An integrated study of photochemical function and expression of a key photochemical gene (psbA) in photosynthetic communities of Lake Bonney (McMurdo Dry Valleys, Antarctica)

Weidong Kong1, Wei Li1, Ingrid Romancova2, Ondřej Prášil2 & Rachael M. Morgan-Kiss1

1Department of Microbiology, Miami University, Oxford, OH, USA and 2Laboratory of Photosynthesis, Algatech, Institute of Microbiology ASCR, Trebon, Czech Republic

Abstract

Lake Bonney is one of several permanently ice-covered lakes in the McMurdo Dry Valleys, Antarctica, which maintain the only year-round biological activity on the Antarctic continent. Vertically stratified populations of autotrophic microorganisms occupying the water columns are adapted to numerous extreme conditions, including very low light, hypersalinity, ultra-oligotrophy and low temperatures. In this study, we integrated molecular biology, microscopy, flow cytometry, and functional photochemical analyses of the photosynthetic communities residing in the east and west basins of dry valley Lake Bonney. Diversity and abundance of the psbA gene encoding a major protein of the photosystem II reaction center were monitored during the seasonal transition between Antarctic summer (24-h daylight) to winter (24-h darkness). Vertical trends through the photic zone in psbA abundance (DNA and mRNA) closely matched that of primary production in both lobes. Seasonal trends in psbA transcripts differed between the two lobes, with psbA expression in the west basin exhibiting a transient rise in early Fall. Last, using spectroscopic and flow cytometric analyses, we provide the first evidence that the Lake Bonney photosynthetic community is dominated by picophytoplankton that possess photosynthetic apparatus adapted to extreme shade.

Introduction

The McMurdo Dry Valleys, Antarctica, are one of the coldest, driest places on earth. Numerous ice-covered lakes are located throughout the dry valleys, providing aquatic habitats for food webs dominated by microbial communities harboring bacteria, algae, and heterotrophic flagellates (Priscu et al., 1998; Roberts et al., 2004; Bielewicz et al., 2011; Kong et al., 2012a, b). Each of the lakes is isolated from the surrounding polar desert by a permanent ice-cover that ranges from 3 to 6 m in thickness, which severely restricts light penetrating the water column that prevents wind-driven turbulence and has a strong influence on the biogeochmistry and associated microbial assemblages. The lakes support stratified populations of photosynthetic microorganisms that are dominated by eukaryotic phytoplankton (Lizotte & Priscu, 1992a; Bielewicz et al., 2011). In the most well-studied dry valley lake, Lake Bonney, recent molecular work has revealed that a cryptophyte population resides in the nutrient-deficient shallow depths under the ice, while haptophytes and stramenopiles reside in the deep chlorophyll maximum (DCM) near the permanent chemocline (13- to 15-m lake depth), and chlorophytes occupy the bottom of the photic zone (18–20 m lake depth), where light is < 1% surface availability (Bielewicz et al., 2011). Recently, we reported using the abundance and diversity of the gene rbcL, which encodes for the large subunit of the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO), to identify photosynthetically active members of the Lake Bonney microbial community (Kong et al., 2012a). Two major isoforms of RubisCO were monitored, which encompassed two distinct groups of autotrophic organisms: those carrying form ID (haptophytes, cryptophytes and stramenopiles) and those with form IA/B (chlorophytes, cyanobacteria and some
This work revealed that isoform ID rbcL related to haptophytes and stramenopiles dominated sequence libraries generated from both environmental DNA and mRNA Lake Bonney extracts. As part of the first study to monitor the response of the microbial communities in the dry valley lakes during the seasonal transition between Antarctic summer and polar night, we also investigated temporal (seasonal) and spatial (lake depth) variability on expression of RubisCO in Lake Bonney (Kong et al., 2012a). This work showed that rbcL expression was strongly correlated with light availability on a seasonal but not a spatial scale. These results indicate that light is a major driver of RubisCO expression in Lake Bonney phytoplankton communities during the polar night transition; however, additional environmental factors including nutrient availability influence vertical trends in rbcL transcript abundance (Kong et al., 2012a).

