Title:

Distribution of micro-eukaryotes during the transition to polar night in a permanently ice-covered Antarctic lake

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Running Title:

Diversity of Antarctic lake micro-eukaryotes

Subject Category

Microbial Ecology and Functional Diversity of Natural Habitats

Abstract:

The McMurdo Dry Valleys of Antarctica harbor numerous permanently ice-covered lakes which provide a year-round oasis for microbial life. Micro-eukaryotes (mainly protists) in these lakes occupy a variety of trophic levels within a relatively truncated food web ranging from primary producers to tertiary predators. Past studies have postulated that protists capable of a mixed trophic mode of growth have an adaptive advantage in these cold, ultra-oligotrophic aquatic environments. Here we report the first molecular study to describe the vertical distribution of the micro-eukaryotic community residing in the photic zone of the east and west lobes of Lake Bonney, a chemically stratified lake in the Taylor Valley. Temporal variation in the micro-eukaryotic populations was also determined in both lobes of the lake during the transition from the 24 h sunlight of the Antarctic summer to complete darkness during the polar night. Clone libraries identified 49 unique phylotypes, which were dominated by clones related to known photosynthetic or mixotrophic protists. Sequence data revealed that protist populations were strongly vertically stratified, with a cryptophyte dominating shallow populations, a haptophyte occupying mid-depths, and chlorophytes residing in the deepest layers of the photic zone. A previously undetected stramenopile related to the nanoplankton, *Nannochloropsis*, occurred throughout the water column. While protist diversity was comparable between the two lobes of Lake Bonney, vertical and temporal variation was evident between the two bodies of water.

Keywords

algae/ice-covered lake/ McMurdo Dry Valleys/mixotrophy/phototrophy/polar night/protists

Introduction

Single-celled eukaryotic organisms (protists) are a diverse and functionally heterogeneous group of microorganisms with a global distribution in aquatic environments. Protists play important and diverse roles in aquatic food webs because they exhibit a range of trophic capabilities related to carbon and energy transformations. The flow of carbon in aquatic ecosystems between primary producers, primary consumers and secondary consumers, is influenced by protists at all levels. Photosynthetic protists contribute significantly to global primary production and incorporate a major portion of inorganic carbon into the food chain (Caron *et al.*, 2009). Phagotrophic protists are important consumers of phytoplankton and bacteria, exerting control over the abundance of these organisms as well as providing a link to higher trophic levels (Sherr and Sherr, 2002). Mixotrophy (combined photosynthetic and heterotrophic ability) is widespread across protists (Boechati *et al.*, 2007; Burkholder *et al.*, 2008; Montagnes *et al.*, 2008; Moorthi *et al.*, 2009), and complicates the early model of the microbial loop (Azam *et al.*, 1983; Fenchel, 2008). The protists also have diverse roles in food web dynamics and the transformation of major nutrients, and individual species can exhibit plasticity in trophic strategies, depending on biotic and abiotic parameters, including light, nutrients and prey availability (Anthony and Fabricius, 2000).

While the advent of molecular tools has dramatically affected our view of microbial biodiversity, much of our knowledge of microorganisms is based on molecular signatures of the bacterial and archaeal domains. Molecular studies are still relatively new for micro-eukaryotes, most habitats are currently undersampled, and little is known about the distribution, diversity, and ecological roles of recently discovered lineages of protists. Molecular surveys in marine environments have revealed a high diversity of 18S rRNA sequences which are unrelated to existing cultured protists (Diez *et al.*, 2001; Lopez-Garcia *et al.*, 2001; Massana *et al.*, 2002; Massana and Pedros-Alio, 2008; Massana *et al.*, 2006; Not *et al.*, 2009; Piganeau *et al.*, 2008). A new interest has produced sequences from a variety of aquatic systems, including temperate marine (Alexander *et al.*, 2009; Countway *et al.*, 2007; Diez *et al.*, 2001; Marie *et al.*, 2006; Not *et al.*, 2007a; Not *et al.*, 2007b; Zhu *et al.*, 2005) and polar oceans (Lovejoy *et al.*, 2006; Moorthi *et al.*, 2009; Piquet *et al.*, 2008), lakes (Lefranc *et al.*, 2005; Lepere *et al.*, 2006; Richards *et al.*, 2005; Wu *et al.*, 2009) as well as hypersaline environments (Alexander *et al.*, 2009; Feazel *et al.*, 2008). More recently, a high throughput pyrosequencing strategy was used to characterize the protistan community residing in anoxic marine ecosystems (Stoeck *et al.*, 2009).

The McMurdo Dry Valleys represent the largest ice-free area in Antarctica (~4000 km^2) and is the coldest and driest desert on Earth. Numerous permanently ice-covered lakes exist within these valleys representing oases for microorganisms in what would otherwise appear to be an inhospitable environment (Priscu *et al.*, 1999). These lakes along with those found in other ice-free areas in Antarctica, represent the only year-round liquid water reservoirs on the continent and differ drastically in their physical, chemical and biological characteristics (Priscu, 1997). Unlike the vast majority of aquatic ecosystems on earth, life in the dry valley lakes is exclusively microbial and food webs are truncated, lacking fish and containing a paucity of metazoans. Each lake is isolated from the outside environment by a permanent 3 to 7 m thick ice cover that prevents wind-driven turbulence and yields highly stable water columns that are often chemically stratified. Gas exchange, allochthonous sediment deposition and well as light penetration are severely limited year round by the permanent ice cover (Fritsen and Priscu, 1999; Howard-Williams *et al.*, 1998). The lakes support stratified populations of phototrophic protists, including cryptophytes, chlorophytes and chrysophytes (DiTullio *et al.*, 1996; Fountain *et al.*, 1999; Lizotte and Priscu, 1998; Priscu *et al.*, 1999), which play a key role in primary productivity and input of inorganic carbon into the food web. Predatory ciliates have also been detected (Laybourn-Parry *et al.*, 1992), and occupy one of the highest trophic levels in the truncated dry valley lake food webs. To date, all studies on phytoplankton species composition have been based on light microscopy or on pigment signatures (Lizotte and Priscu, 1990; Lizotte and Priscu, 1992a; Lizotte and Priscu, 1998; Lizotte *et al.*, 1996). There have been no genetically-based studies except for phylogenetic analyses of a single chlorophyte species, *Chlamydomonas raudensis* UWO 241 (Neale and Priscu, 1995; Pocock *et al.*, 2004).

The McMurdo Dry Valley lakes are the site of an NSF-funded Long Term Ecological Research Project (LTER; http://www.mcmlter.org/) which has provided an intensive set of water column limnological data over the past 20 years. Owing to logistical constraints, most of these studies have been restricted to the austral summer months (November to February); little is known about biodiversity and metabolic processes during the dark winter months. In an effort to understand physiological adaptation, biodiversity and ecosystem function during prolonged exposure to low temperatures and darkness, we conducted the first study on these lakes that encompassed the transition from 24 hour sunlight to the polar night (November 2007 to April 2008). We hypothesized that this solar transition causes a cascade of physiological changes that alter the functional roles of autotrophic and heterotrophic planktonic microorganisms. Extensive sampling of the water column during this transition allowed us to describe for the first time the phylogenetic diversity of the protistan population in the east and west lobes of Lake Bonney.

Materials and methods

Sampling

Temperature and conductivity were measured with a Seabird model 25 profiler as described by Spigel and Priscu (Spigel and Priscu, 1998). Chlorophyll-a was determined with a bbe Moldaenke profiling spectrofluorometer. This instrument was lowered at a rate that produced ~10 measurements m^{-1}, which was adequate to define the highly layered phytoplankton species. Discrete water samples for nutrient analysis were collected at 1-3 m intervals throughout the water column, immediately filtered through Whatman GF/F filters, and stored at -20 ^{o}C for no longer than 2 months before processing. All inorganic nitrogen species were determined with a Lachat autoanalyzer using methods described by Priscu (Priscu, 1997). Owing to the low levels of soluble reactive phosphorus (SRP) in these systems, SRP was analyzed manually using the antimony-molybdate method (Stickland and Parsons, 1972) with a 10 cm pathlength cuvette. Dissolved oxygen was measured on an unfiltered aliquot of the discrete samples immediately upon collection using Winkler titrations.

Samples for phylogenetic analysis were collected at 6, 13, 18, and 20m in the east lobe (ELB) and 10, 13, 15, and 20m in the west lobe (WLB) of Lake Bonney from February to April 2008. All collection depths are relative to the piezometric water level in the ice borehole and represent microbial populations throughout the trophogenic zone (Priscu, 1995). Samples (1 liter) were vacuum concentrated (5 kPa) onto 47 mm 0.45 µm Durapore polyvinylidene fluoride membrane filters (Millipore). The filters were frozen immediately in liquid nitrogen and transported frozen to McMurdo Station, where they were stored at -80^{o}C until DNA extraction. DNA was isolated from a whole filter using the MP DNA kit (MP Biomedicals, CA) following the kit's instruction.

PCR amplification and RFLP

18S rRNA genes were amplified from DNA with universal eukaryote primers: EK-82F (5′-GAAACTGCGAATGGCTC) and EK-1520R (5′-CYGCAGGTTCACCTAC) (Lopez-Garcia *et al.*, 2001) to generate products for cloning. PCR was performed in triplicate using 25 cycles of 95°C for 1 min, 52°C for 1 min, and 72°C for 2 min. Gel purified PCR products were ligated into pGEM-T Easy Vector (Promega, WI) and transformed into TOP 10 cells. Colonies were selected for PCR amplification to screen the presence of inserts using standard M13 primers and the products were subjected to restriction fragment length polymorphism (RFLP) analysis. Positive PCR products were digested with the restriction enzyme *Hae*III (Fermentas, MD) for 3 h at 37ºC. RFLP patterns were visualized in 2.5% agarose gels. Each unique RFLP pattern was sequenced at least once for phylogenetic identification.

