffries MO (2003) Break-up of the largest Arctic ice shelf and associated loss of an epishelf lake. *Geophys Res Lett* **30**: e2031.

Mueller DR, Van Hove P, Antoniades D, Jeffries MO & Vincent WF (2009) High Arctic lakes as sentinel ecosystems: cascading regime shifts in climate, ice cover, and mixing. *Limnol Oceanogr* **54**: 2371–2385.

Nicholls KH (2009) Chrysophyte blooms in the plankton and neuston of marine and freshwater systems. *Chrysophyte Algae: Ecology, Phylogeny and Development* (Sandgren C, Smol JP & Kristiansen J, eds), pp. 181–213. Cambridge University Press, New York, NY.

Nielsen KM, Johnsen PJ, Bensasson D & Daffonchio D (2007) Release and persistence of extracellular DNA in the environment. *Environ Biosafety Res* **6**: 37–53.

Not F, del Campo J, Balagué V, de Vargas C & Massana R (2009) New insights into the diversity of marine picoeukaryotes. *PLoS ONE* **4**: e7143.

Porter KG & Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* **25**: 943–948.

Poulsen LK, Ballard G & Stahl DA (1993) Use of rRNA fluorescence *in situ* hybridization for measuring the activity of single cells in young and established biofilms. *Appl Environ Microbiol* **59**: 1354–1360.

Prescott LM, Harley JP & Klein DA (2002) *Microbiology*, 5th edn. McGraw-Hill, New York, NY.

Price MN, Dehal PS & Arkin AP (2010) FastTree 2-approximately maximum-likelihood trees for large alignments. *PLoS ONE* **5**: e9490.

Ptacnik R, Sommer U, Hansen T & Martens V (2004) Effects of microzooplankton and mixotrophy in an experimental planktonic food web. *Limnol Oceanogr* **49**: 1435–1445.

Raven J (1997) Phagotrophy in phototrophs. *Limnol Oceanogr* **42**: 198–205.

Raven J (2009) Comparative aspects of chrysophyte nutrition with emphasis on carbon, phosporus and nitrogen. *Chrysophyte Algae: Ecology, Phylogeny and Development* (Sandgren C, Smol JP & Kristiansen J, eds), pp. 95–118. Cambridge University Press, New York, NY.

Rengefors K, Laybourn-Parry J, Logares R, Marshall WA & Hansen G (2008) Marine-derived dinoflagellates in Antarctic saline lakes: community composition and annual dynamics. *J Phycol* **44**: 592–604.

Rintala J-M, Spilling K & Blomster J (2007) Temporary cyst enables long-term dark survival of *Scrippsiella hangoei* (Dinophyceae). *Mar Biol* **152**: 57–62.

Rothhaupt KO (1996) Laboratory experiments with a mixotrophic chrysophyte and obligately phagotrophic and phototrophic competitors. *Ecology* **77**: 716–724.

Sanders RW (2011) Alternative nutritional strategies in protists: symposium introduction and a review of freshwater protists that combine photosynthesis and heterotrophy. *J Eukaryot Microbiol* **58**: 181–184.

Schindler DW & Smol JP (2006) Cumulative effects of climate warming and other human activities on freshwaters of Arctic and Subarctic North America. *Ambio* **35**: 160–168.

Schloss P, Westcott SL, Ryabin T *et al.* (2009) Introducing mothur: open-source, platform-independent,

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved

community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**: 7537–7541.

Sogin ML & Gunderson JH (1987) Structural diversity of eukaryotic small subunit ribosomal RNAs. *Ann NY Acad Sci* **503**: 125–139.

Spencer MA, Vick MC & Dick MW (2002) Revision of *Aplanopsis*, *Pythiopsis*, and "subcentric" *Achlya* species (Saprolegniaceae) using 18S rDNA and morphological data. *Mycol Res* **106**: 549–560.

Stoeck T, Zuendorf A, Breiner H-W & Behnke A (2007) A molecular approach to identify active microbes in environmental eukaryote clone libraries. *Microb Ecol* **53**: 328–339.

Stoecker DK (1998) Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *Eur J Protistol* **34**: 281–290.

Terrado R, Medrinal E, Dasilva C, Thaler M, Vincent WF & Lovejoy C (2011) Protist community composition during spring in an Arctic flaw lead polynya. *Polar Biol* **34**: 1901–1914.

Tittel J, Bissinger V, Zippel B, Gaedke U, Bell E, Lorke A & Kamjunke N (2003) Mixotrophs combine resource use to outcompete specialists: implications for aquatic food webs. *PNAS* **100**: 12776–12781.

Veillette J, Martineau M-J, Antoniades D, Sarrazin D & Vincent WF (2011) Effects of loss of perennial lake ice on mixing and phytoplankton dynamics: insights from high Arctic Canada. *Ann Glaciol* **51**: 56–70.

Vincent WF (2010) Microbial ecosystem responses to rapid climate change in the Arctic. *ISME J* **4**: 1087–1090.

Vincent WF, MacIntyre S, Spigel RH & Laurion I (2008) Physical limnology of high-latitude lakes. *Polar Limnology* (Vincent WF & Laybourn-Parry J, eds), pp. 65–81. Oxford University Press, Oxford.

Vincent WF, Fortier D, Lévesque E, Boulanger-Lapointe N, Tremblay B, Sarrazin D, Antoniades D & Mueller DR (2011) Extreme ecosystems and geosystems in the Canadian High Arctic: Ward Hunt Island and vicinity. *Ecoscience* **18**: 236–261.

Webb CO, Ackerly DD, McPeek MA & Donoghue MJ (2002) Phylogenies and community ecology. *Annu Rev Ecol Syst* **33**: 475–505.

Werner JJ, Koren O, Hugenholtz P, DeSantis TZ, Walters WA, Caporaso JG, Angenent LT, Knight R & Ley RE (2012) Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys. *ISME J* **6**: 94–103.

White J, Nagarajan N & Pop M (2009) Statistical methods for detecting differentially abundant features in clinical metagenomic samples. *PLoS Comput Biol* **5**: e1000352.

Zhang X & Watanabe MM (2001) Grazing and growth of the mixotrophic Chrysomonad *Poterioochromonas malhamensis* (Chrysophyceae) feeding on algae. *J Phycol* **37**: 738–743.

© 2014 Federation of European Microbiological Societies.
Published by John Wiley & Sons Ltd. All rights reserved