In addition to the gene expression data, our polar night study also showed that seasonal trends in chlorophyll (Chl) a levels in Lake Bonney were negatively correlated with rates of primary productivity and light levels, suggesting that dry valley lake phytoplankton may acclimate to declining light availability by increasing their light capture capability by accumulating Chl (Kong et al., 2012a). This hypothesis is supported by earlier work on a green algal isolate from Lake Bonney (Chlamydomonas raudensis UWO241), which possesses a large light harvesting antenna for efficient light energy capture in its natural environment of extreme shade (Morgan et al., 1998; Morgan-Kiss et al., 2006). In cyanobacteria, microbial eukaryotes and plants light harvesting pigments (chlorophylls) are arranged in two membrane-bound pigment-protein complexes named Photosystem I and Photosystem II (PSI and PSII, respectively). Absorbed light energy via the pigment antenna is transferred to reaction centers where the conversion of light to chemical energy occurs. Thus, structural and functional alterations in PSII play an important role in survival of C. raudensis under year-round low light levels in the deep photic zone (Morgan-Kiss et al., 2006).

The psbA gene, a major PSII reaction center protein, has been utilized as a proxy for evidence of photosynthetic activity in a variety of natural environments (Zeidner et al., 2003; Zeidner & Beja, 2005; Sharon et al., 2007; Eriksson et al., 2009). Given our earlier studies showing that photochemical function and specifically PSII play critical roles in environmental adaptation in the Lake Bonney isolate, C. raudensis UWO241, we investigated: (1) spatial and temporal trends in diversity and expression of the major PSII reaction center gene (psbA), and (2) photochemical function using low temperature Chl spectroscopy, in both lobes of the Antarctic Lake Bonney. This current study extends our understanding of environmental adaptation from laboratory isolates to the natural communities residing in these unique aquatic systems and provides new functional and molecular data for a major energy acquisition system in the dry valley lake primary producer community.

Materials and methods

Site description and field sample collection

Samples were collected from the water column of the east and west lobes of Lake Bonney (ELB and WLB, respectively), McMurdo Dry Valleys, Antarctica. Lake water samples were collected during mid February to early April (2008), a period representing the seasonal transition from 24 h of sunlight to complete darkness. Samples (2 L) at varying depths were collected weekly during the sampling period (24 February, 2 March, 9 March, 16 March, 24 March, 19 March and 10 April). Sampling depths measured from the piezometric water level (6, 13, 15, 20 m for ELB; 10, 13, 15, 18, 20 m for WLB) coincided with Chl a maxima and spanned the breath of the photic zone (Bielewicz et al., 2011). Lake water was collected from the water column at the onset of dawn (06:00 hours) using a 5-L Niskin bottle and stored in 1-L amber bottles until processing. Samples were processed on site within 30 min of collection. These samples were part of a larger study to investigate dynamics in the plankton community during the polar night transition. Major physicochemical parameters are summarized in Supporting information, Table S1. Other parameters (Chl a, primary productivity) are reported in two earlier papers (Bielewicz et al., 2011; Kong et al., 2012a).

Microscopy

Lake samples were collected at depths of maximum primary productivity and the DCM (13–15 m) from both lobes of Lake Bonney during the 2012 field season. Water samples were concentrated c. 100-fold using tangential flow filtration (TFF, Millipore Pellicon XL 0.45 μm) within 5 h of collection, and concentrated samples were stored in the dark at 4 °C until processing. Concentrated samples were viewed using a Zeiss Axioskop 10 microscope equipped with differential interference contrast, epifluorescence capability, and a Zeiss AxioCam MRC5 digital camera. Autofluorescence from photosynthetic microorganisms was visualized using specific filter sets (425-nm excitation, 600-nm emission; 365-nm excitation, 420-nm long-pass emission).
Flow cytometry

Distribution of phytoplankton size classes was determined by flow cytometry. Lake water samples were collected as described above at 13 and 15 m from ELB and WLB and preserved for flow cytometric analyses using the protocol for aquatic cryopreservation from Bigelow Laboratory Single Cell Genomics Center (http://scgc.bigelow.org/). Filter-sterilized GlyTE stock (1 mL; 10 × TE buffer, pH 8; 50% molecular-grade glycerol) was added to 9-mL aliquots of lake water and immediately frozen in liquid nitrogen. Samples were analyzed with a flow cytometer equipped with a 488-nm laser (FACScan, BD Biosciences) following the method described by Olson et al. (1993). Size classes of phytoplankton were determined on the basis of forward scattering vs. chlorophyll fluorescence cytograms (Fig. S1). Cells were classified into three populations of phytoplankton: 1–5 μm, 5–10 μm, and > 10 μm. Cell sizes were determined using a series of internal standards of polystyrene microspheres (Life Technologies). Forward scattering and autofluorescence (FL3, 650LP) signals were acquired and analyzed with FLOWJO v9.3 software (TreeStar Inc.) to define size populations. Results represent three biological replicates.