Phylogenetic analysis

Sequencing reactions were performed using the BigDye Terminator v3.1 cycle sequencing kit (ABI, CA) with M13R primer and the fragments were sequenced on an Applied Biosystems 3730×l DNA Analyzer (ABI, CA). Nucleotide-nucleotide BLAST (www.ncbi.nlm.nih.gov/BLAST/) was used to search GenBank for nearest relative sequences. BLAST results and representatives for each archive were aligned by using CLUSTAW from the MEGA 4.1. Alignment was hand corrected when necessary. Phylogenetic trees were constructed by Neighbor-joining method with a Kimura two-parameter distance model using MEGA 4.1 software. Bootstrapping was used to estimate reliability of phylogenetic trees with 1,000 replicate trees. The 18S rRNA gene sequences reported in the current study have been deposited in GenBank under accession numbers GU969060 to GU969102.

Operational taxonomic unit (OTU) richness was compared by rarefaction analysis according to Kenneth et al. (Kenneth *et al.*, 1975) within each 18S rRNA clone library as well as within libraries pooled by lake depth. An OTU was defined as a clone exhibiting a unique RFLP pattern. All unique OTUs were confirmed by sequencing. Species diversity was calculated using the Shannon-Wiener index (Krebs, 1989).

Real Time PCR

Quantification of 18S rRNA was performed using real-time quantitative PCR (qPCR) a Bio-Rad iCycler iQ detection system using the primers EUK345f (AAGGAAGGCAGCAGGCG) and EUK499r (CACCAGACTTGCCCTCYAAT) (Zhu *et al.*, 2005). QPCR was performed in duplicate in a 25-μL reaction mixture containing 1 μL of cDNA, 1.5 μL of each primer (10 pmol μL^{-1}) and 12.5 μL iQ SYBR Green Supermix (Bio-Rad, CA). Amplification conditions were 5 min at 95°C followed by 35 cycles of 1 min at 94°C, 20 s at 60°C**,** and 30 s at 72°C. To determine the melting temperature and PCR product specificity, a melting curve was acquired by heating from 50°C to 95°C. The threshold cycle (Ct) was defined as the cycle number at which a statistically significant increase in fluorescence was detected. Standard curves for real-time PCR were developed from plasmids containing the target insert.

Results

Environmental description

Lake Bonney is a chemically stratified lake in the McMurdo Dry Valleys (77°00′S, 162°52′E) of southern Victoria Land, Antarctica, which is typically covered by 3 to 5 m of permanent ice.

Lake Bonney has two 40 m deep lobes, which are separated by a 13 m deep bedrock sill that allows exchange of the fresh surface waters, but prevents mixing of the deep saline waters between the two basins. The deep waters of each basin are chemically distinct as a result of this isolation (Priscu, 1997; Priscu *et al.*, 2008). Physical and chemical profiles in Lake Bonney reveal the highly stratified vertical structure of the water columns within each basin. The water temperature reaches a maximum in the middle of the water column and never exceeds 4.9 ^{o}C in ELB and 2.2 ^{o}C in the WLB. The lowest temperatures recorded during this study in ELB and WLB were -2.1 and -4.3 ^{o}C, respectively, and occurred just above the bottom (~38 m) (Figure 1 A, B). ELB and WLB both have steep mid-depth conductivity gradients with values ranging from 0.36 to 109.13 mS cm^{-1} and 0.74 to 79.60 mS cm^{-1}, respectively. The water column of Lake Bonney does not mix on an annual scale (mixing time is about 50,000 years; (Spigel and Priscu, 1996) and probably has not done so for more than 1000 years (Lyons *et al.*, 2006; Spigel and Priscu, 1998). Both lobes of Lake Bonney showed distinct chlorophyll-a maxima at 13.5 m, just above the chemocline (Figure 1 C, D). Chlorophyll levels in WLB reached 15.5 µg l^{-1}, which was almost twice those in the deep maxima of ELB reflecting higher levels of phytoplankton primary production in the former (Priscu and Neale, 1995). Relatively little chlorophyll-a was present below 20m, a depth where insufficient light exists to support photosynthesis (i.e., the bottom of the trophogenic zone). The dissolved oxygen in the trophogenic zone of both lobes of Lake Bonney was highly supersaturated with respect to the mixing ratio in air above the lake and show maxima that coincides with the chlorophyll-a maxima (Figure 1 C, D). Oxygen levels decreased precipitously beneath the chemoclines. The sub-oxic water in WLB supports active denitrification (Priscu, 1997; Priscu *et al.*, 2008; Priscu *et al.*, 1996), whereas bulk denitrification is absent in the sub-oxic waters of ELB, presumably due high and potentially toxic levels of certain salts (Ward *et al.*, 2003; Ward *et al.*, 2005; Ward and Priscu, 1997). Differential rates of denitrification between the two lobes led to large difference in nitrate concentration observed beneath the chemoclines, whereas ammonium remained high in both lobes (Figure 1 C, D). Phytoplankton populations in the trophogenic zone above the chemocline have been shown to be P-deficient and supported to a large extent by upward diffusing nutrients across the chemocline (Dore and Priscu, 2001; Priscu, 1995).

Micro-eukaryote diversity

18S rDNA clone libraries were generated from 21 samples collected from each lobe of Lake Bonney at weekly time-points during the polar night transition (late February to early April) (Table 1). Based on a cut-off value of <97% similarity, a total of 49 phylotypes were identified. More than 90% of our partial 18S rRNA sequences (800 to 1024 bp) aligned with taxonomically assigned known groups. Clones were organized into six eukaryotic supergroups; the majority (~85%) of the clones aligned with known phototrophic microbial eukaryotes. These data agreed well with previously published reports based on microscopy showing that phototrophic nanoflagellates dominate the phytoplankton biomass, while heterotrophic nanoflagellates were relatively low (Laybourn-Parry and Pearce, 2007; Parker *et al.*, 1982). The two most dominant protist groups, Cryptophyta and Chlorophyta, represented more than half of the total clones (Figure 2). Stramenopiles were also prevalent in both lobes, representing 22.1% of the total clones. Haptophytes were more abundant in ELB (10.5%) than WLB (7.9%). The remainder of the clones were associated with known heterotrophic or mixotrophic groups (alveolates, ciliates, fungi and choanoflaellids).

Phototrophic protists dominated samples collected from either lobe both at the level of clone representation and number of phylotypes. Four phylotypes were affiliated with nuclear sequences of Cryptophyceae (Figure 3A). The vast majority of the sequences derived from samples collected from either lake were related to the marine picoplankton, *Geminigera cryophila* (Figure 3A). The highest phylogenetic diversity was observed in the stramenopile group (Figure 3B). The most prevalent stramenopile detected was related to *Nannochloropsis* sp. (Figure 3B), a photosynthetic stramenopile with cosmopolitan distribution (Fawley and Fawley, 2007). A number of phylotypes were related to chrysophytes, but had no cultivated representatives (Figure 3B). Haptophytes were represented by two phylotypes (Figure 2A). The majority of haptophyte sequences were most closely related to the prymnesiophyte, *Isochrysis galbana* (Figure 3A). Chlorophytes were also highly represented in clones libraries of both lakes, and seven phylotypes were identified in total. Several sequences were related to known polar *Chlamydomonas* species (Figure 3C). In addition, we detected several 18S rRNA clones of the Lake Bonney green alga, *Chlamydomonas raudensis* UWO 241, one of the few phototrophic protists isolated from Lake Bonney more than 20 years ago (Neale and Priscu, 1995).

Approximately 15% heterotrophic eukaryote sequences were detected in the libraries, including two phylotypes belonging to the alveolate supergroup. Most of the phylotypes detected were ciliates (Figures 2A, 3A). Lastly, we identified seven phylotypes related to Fungi, including two phylotypes related to cercozoans and four choanoflagellids (Figure 3A).

Vertical distribution of Lake Bonney micro-eukaryotes

Total clones across all libraries were pooled by sampling depth to examine the vertical distribution of major eukaryotic groups (Figure 4). Cryptomonad sequences represented >60%

of all sequences in the shallow waters of both lobes. Haptophytes were detected in mid-depths (13-m) of ELB (37% total clones) and WLB (17% total clones), as well as the deeper water column of WLB (Figure 4B). Chlorophyte sequences dominated the libraries generated from 18m and 20m lake samples of ELB (64% and 94% total clones, respectively), and 13m (40% total clones), 15m (62% total clones) and 20m (43% total clones) depths of WLB. Stramenopile sequences were detected in libraries generated at all sampling depths in both lobes of Lake Bonney, comprising 4 to 41% of the total clones in ELB and 15 to 33% in WLB. Last, sequences related to fungi (<10% total clones) were detected in only the shallow depths of both lobes, while alveolate sequences (2 to 11% total clones) were recovered throughout the water column. The majority of these were related to known choanoflagellids (Figure 2).

Eukaryote diversity in Lake Bonney was estimated by constructing rarefaction curves (Figure 5). Only the curves constructed from the deep layer populations (20 m) approached the asymptote, indicating that for the majority of the sampling depths, we did not saturate the assemblage diversity with our sequencing efforts. Species diversity, estimated as Shannon-Wiener index (H), and species evenness (E) varied between sampling depths (Table 1). Eukaryotic populations residing at 13m in both lobes exhibited the highest species diversity and evenness. ELB 13m populations exhibited a higher species diversity (H=2.18) compared with WLB 13m (H=1.84), while both sites exhibited comparable species evenness (E=0.73-0.74). Deeper layers of both lobes exhibited low species diversity (H=0.69-0.99 in ELB; H=1.2-1.45 in WLB) relative to shallower depths.