Fluorescence emission spectroscopy

Low temperature Chl fluorescence emission spectroscopy was collected from 15-m sampling depths in ELB and WLB using a custom built portable spectrophotometer (Kupper et al., 2008; Prášil et al., 2009). TFF-concentrated samples (5 mL) were stored in the dark for 10 min (dark adaptation) and then filtered under very low illumination on to 25 mm GF/F filters and placed in a glass Dewar containing liquid nitrogen. Samples were illuminated with narrow-band excitation using a 455-nm light emitting diode, and emission spectra were collected over the spectral range from 650 to 800 nm. Decompositional analysis of fluorescence emission spectra in terms of five Gaussian bands was performed by a nonlinear least squares algorithm according to Morgan-Kiss et al. (2002) using ORIGINPRO 8.5 (OriginLab Corp., Northampton, MA). The fitting parameters for the Gaussian components (center max, area) were free-running parameters.

Nucleic acid isolation and quantification of the psbA gene

Lake water for molecular analyses was processed on site within 30 min of collection by filtration onto sterile 47 mm, 0.45-μm Durapore polyvinylidene fluoride membrane filters (Millipore) using a vacuum of 25 kPa. Filters were immediately flash-frozen in liquid nitrogen and stored in a liquid nitrogen Dewar until they were shipped to the US laboratories on dry ice. Samples were stored at −80 °C until nucleic acid extraction. Frozen filters were cut in half for extraction of environmental RNA and DNA from same filter. Total environmental DNA or RNA was isolated using FastDNA spin kit for soil (MP Biomedicals, OH) or a combination of RNasea Mini (Qiagen, CA) and FastRNA Pro Soil-Direct (MP Biomedicals, OH) kits, respectively, according to Kong et al. (2012a). Total RNA was converted to cDNA using an iScript cDNA synthesis kit (Bio-Rad, CA). Gene copy number (DNA) and transcript abundance (mRNA) were quantified using quantitative real-time PCR (qPCR). Due to a shortage of samples, psbA gene copy number was determined for only one sampling time point (March 9, 2008). Transcript abundance was quantified for all sampling dates. The psbA gene was targeted using the primer set psbA-F/psbA-R (forward: GTTITYARGCIGARCRY AAYATYTIATGCAYCC and reverse: CRRTTARRTRRA AIGCCATIGT) (Eriksson et al., 2009). In addition, this primer set was validated in several isolated strains in our laboratory for specificity and ability to amplify the psbA gene from a variety of algal species (data not shown). The qPCR conditions were an initial 5-min period at 95 °C, followed by 40 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s, and 78 °C for 10 s to collect data (Kong et al., 2012a). To ensure valid qPCR results, all qPCR assays generated in this study were compliant with the guidelines for Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) (Bustin et al., 2009). All samples and standards were run in duplicate. Negative control reactions without template were run on all plates. PCR amplification efficiency and inhibition testing of environmental samples were verified by running Cq dilution series (Bustin et al., 2009). DNA contamination was checked in controls lacking reverse transcriptase. Standard curves were made using known concentrations of a plasmid harboring the psbA gene according to Kong & Nakatsu (2010).

Clone library construction and sequencing

The psbA gene was amplified by PCR from cDNA and environmental DNA from samples collected from both ELB and WLB and various sampling time points (for DNA, March 9; for RNA, March 2, 9 and 29) using the same primer set and PCR conditions as described for qPCR. Gel-purified PCR products were ligated into the pGEM-T Easy vector (Promega, WI) and transformed into chemically competent Escherichia coli TOP10 cells. 24 transformants from each library were randomly selected and sequenced on an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, CA) in the Center
for Bioinformatics and Functional Genomics (Miami University, OH). Representative sequences generated from each library and closest sequences from GenBank were aligned using MUSCLE. A maximum-likelihood tree was obtained using the general time reversal model with gamma distribution using MEGA 5.2 software (Tamura et al., 2011). Bootstrapping was used to estimate node support with 500 replicate trees. All sequences generated in this study have been deposited in the National Center for Biotechnology Information GenBank database under Accession Numbers (JQ924353 to JQ924385).

**Results and discussion**

A green algal isolate from the deep phytoplankton population (17 m) of ELB, named *C. raudensis* UWO241, is one of the best studied cold-adapted photosynthetic organisms and is a model for adaptation to permanent low temperatures and extreme shade conditions (Morgan-Kiss et al., 2006; Dolhi et al., 2013). Past studies have shown that this photopsychrophile possesses specialized adaptations to the extreme shade conditions at the level of its photochemical apparatus to efficiently harvest light energy enriched in blue-green wavelengths (Morgan et al., 1998). A model of the photochemical apparatus for *C. raudensis* UWO241 predicts that this shade-adapted alga has a relatively large PSII-absorption cross section, a functionally downregulated PSI, as well as an unusually high PSII : PSI ratio (Morgan-Kiss et al., 2006). To validate these findings in the natural phytoplankton communities of Lake Bonney, we applied in situ spectroscopic analyses using a novel portable spectrophotometer to phytoplankton communities residing in both basins (Fig. 1). Low temperature (77 K) fluorescence emission spectra were generated from samples collected at the DCM (13 m) of ELB and WLB using a blue excitation wavelength, which preferentially excites Chl a. Extreme shade adaptation was evident in the spectroscopic properties of the natural phytoplankton communities residing in both lobes of Lake Bonney (Fig. 1a and b). Dominant Chl a fluorescence emission peaks in both lobes were 686–689 nm and 699–701 nm, representing fluorescence associated with light harvesting II and PS II core complexes, respectively (Fig. 1). Emission peaks associated with PS I (700–720 nm) were absent; although, a minor emission peak at longer wavelengths (730–732 nm) was observed in both samples. This near-infrared peak was observed in previous studies and has been attributed to small vibrational transitions (Morgan-Kiss et al., 2002). The emission spectra of the natural phytoplankton samples closely resembled that of the shade-adapted Lake Bonney *C. raudensis* UWO241, which also possesses reduced PSI fluorescence and high PSII core complex emission yields, indicative of unbalance PSII : PSI ratios combined with a tight coupling of energy transfer between LHCII and PSII core (Morgan et al., 1998; Morgan-Kiss et al., 2002, 2005). Thus, these results validate our models based on laboratory-controlled studies and indicate that the photochemical apparatus of Lake Bonney phytoplankton exhibits extreme shade adaptation to a permanent low-light environment enriched in blue-green wavelengths. Last, this unusual organization of the photosynthetic apparatus would have important implications on energy acquisition in dry valley lake phytoplankton communities, favoring high production of ATP relative to reducing equivalents (NADPH; Dolhi et al., 2013).

Recent molecular evidence indicates that cryptophytes (related to *Gemingera cryophila*), haptophytes (related to *Isochrysis* sp.), and a stramenopile (related to *Nannochloropsis* sp.) appear to be the major phytoplankton species occupying the zone of maximum primary productivity (i.e. at 13- to 15-m lake depth) in the dry valley lakes (Bielewicz et al., 2011; Vick-Majors et al., 2013). We confirmed these conclusions in this study by both micro-

![Fig. 1. Low temperature (77 K) fluorescence and decomposition in Gaussian sub-bands from natural lake water phytoplankton collected at depths of maximum productivity (13 m) in the (a) east and (b) west lobes of Lake Bonney. Experimental curves represent the average of ten scans —, experimental curve; ——, Gaussian sub-band. Insets: Gaussian fitting parameters for sub-band decompositions. Percentage areas were calculated from the total area given by the sum of all bands reported.](image-url)
scopic and molecular methods, and quantified major size phytoplankton size classes using flow cytometry. Microscopic investigation of samples collected from sampling depths of 13 m from ELB and WLB revealed that both sampling depths are dominated by picophytoplankton (i.e. < 5 μm) of two distinct cell morphologies: a coccoid cell of 2–3 μm and a biflagellate asymmetrical cell of 5 μm, identified as *Nannochloropsis* and *Isochrysis* spp., respectively (Fig. 2a and b). Nanoplankton that resembled cryptophytes (Fig. 2c and d) as well as a chlorophyte (*Chlorella* sp.; Fig. 2e) were also observed in both samples. In contrast, larger phytoplankton (> 10 μm) such as the chlorophyte *Chlamydomonas* sp. (Fig. 2f) were rarely observed. Last, predatory protists resembling the dinoflagellate *Gymnodinium* were frequently observed harboring phytoplankton prey (Fig. 2g). The identity of these phytoplankton species was confirmed by cultivation and a combined microscopy and sequencing of isolates.