Influence of polar night on micro-eukaryote populations

The transition from 24 h full sunlight to complete darkness during the summer-winter transition represents one of the most variable climatic events that the microbial communities are exposed to in the dry valley lakes. To investigate the effects of the polar night transition on the eukaryotic population as a whole, we quantified 18S rRNA transcript levels via qPCR at weekly sampling points throughout the polar night transition (Figure 6). Early in the polar transition, PAR declined rapidly as a function of lake depth. This effect was most pronounced in our earliest sampling date (Feb 27, 2008) in ELB (Figure 6A). PAR declined gradually throughout the transition from summer to polar winter, and time-points later than March 29, 2008 exhibited PAR levels that were <1 μmol photons m^{-2} s^{-1} throughout the water column of both lobes (Figure 6A,B).

Both ELB and WLB samples exhibited maximum 18S rRNA levels at a depth of 13m in the water column (Figure 6C,D). Populations residing at 13m in both lobes also exhibited high variability in response to the transition to polar night. Highest transcript levels (up to 8×10^{10} 18S rRNA copies/L) were observed early in the transition, and declined (down to 2×10^{9} 18S rRNA copies/L) as the polar transition progressed. However, an eight- and three-fold transient rise in 18S rRNA levels were observed in 13m populations of WLB and ELB, respectively, as well as a 10-fold increase in 18S rRNA in WLB-15m at the 29 March 2008 timepoint (Figure 6C,D). Levels of 18S rRNA in 20m populations of WLB as well as 18m and 20m in ELB were 10-100 fold-lower compared with 13m populations, and generally remained comparable low (<1 x 10^{10} 18S rRNA copies/L) throughout the polar transition.

Alterations in distribution of major protist groups during the polar night transition were monitored by sequencing/RFLP screening of 18S clone libraries generated from samples collected weekly in ELB and WLB during the transition from summer to polar winter (Figure 7).

We focused our investigation on 6m, 13m and 18m for ELB, and 10m, 13m and 15m for WLB, as our qPCR results indicated these depths exhibited the highest 18S rRNA levels in both lakes as well as the highest variability in response polar night (Figure 6C,D). Chlorophytes and haptophytes remained below 10% in all clone libraries generated from shallow sampling depths (6m or 10m in ELB and WLB, respectively), while cryptophytes (dominated by *G. cryophila*) were prevalent (40 to 85% vs. 63 to 74% total clones in ELB vs. WLB, respectively) throughout the polar transition in shallow depths of both lobes (Figure 7A,B). In contrast, stramenopiles increased by 2-fold during the transition in shallow waters of ELB, but remained low (10-15% total clones) in WLB. While the trends between the 13m populations were similar between ELB and WLB at the level of 18S rRNA abundance (Figure 6C,D), the distribution of the algal groups was not comparable between the two lakes at this depth. Haptophytes related to *Isochrysis* made up 61% of the clones in 13 m ELB early in the polar transition (Figure 7C), but were replaced by stramenopiles during the polar transition. In contrast, 13m samples of WLB exhibited a 4-fold increase in haptophytes in response to the polar transition, while the abundance of stramenopile sequences remained low (15-20% total clones; Figure 7D). Most notably, chlorophytes were more abundant in WLB 13m and made up 40-62% of sequences in the clone libraries in the early and late timepoints (Figure 7D). Clone libraries from 18m ELB and15 m WLB clone libraries (90 vs. 77%, respectively) were dominated by chlorophytes early in the transition, but were replaced by stramenopiles as the sunlight decreased (Figure 5E,F). The abundance of sequences related to alveolates and fungi were variable throughout the polar transition, but generally represented below 15% of the total sequences.

Discussion

While molecular analyses on the bacterial communities residing in Antarctic lakes has begun to describe the phylogeny and functionality of the prokaryotic community (Glatz *et al.*, 2006; Karr *et al.*, 2006; Karr *et al.*, 2003), our understanding of the diversity and ecological roles the eukaryotic microorganisms remains limited. Microbial eukaryotes play critical roles in the dry valley lake food web, as they occupy multiple trophic levels ranging from primary producers to tertiary consumers. (Laybourn-Parry, 2002; Laybourn-Parry *et al.*, 1997; Laybourn-Parry and Pearce, 2007). We showed that photosynthetic protists dominated the clones libraries generated from the trophogenic zones of Lake Bonney and comprised more than 80% of the 18S rDNA clones from either the east or west lobe water columns despite low light. Photoautotrophs were dominated by phylotypes related to *Geminigera cryophila* (~30%), *Isochrysis* sp. (~10%), *Nannochloropsis* sp. (~15%) and *Chlamydomonas* (~16%), representing each of the major photosynthetic groups (cryptophytes, stramenopiles, haptophytes, and chlorophytes, respectively). The phylotype diversity in Lake Bonney is low (H=0.69 to 2.18) given the diversity across phyla when compared with molecular studies based on 18S rDNA in other oligotrophic aquatic systems in lower latitudes (Diez *et al.*, 2001; Lopez-Garcia *et al.*, 2001; Moon-van der Staay *et al.*, 2001; Richards *et al.*, 2005; Romari and Vaulot, 2004). However, the diversity indices in Lake Bonney were comparable to diversity indices reported for marine Antarctic microbial eukaryote community (2.12-2.26; Piquet *et al.*, 2008). Thus, micro-eukaryote diversity in high latitude aquatic systems may be generally lower compared with lower latitude ecosystems.

Cryptophytes have been observed to rely heavily on mixotrophy for carbon supplementation in high latitude lakes, even during the summer when primary productivity peaks (Marshall and Laybourn-Parry, 2002). One of the most abundant organisms we detected in the shallow populations of both lobes of Lake Bonney throughout the polar night transition was a cryptophyte related to *Geminigera cryophila*. *G. cryophila* is a polar cryptophyte originally isolated from the Ross Sea, Antarctica. However, nanoplankton related to *G. cryophila* have been detected in other low latitude freshwater and marine systems (Chen *et al.*, 2008; Richards *et al.*, 2005; Romari and Vaulot, 2004), indicating that it is not restricted to low temperature aquatic systems. *G. cryophila* is a source of prey for plastid-sequestering ciliates, such as *Mesodinium rubrum* (Johnson *et al.*, 2006). While *G. cryophila* has not been previously observed in the McMurdo Dry Valley lakes, it seems probable that it is the same abundant cryptophyte in Lakes Fryxell, Hoare, and Bonney previously identified by microscopic methods (Laybourn-Parry *et al.*, 1997; Laybourn-Parry *et al.*, 1992; Lizotte and Priscu, 1992c), and we have molecular evidence that an organism related to *G. cryophila* was highly abundant in samples collected from all three MCM LTER lakes during the summer of 2005 (Bielewicz and Morgan-Kiss, unpub. data). Thus, *G. cryophila* is likely a successful protist that dominates year-round in all of the dry valley lakes. Mixotrophic ability via phagotrophic feeding on bacteria presumably contributes to the adaptive success of this cryptophyte and allows for heterotrophy when available light is extremely limited (Laybourn-Parry, 2002; Laybourn-Parry *et al.*, 2005; Roberts and Laybourn-Parry, 1999; Roberts and Laybourn-Parry, 2000).

Stramenopiles are a diverse group of protists, many of which are uncultured and encompass both autotrophic and heterotrophic members (Massana *et al.*, 2004). In contrast with the recently discovered stramenopile diversity in other temperate environments, stramenopiles in both lobes of Lake Bonney were dominated by one phylotype, a close relative of a marine *Nannochloropsis.* Despite the high abundance of sequences related to this organism in our clone libraries, stramenopiles have not been previously observed in the dry valley lakes. This is likely because of the small size (<2 μm) and indistinguishable morphology characteristic of this genus, as well as potential problems caused by preservation of samples for subsequent light microscopic examination. *Nannochloropsis* is a cosmopolitan picoplankton capable of incorporating dissolved organic carbon in laboratory cultures (Xu *et al.*, 2004) that lacks chlorophyll pigments other than chlorophyll-a and accumulates high levels of a variety of carotenoids (Fisher *et al.*, 1998). Production of high levels of carotenoids over other chlorophylls would be an advantage for photochemically derived energy production in the extreme shade and blue-green light environment of the ice-covered lakes (Lizotte and Priscu, 1992b). *Nannochloropsis* sp. also produces high levels of unsaturated fatty acids, including the very long chain polyunsaturated fatty acid, eicosapentaenoic acid, EPA (20:5ω3) (Rocha *et al.*, 2003). We detected EPA in lipid extracts from samples collected from 5m (7.53% total lipids), 10m (5.29% total lipids) and 15m (1.13% total lipids) depths in WLB and 6m (5.34% total lipids) from ELB in samples collected from summer 2005 (Morgan-Kiss, unpubl. data). *Nannochloropsis*-dominated algal blooms have been detected in other aquatic systems during cold-water periods (Fawley and Fawley, 2007; Fietz *et al.*, 2005; Krienitz *et al.*, 2000), suggesting that they are well adapted to low temperatures. While it has been hypothesized that flagellated protists dominate the dry valley lake microbial food webs (Laybourn-Parry, 2009), *Nannochloropsis* is a nonflagellated, nonmotile alga. Our 18S rDNA sequence data show that *Nannochloropsis* is abundant throughout the photic zone of Lake Bonney and may play an important role in the production and biogeochemical dynamics in this ecosystem, particularly during the polar night.

Prymnesiophytes related to the genus *Isochrysis* were abundant in the clone libraries constructed from the mid-depths (13 m) of both lobes of Lake Bonney, and persisted throughout the transition to polar night. Molecular and lipid biomarkers for *Isochrysis* have also been detected in the sediments of Lake Fryxell (Jaraula *et al.*, 2010) and Ace Lake, Antarctica (Volkman *et al.*, 1986) indicating that *Isochrysis* has a wide distribution among Antarctic lakes. A previous study based on inverted light microscopy reported that a chrysophyte identified as *Ochromonas* sp. was present at very high biomass in upper and mid-depths of the east lobe of Lake Bonney (Morgan-Kiss *et al.*, 2006; Tursich, 2002), which is contrary to our present study where we detected only a few chrysophyte 18S rDNA sequences. This discrepancy could be the result of seasonal variation in phytoplankton species (the microscopic study reporting *Ochromonas* sp was based on samples collected between 1989 and 2000) or the fact that past microscopic observations may have misidentified *Isochrysis* as an *Ochromonas* sp.

One of the few phototrophic protists from the dry valley lakes that has been cultivated is the chlorophyte *Chlamydomonas raudensis* UWO241, which was isolated from the deep photic zone (18m) of the east lobe of Lake Bonney (Neale and Priscu, 1995). *C. raudensis* possesses a unique organization of the photochemical apparatus to efficiently harvest blue wavelength light under extreme shade conditions; however, it is incapable of supplementing inorganic carbon fixation with uptake/breakdown of dissolved organic carbon (Morgan-Kiss *et al.*, 2005; Morgan *et al.*, 1998). This inability to utilize organic carbon supports our observed decline in the green algal clones as sunlight decreases in late March and early April (Figure 7). Thus, while cryptophytes related to *G. cryophila* could rely on strict heterotrophy throughout the polar night, dry valley *Chlamydomonas* sp. residing in the deep photic layers may not supplement photochemically-driven energy acquisition with heterotrophy.

Heterotrophic protists acquire carbon and energy mainly by grazing on bacteria or small phytoplankton and play important roles in microbial food webs by maintaining picoplankton abundance and remineralization of nutrients. Alveolates, particularly the ciliates in this group, are consumers of bacteria and other protists. Laybourn-Parry and colleagues (Ellis-Evans *et al.*, 1998; James *et al.*, 1998; Laybourn-Parry *et al.*, 1995; Laybourn-Parry *et al.*, 1992; Roberts and Laybourn-Parry, 1999) have described several ciliates in Lake Fryxell as well as other ice-covered Antarctic lakes. These authors also showed that Lake Bonney has reduced numbers of ciliates compared with other Antarctic lakes in the region, a result supported by our clone library results.

Little data exists on the presence of fungi in the lakes of the McMurdo Dry Valleys. We detected the Cercozoan *Crythecomonas* in both lobes of Lake Bonney. *Crythecomonas* is a colourless nanoflagellate that is known to feed on diatoms in marine environments (Slapeta *et al.*, 2005). Several clones were related to Chytridiomycota, which contains phytoplankton parasites. Choanoflagellates are single-celled or colony forming microbial eukaryotes that are ubiquitously distributed in aquatic environments. There is often a positive correlation between primary producers and choanoflagellate densities, indicating that they play an important role in food web dynamics in polar aquatic systems (Buck and Garrison, 1988). Thus, Lake Bonney harbors a variety of predatory micro-eukaryotes, which could play an important role in top-down control of the primary producer diversity and abundance. Interestingly, the fungal phylotypes observed in Lake Bonney were distinct from those observed in the surrounding soils of the lake (Connell *et al.*, 2006), presumably because the permanent ice cover presents Aeolian sediment deposits reaching the water column.

Our 18S rDNA clone library data provides the first comprehensive genomic data set on the presence and distribution of microbial eukaryotes in a McMurdo Dry Valley lake. These results reveal that micro-eukaryotes are a diverse group within this lake and play a significant role in water column trophic dynamics. Importantly, we show that many of the phototrophic microalgae possess the potential to obtain energy from organic matter during the transition from 24 h sunlight to the darkness of the Polar Night, a key strategy for surviving in these and other Antarctic lakes.

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Titles and Legends to Figures

Figure 1. Environmental data for the water columns of the east and west lobes of Lake Bonney collected on 12 and 16 March 2008, respectively. All depths are from the piezometric water level within the sampling hole. SRP = Soluble Reactive Phosphorus; chlorophyll-a levels are based on high resolution (~10 cm depth intervals) *in situ* spectrofluorometry. The ice cover at this time was ~ 3.5 m thick.

Figure 2. Distribution of total 18S rRNA clones from ELB and WLB. Clone libraries were generated from a variety of sampling depths and times as described in the Methods. Data represents a total of 860 clones screened by a combined RFLP and sequencing approach.

Figure 3. Neighbor-Joining tree based on 18S rRNA partial sequences inferred with MEGA 4 software. The numbers at the notes show bootstrap support at percentages based on 1,000 replicates. Only bootstrap values greater than 50% are given. A. All 18S rRNA sequences. B. Sub-tree showing stramenopiles. C. Sub-tree showing chlorophytes.

Figure 4. Depth distribution of 18S rRNA clones from major photosynthetic groups in the east (A) and west (B) lobes of Lake Bonney. Clone libraries were screened by a combined RFLP/sequencing approaching using samples collected at various times throughout the transition from summer to polar night. Data represents the combined set of sequences across all time points. Arrows denote the chemocline.

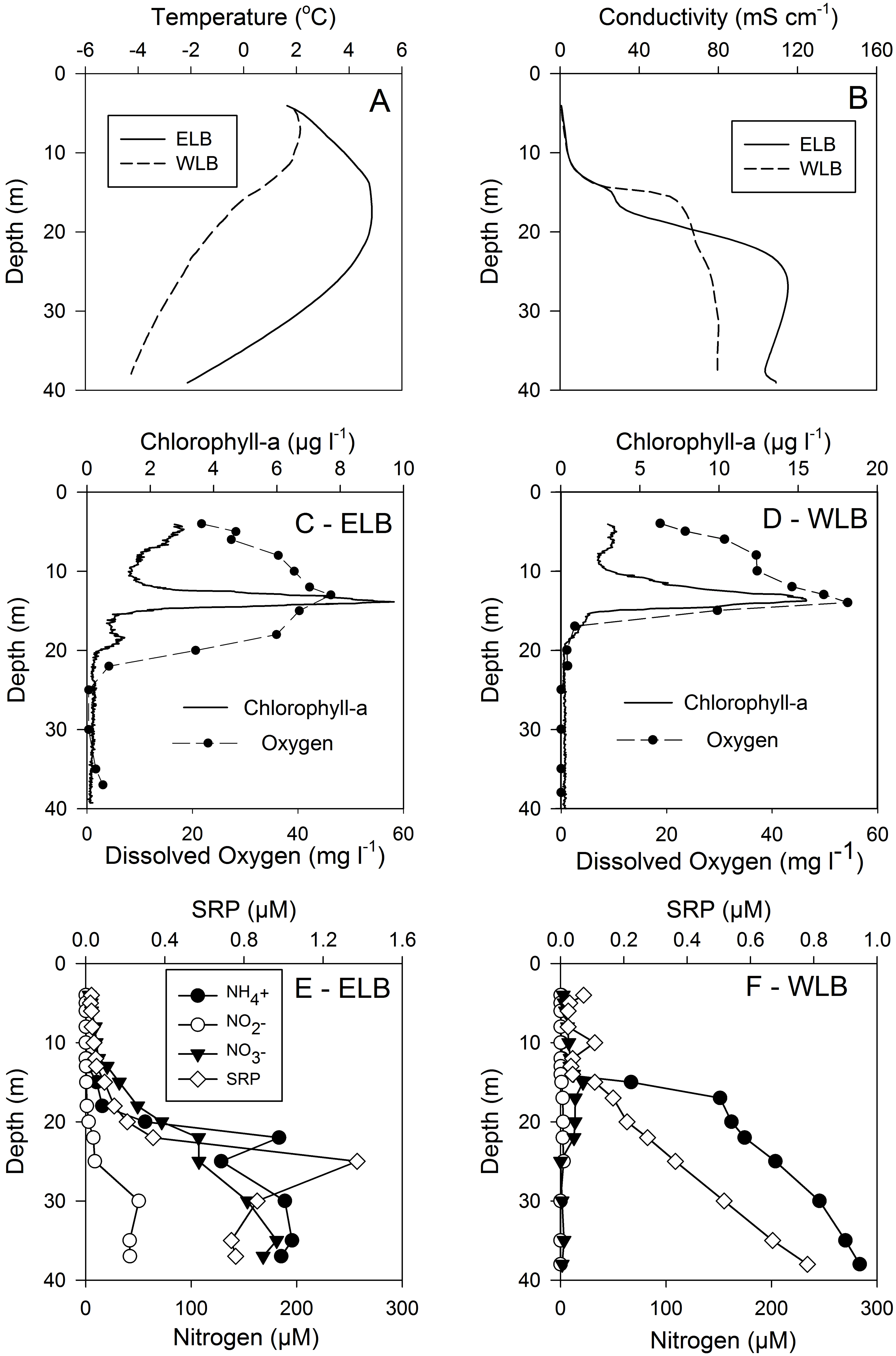
Figure 5. Rarefactions curve analysis for 18S rRNA clone libraries for the combined set of sequences detected across all timepoints from east (A) and west (B) lobes of Lake Bonney. A unique OTU was defined as a distinct RFLP pattern. All unique RFLPs were confirmed by sequencing.