Flow cytometry was used to size fractionate phytoplankton communities residing in the zone of maximum productivity (13- to 15-m sampling depths) into three size classes (1–5 μm; 5–10 μm; > 10 μm). The smallest size class (1–5 μm) made up the vast majority of phytoplankton cells, representing 74–81% of the total phytoplankton population, while phytoplankton > 10 μm made up < 10% of the phytoplankton populations across all samples (Fig. 3). These data supported our microscopy observations that larger phytoplankton such as *Chlamydomonas* are rare. Low temperature and oligotrophic ecosystems are often dominated by picoplankton; their small cell size which confers greater capacity to acquire nutrients as well as efficient nutrient use for growth (Agawin et al., 2000; Not et al., 2007; Li et al., 2009; Shi et al., 2009).

The diversity and abundance of *psbA*, the gene encoding the PSII reaction center protein D1, was investigated in both lobes of Lake Bonney. We amplified a 344-bp fragment of the *psbA* gene from environmental DNA and mRNA (cDNA), which were then used to construct a phylogenetic tree (Fig. 4). In agreement with our microscopy observations, sequences related to the stramenopiles *Nannochloropsis* sp. (96% identity) and *Ochromonas* sp. (90% identity), and a haptophyte related to *Isochrysis* sp. (95% identity) dominated clone sequence libraries (Fig. 4; Fig. S2). *PsbA* sequences related to the cryptophyte *G. cryophila* (92% identity) were also recovered, but made up < 10% of the phytoplankton community (Fig. S2). Not surprisingly, we did not recover *psbA* sequences
related to chlorophytes, which supported the microscopic and flow cytometric analyses (Figs 2 and 3). An earlier study also reported that chlorophyte rbcL transcript and gene copy abundance were 100–10 000 lower compared with haptophyte/stramenopile rbcL levels (Kong et al., 2012a).

Spatial trends for psbA gene copy number (DNA) vs. gene expression (mRNA) were determined for the photic zones of both lobes of Lake Bonney. Coinciding with the peaks of maximum Chl a levels and primary productivity, maximum levels of psbA gene copy and expression were observed at 13-m water columns in both lobes (Fig. 5a and b); however, psbA transcriptional activity (i.e. psbA mRNA : DNA) increased with depth in ELB and was c. twofold higher at deeper sampling depths compared with WLB (Fig. 5c). Furthermore, psbA transcriptional activity was at or close to 1 at all sampling depths, while transcriptional activity of RubisCO was reported to be far below 1 (Kong et al., 2012a), indicating that transcriptional activity of the photochemistry gene is significantly higher than that of carbon fixation. Low RubisCO transcriptional activity agrees well with low rates of primary production that have been reported in earlier studies (Lizotte et al., 1996; Kong et al., 2012a), while high psbA transcriptional activity may reflect adaptation to the extreme shade conditions in the water column of Lake Bonney, which is supported by the spectroscopic results (Fig. 1). Alternatively, high psbA transcript levels may be needed to support high rates of PsbA turnover via the PSII repair cycle under permanent oxidative stress conditions in the dry valley lakes (i.e. low temperature, high salinity, increased oxygen levels; Table S1). High rates of D1 repair were reported in cultures of C. raudensis UWO241 when exposed to oxidative stress (Pocock et al., 2007). Last, lower rbcL expression may also reflect a reduced need for carbon fixation in mixotrophic species, such as Isochrysis and Nannochloropsis (Kong et al., 2012a).