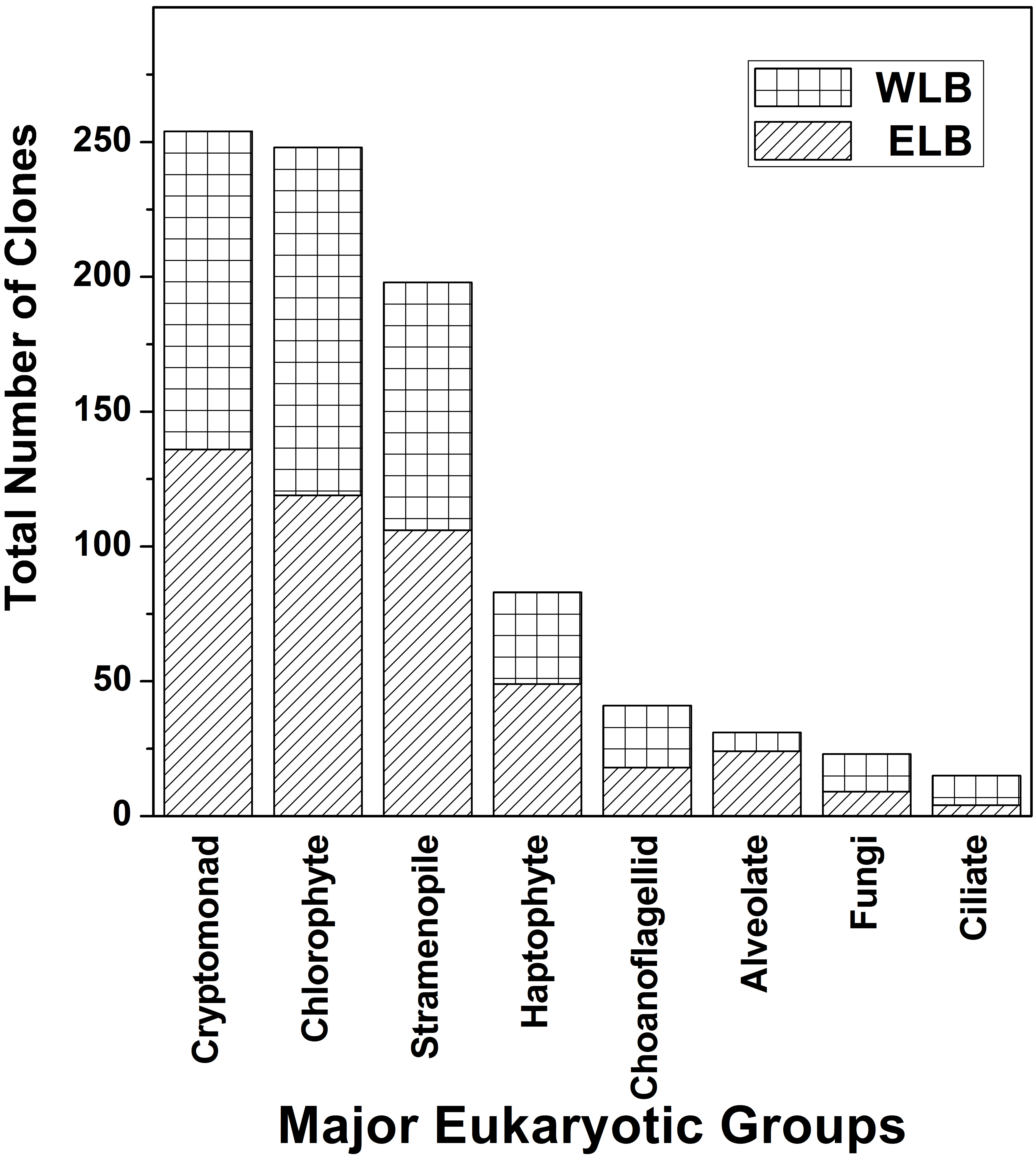
Figure 6. Variations in light and 18S rRNA abundance in the east (A,C) and west (B,D) lobes of Lake Bonney during the polar night transition. A,B. Changes in photosynthetically available radiation, PAR. C,D. 18S rRNA abundance. Environmental RNA was extracted from lake samples collected at various depths and weekly time points during the polar night transition. Transcript levels were quantified using qPCR as described in the methods. Data points represents 4 replicates from two qPCR runs.

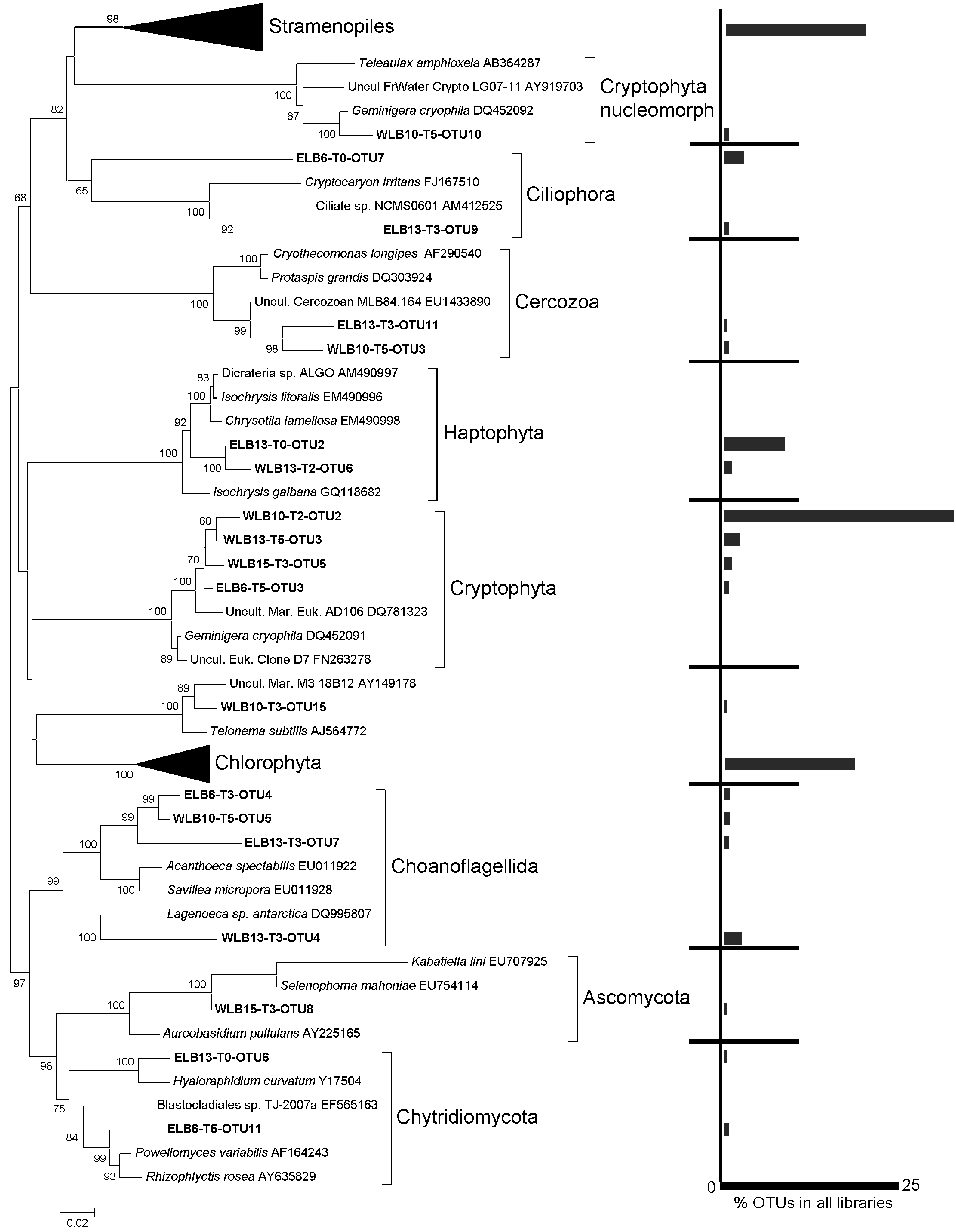
Figure 7. Distribution of major algal groups based on distribution of clones in 18S rRNA clone libraries generated from 6m and 13m depths in the east and 10m and 13m depths in the west lobes of Lake Bonney. **A**, ELB 6m; **B**, WLB 10m; **C**, ELB 13m; **D**, WLB 13m.

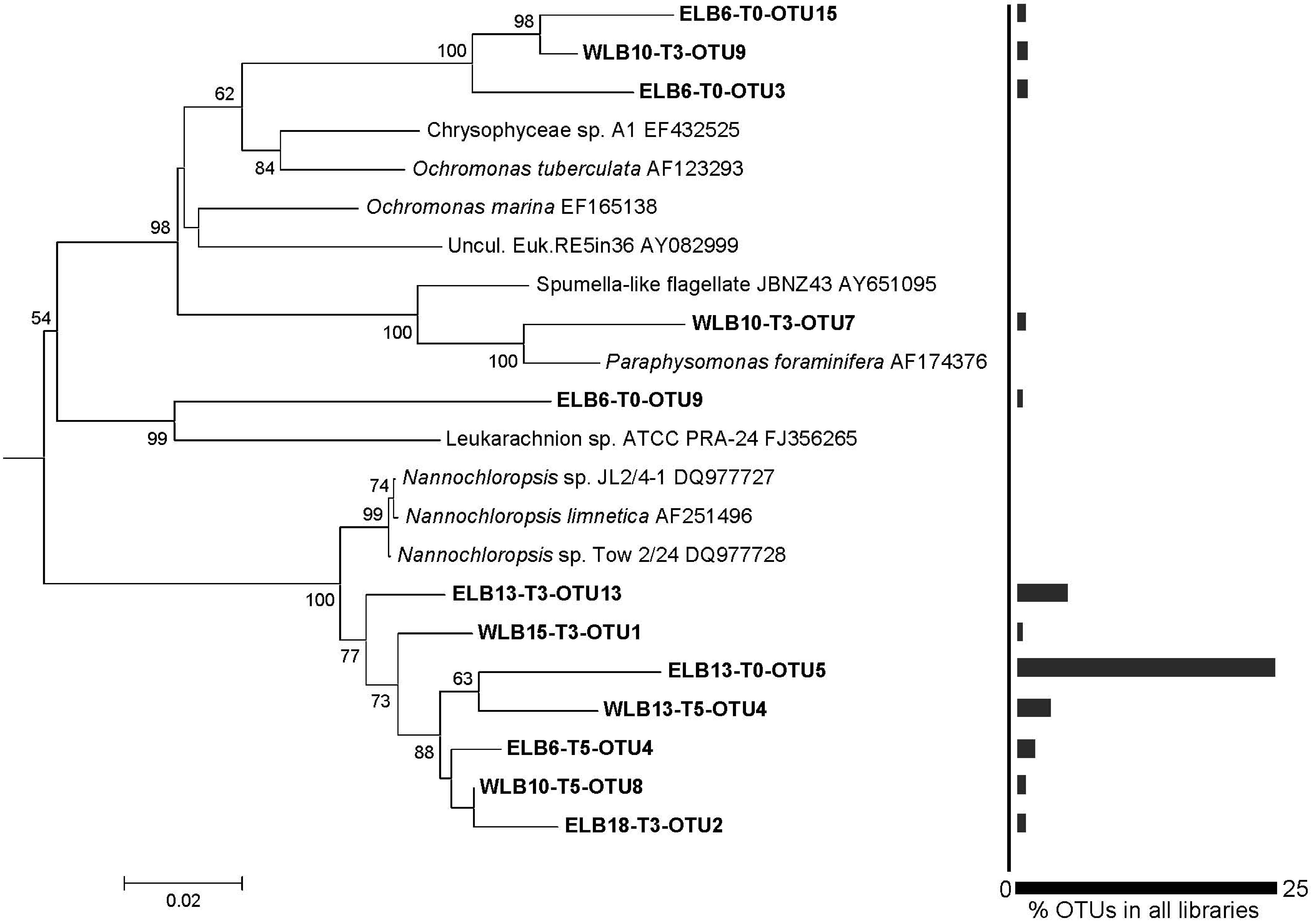
Table 1. Richness and diversity estimates from 18S rRNA clone libraries from lake samples collected during the transition to polar night. No. of clones, number of 18S rRNA clones from each library screened by RFLP; SW, the Shannon-Wiener diversity index, calculated as described in (Krebs, 1989) ; MaxSW, maximum SW diversity, calculated as ln(number of OTUs); Evenness, the product of SW/MaxSW.

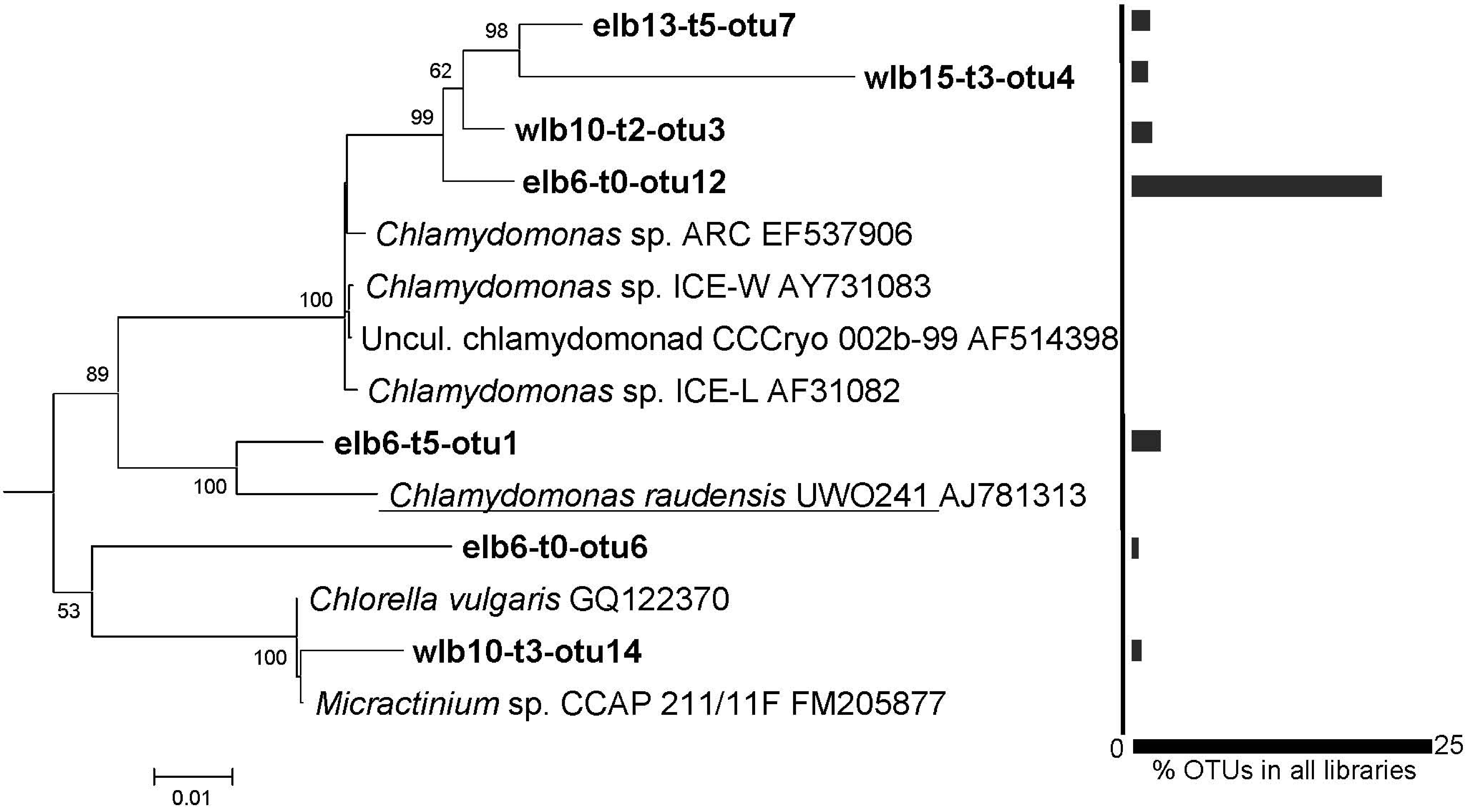
Lake	**Depth (m)**	**No. of clones**	**No. of libraries**	**OTUs**	**SW**	**Max-SW**	**Evenness**
ELB	6	210	4	20	1.48	3	0.49
ELB	13	129	4	20	2.18	3	0.73
ELB	18	93	2	6	0.69	1.79	0.39
ELB	20	49	1	4	0.99	1.39	0.71
WLB	10	109	3	21	1.66	3.04	0.55
WLB	13	144	4	12	1.84	2.48	0.74
WLB	15	65	2	8	1.2	2.08	0.58
WLB	20	61	1	6	1.45	1.79	0.81

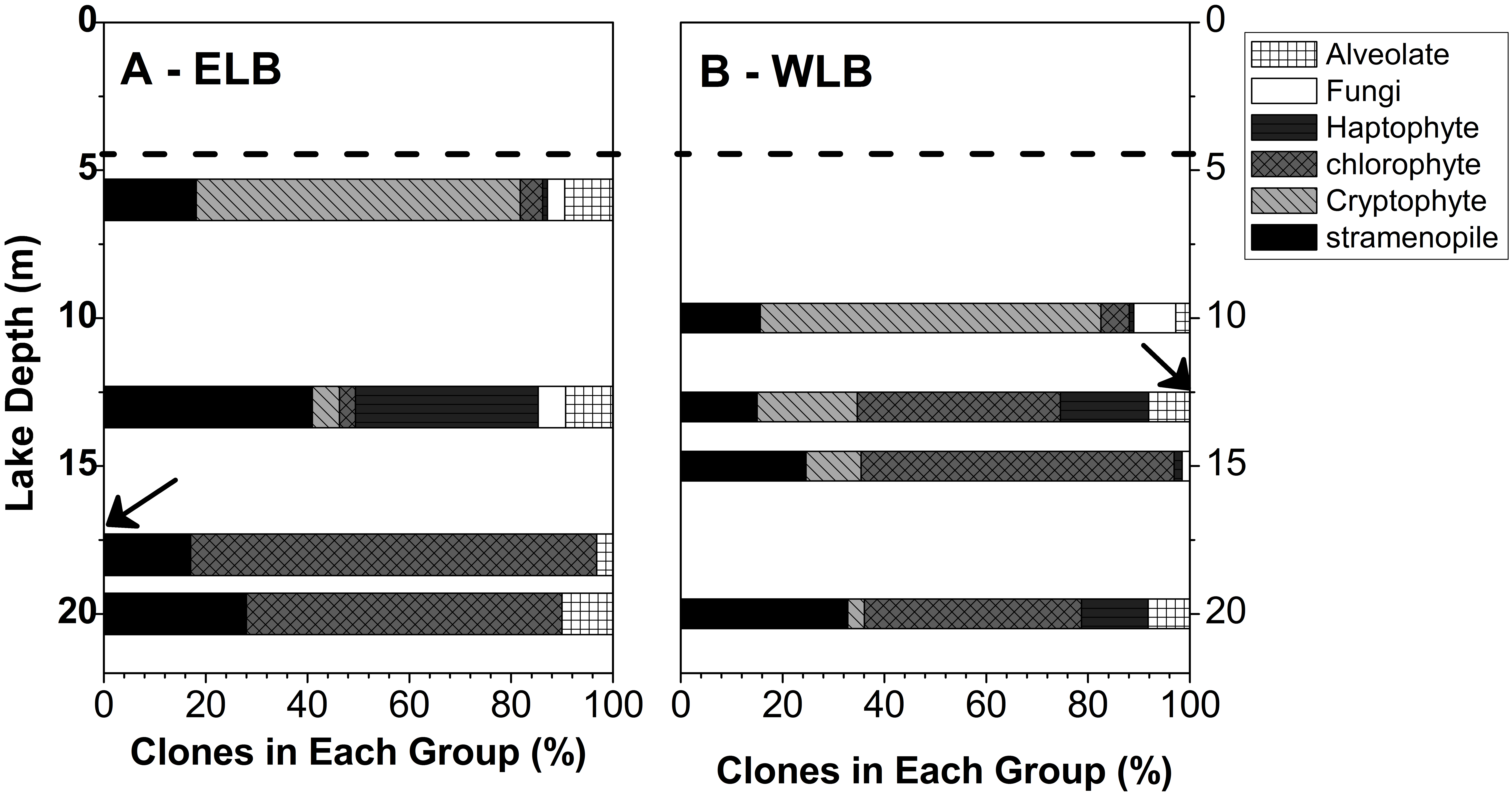

Temperature (°C)
-6 -4 -2 0 2 4 6
A
ELB
WLB
Depth (m)
0 10 20 30 40
Conductivity (mS cm^{-1})
0 40 80 120 160
B
ELB
WLB
Chlorophyll-a (µg l^{-1})
0 2 4 6 8 10
C - ELB
Chlorophyll-a
Oxygen
Dissolved Oxygen (mg l^{-1})
0 20 40 60
Chlorophyll-a (µg l^{-1})
0 5 10 15 20
D - WLB
Chlorophyll-a
Oxygen
Dissolved Oxygen (mg l^{-1})
0 20 40 60
SRP (µM)
0.0 0.4 0.8 1.2 1.6
E - ELB
NH_4+
NO_2-
NO_3-
SRP
Nitrogen (µM)
0 100 200 300
SRP (µM)
0.0 0.2 0.4 0.6 0.8 1.0
F - WLB
Nitrogen (µM)
0 100 200 300

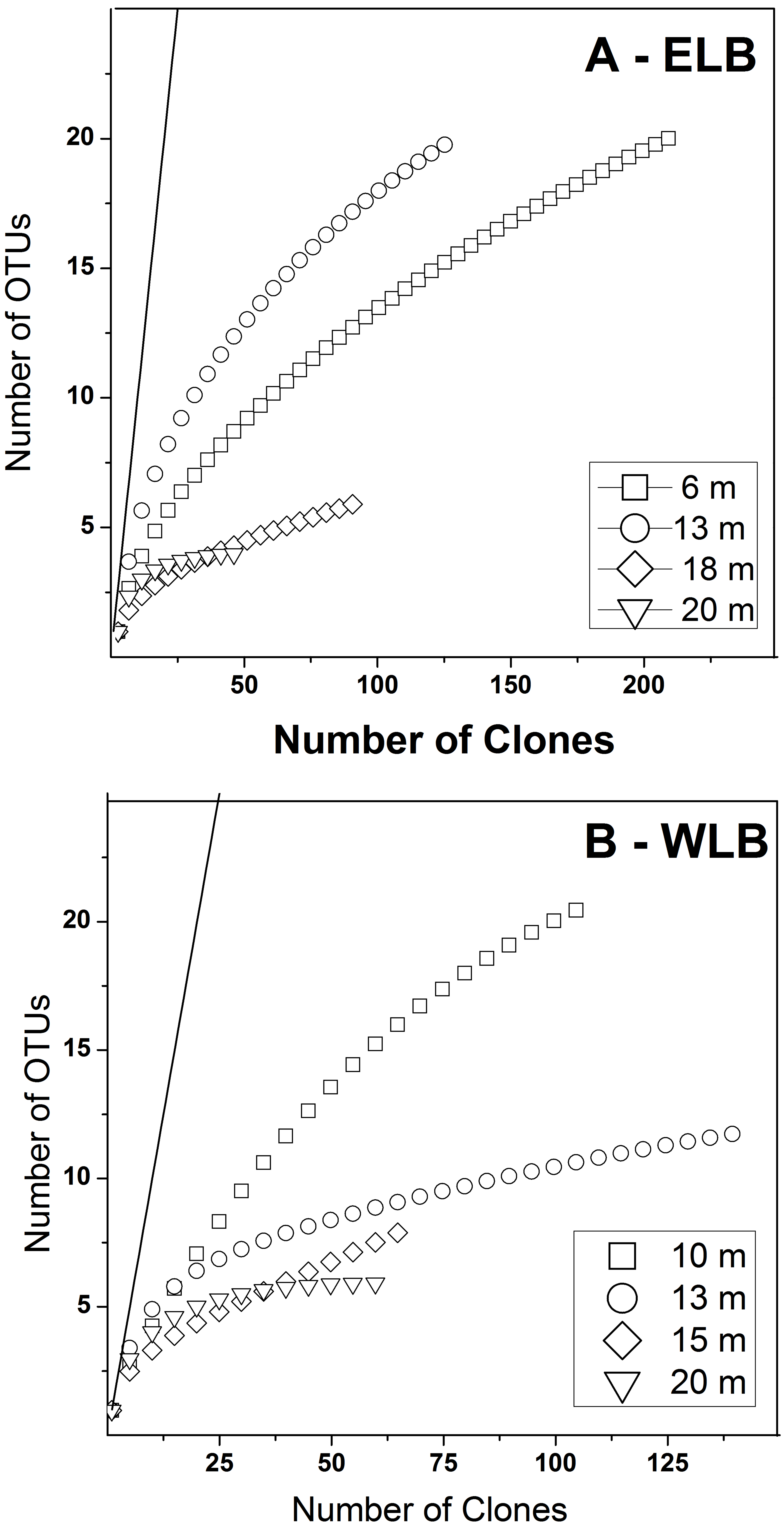

WLB
ELB
Total Number of Clones
0
50
100
150
200
250
Cryptomonad
Chlorophyte
Stramenopile
Haptophyte
Choanoflagellid
Alveolate
Fungi
Ciliate
Major Eukaryotic Groups

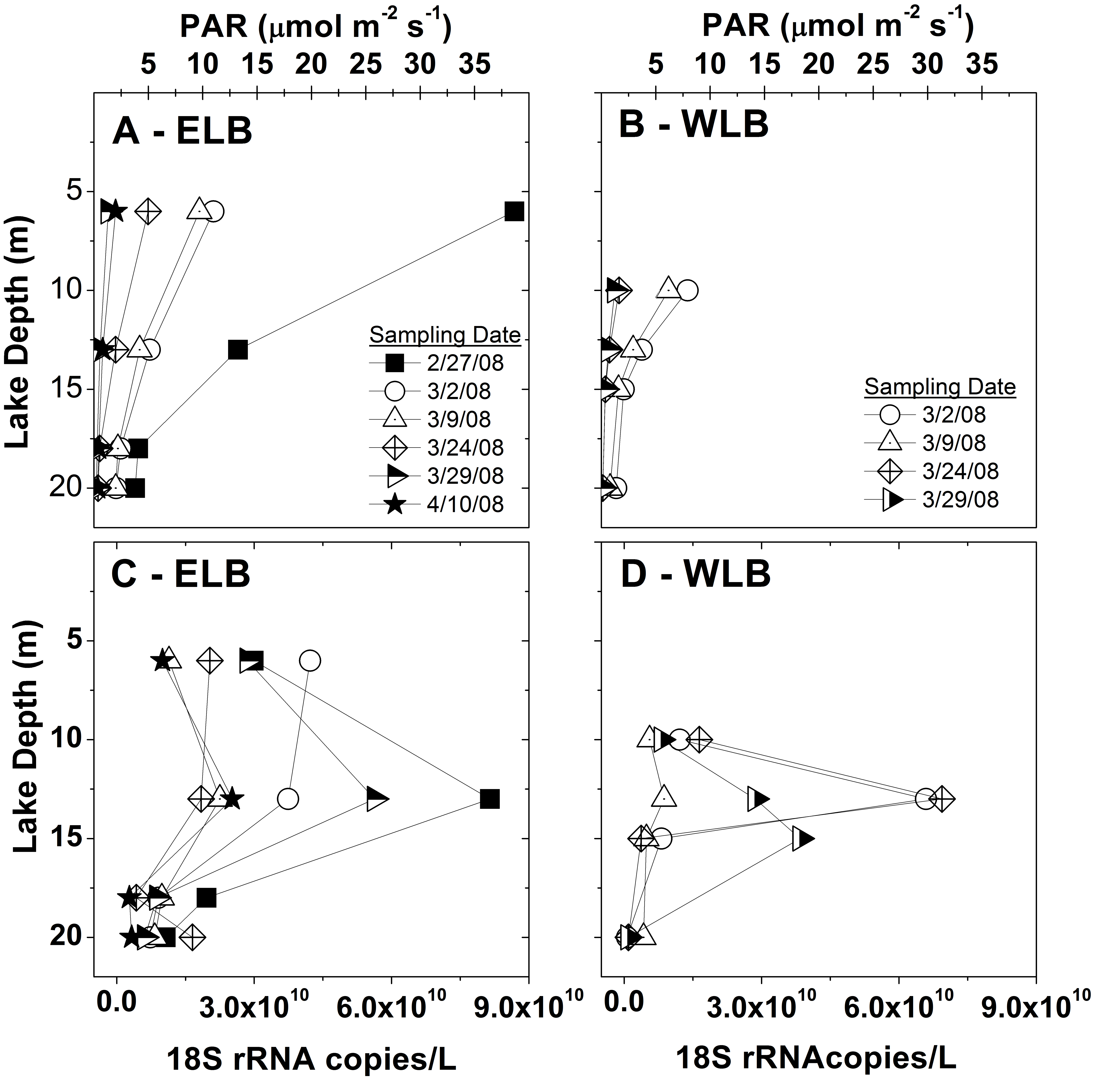

98
Stramenopiles
Teleaulax amphioxeia AB364287
Uncul FrWater Crypto LG07-11 AY919703
Geminigera cryophila DQ452092
WLB10-T5-OTU10
100
67
100
Cryptophyta nucleomorph
82
ELB6-T0-OTU7
Cryptocaryon irritans FJ167510
Ciliate sp. NCMS0601 AM412525
ELB13-T3-OTU9
65
100
92
Ciliophora
68
Cryothecomonas longipes AF290540
Protaspis grandis DQ303924
Uncul. Cercozoan MLB84.164 EU1433890
ELB13-T3-OTU11
WLB10-T5-OTU3
100
100
99
98
Cercozoa
Dicrateria sp. ALGO AM490997
Isochrysis litoralis EM490996
Chrysotila lamellosa EM490998
ELB13-T0-OTU2
WLB13-T2-OTU6
Isochrysis galbana GQ118682
83
100
92
100
100
Haptophyta
WLB10-T2-OTU2
WLB13-T5-OTU3
WLB15-T3-OTU5
ELB6-T5-OTU3
Uncult. Mar. Euk. AD106 DQ781323
Geminigera cryophila DQ452091
Uncul. Euk. Clone D7 FN263278
60
70
100
100
89
Cryptophyta
Uncul. Mar. M3 18B12 AY149178
WLB10-T3-OTU15
Telonema subtilis AJ564772
89
100
Chlorophyta
100
ELB6-T3-OTU4
WLB10-T5-OTU5
ELB13-T3-OTU7
Acanthoeca spectabilis EU011922
Savillea micropora EU011928
Lagenoeca sp. antarctica DQ995807
WLB13-T3-OTU4
99
99
100
100
99
100
Choanoflagellida
Kabatiella lini EU707925
Selenophoma mahoniae EU754114
WLB15-T3-OTU8
Aureobasidium pullulans AY225165
100
100
Ascomycota
97
ELB13-T0-OTU6
Hyaloraphidium curvatum Y17504
Blastocladiales sp. TJ-2007a EF565163
ELB6-T5-OTU11
Powellomyces variabilis AF164243
Rhizophlyctis rosea AY635829
100
98
75
84
99
93
Chytridiomycota
0.02
0
25
% OTUs in all libraries


98
ELB6-T0-OTU15
100
WLB10-T3-OTU9
ELB6-T0-OTU3
62
Chrysophyceae sp. A1 EF432525
84
Ochromonas tuberculata AF123293
Ochromonas marina EF165138
98
Uncul. Euk.RE5in36 AY082999
Spumella-like flagellate JBNZ43 AY651095
54
100
WLB10-T3-OTU7
100
Paraphysomonas foraminifera AF174376
ELB6-T0-OTU9
99
Leukarachnion sp. ATCC PRA-24 FJ356265
74
Nannochloropsis sp. JL2/4-1 DQ977727
99
Nannochloropsis limnetica AF251496
Nannochloropsis sp. Tow 2/24 DQ977728
ELB13-T3-OTU13
100
WLB15-T3-OTU1
77
63
ELB13-T0-OTU5
73
WLB13-T5-OTU4
88
ELB6-T5-OTU4
WLB10-T5-OTU8
ELB18-T3-OTU2
0.02
0
25
% OTUs in all libraries

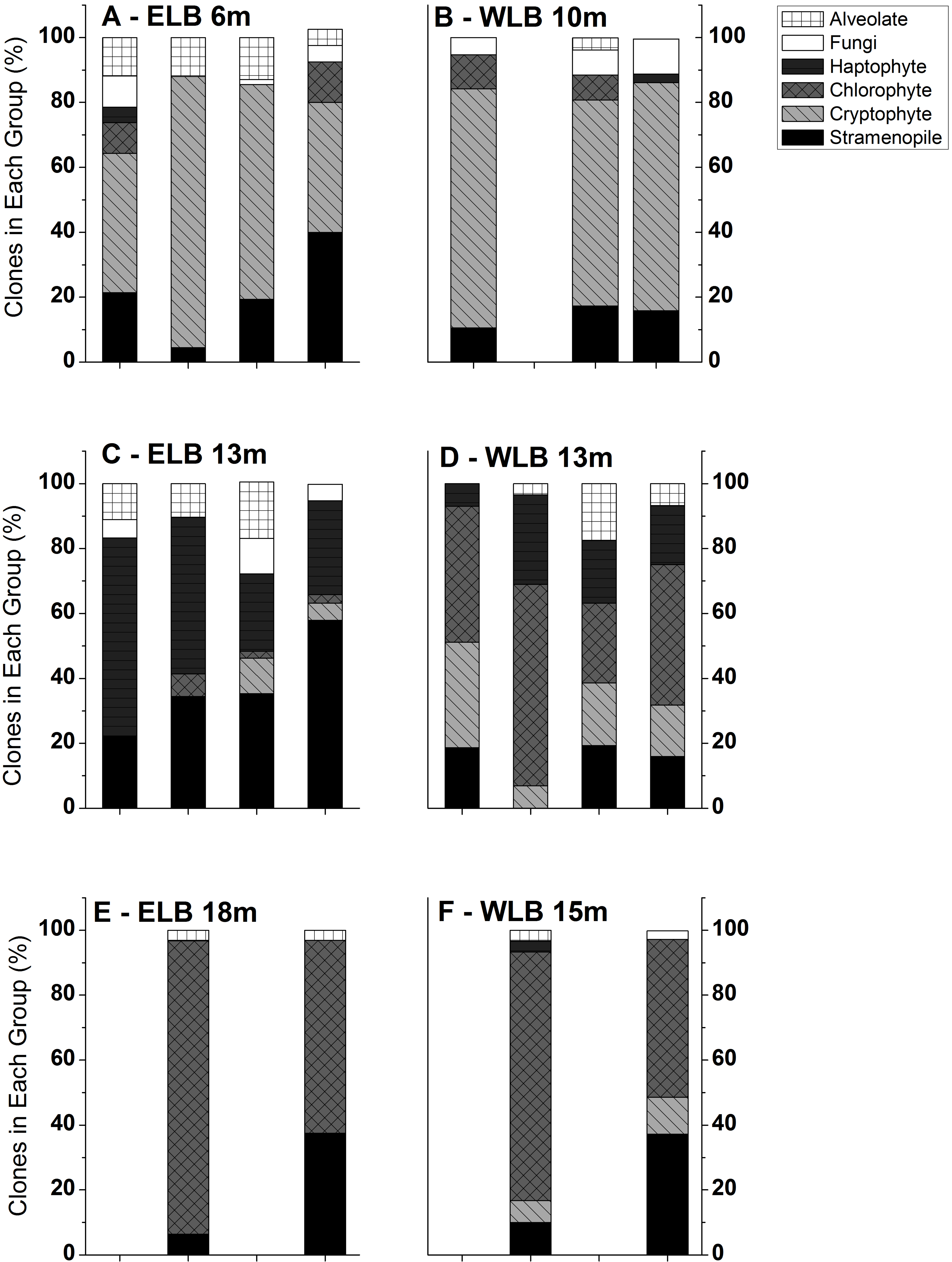

98
elb13-t5-otu7
62
wlb15-t3-otu4
99
wlb10-t2-otu3
elb6-t0-otu12
Chlamydomonas sp. ARC EF537906
100
Chlamydomonas sp. ICE-W AY731083
Uncul. chlamydomonad CCCryo 002b-99 AF514398
89
Chlamydomonas sp. ICE-L AF31082
elb6-t5-otu1
100
Chlamydomonas raudensis UWO241 AJ781313
elb6-t0-otu6
53
Chlorella vulgaris GQ122370
100
wlb10-t3-otu14
Micractinium sp. CCAP 211/11F FM205877
0.01
0
25
% OTUs in all libraries


A - ELB
B - WLB
Lake Depth (m)
0
5
10
15
20
0
20
40
60
80
100
Clones in Each Group (%)
Alveolate
Fungi
Haptophyte
chlorophyte
Cryptophyte
stramenopile


A - ELB
Number of OTUs
5
10
15
20
50
100
150
200
Number of Clones
6 m
13 m
18 m
20 m
B - WLB
Number of OTUs
5
10
15
20
25
50
75
100
125
Number of Clones
10 m
13 m
15 m
20 m



PAR (μmol m-2 s-1)
5
10
15
20
25
30
35
PAR (μmol m-2 s-1)
5
10
15
20
25
30
35
A - ELB
B - WLB
Lake Depth (m)
5
10
15
20
Sampling Date
2/27/08
3/2/08
3/9/08
3/24/08
3/29/08
4/10/08
Sampling Date
3/2/08
3/9/08
3/24/08
3/29/08
C - ELB
D - WLB
Lake Depth (m)
5
10
15
20
0.0
3.0x10^10
6.0x10^10
9.0x10^10
0.0
3.0x10^10
6.0x10^10
9.0x10^10
18S rRNA copies/L
18S rRNAcopies/L


A - ELB 6m
B - WLB 10m
C - ELB 13m
D - WLB 13m
E - ELB 18m
F - WLB 15m
Clones in Each Group (%)
0
20
40
60
80
100
3/2/08
3/8/08
3/16/08
3/29/08
Alveolate
Fungi
Haptophyte
Chlorophyte
Cryptophyte
Stramenopile