Temporal dynamics of psbA mRNA were monitored through the photic zone of both lobes of Lake Bonney from mid February to early April, representing the transition from Antarctic summer (i.e. 24-h daylight) to winter (i.e. 24-h darkness). Maximum psbA expression at 13-m sampling depths was maintained in both lobes throughout the polar night transition (Fig. 6a and b). Both lobes exhibited a decline in psbA expression at the onset of the polar night transition; however, during the fall (late March), a transient rise in psbA transcript abundance was observed. This increase in psbA expression was most pronounced in WLB (Fig. 6b). We investigated whether there was a relationship between psbA abundance and light availability (photosynthetically active radiation, PAR). As stated above, the depth profile of psbA expression matched trends in light-dependent primary productivity (PPR) and Chl a levels (reported in Kong et al., 2012a), and exhibited no dependence on PAR availability. In contrast with spatial trends, psbA mRNA abundance showed a significant positive association with PAR at sampling depths of 13 and 20 m of ELB (r = 0.82 and 0.90, P < 0.05; Table S2). In contrast with ELB, temporal trends in psbA mRNA showed no correlation with PAR at any sampling depths in WLB (Fig. 5b and Table S2). Thus, despite the yearly mixing between the upper layers of the two lobes of Lake Bonney (the water column of WLB flows into ELB during the summer months) as well as similarities in phytoplankton diversity between the two lobes (see Figs 2–4), there are clearly distinct differences in the regulation of expression of key photosynthetic genes in the two lobes of Lake Bonney. Most striking is the transient rise in psbA expression to almost summer expression levels in WLB late in the season (Fig. 6b). Primary productivity in the dry valley lakes is controlled by not only light, but also nutrient availability (Lizotte et al., 1996). The upper layers of Lake Bonney are ultra-oligotrophic and phytoplankton blooms in the early summer are dependent upon replenishment of nutrients during the previous winter (Priscu, 1995). New nutrients are quickly depleted during the early summer, phytoplankton populations become nutrient deprived (particularly phosphorus; see Table S1), and primary production declines in mid- to late-summer (Lizotte et al., 1996). One possible environmental driver for the transient rise in psbA expression in WLB is a pulse of nutrients in the autumn.
Priscu (1995) hypothesized that phytoplankton biomass accumulation in the DCM of Lake Bonney is driven by upward nutrient fluxes and estimated vertical nutrient fluxes of 6.44 and 1.00 μmol m\(^{-2}\) day\(^{-1}\) for dissolved inorganic nitrogen and soluble reactive phosphorus (SRP), respectively, in mid-depths (14 m) of WLB. In contrast, SRP flux at the DCM of ELB (14 m) was predicted to be negative, indicating that phosphorus flux was downwards at this depth in the water column (Priscu, 1995). As the phytoplankton in Lake Bonney exhibit a high degree of phosphorus deficiency, differences in phosphorus flux from the deep waters to the DCMs between the west and east lobes may contribute to the differences in the seasonal trends psbA expression between the two basins of Lake Bonney.

**Conclusions**

Antarctic lakes covered by permanent ice harbor microorganism-dominant food webs that are exposed to minimal...
direct human impact and low allochthonous input. Therefore, the majority of the organic carbon that supports the lake ecosystem is provided by autotrophic microorganisms by fixing carbon. Past studies have shown that primary productivity in the austral summer is limited by low PAR (Lizotte & Priscu, 1992b, 1994) as well as nutrient availability (Priscu, 1995). Our current study extends these earlier findings by providing new functional and molecular data for the major energy acquisition systems (i.e. the photochemical apparatus) in the dry valley lake primary producer community. The photic zones of both lobes of Lake Bonney are dominated by picophytoplankton, which possess unique adaptive strategies to optimize light energy acquisition under extreme shade conditions. However, despite the similarities in phytoplankton diversity and photochemical function, expression patterns of the psbA gene exhibited seasonal differences between the two lobes of Lake Bonney. One possible scenario to account for these differences is differential supply of phosphorus from deep waters to the DCM. As the Antarctic continent slowly warms and more liquid water flows into the McMurdo Dry Valley lakes during episodic summer flood events such as one observed in 2001/02 (Doran et al., 2008), phytoplankton communities will be exposed to more frequent pulses in nutrients. Perturbations in major environmental drivers, including light and nutrient availability, are likely to not only influence phytoplankton diversity.

Fig. 5. Depth profiles in the east and west lobes of Lake Bonney for gene copy number (DNA) and transcript (mRNA) abundance of the photosystem II gene, psbA. (a) Average transcript abundance of psbA across sampling times between late February and early April. (b) Gene copy number of psbA was sampled on 3/9/2008. (c) Transcriptional activity expressed as the ratio of psbA mRNA to DNA. Abundance was quantified using qPCR (n = 2–4).

Fig. 6. Spatio-temporal distribution patterns of psbA mRNA levels in ELB (a) and WLB (b) during the polar night transition. Dots indicate sampling times. Right hand scale indicates psbA expression levels (10^8 copies psbA mRNA L^-1). Abundance was quantified using qPCR.
and physiology, but also carbon and energy acquisition by mixotrophic species that are important players in the dry valley aquatic food web.

Acknowledgements

The authors thank the McMurdo LTER limnology team for collection and preservation of the samples in Antarctica. We thank Ratheon Polar Services and PHI helicopters for logistical support. We thank the Center for Bioinformatics and Functional Genomics at Miami University for assistance with sequencing. This work was supported by NSF Office of Polar Programs Grants OPP-0631659 and OPP-1056396. O.P. and I.R. were supported by project Algatech (MSMT Grant CZ.1.05/2.1.00/03.0110).

References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Flow cytometry analysis of photosynthetic plankton from East Lobe Bonney (sampling depth 13 m).

**Fig. S2.** Distribution of *psbA* sequences generated from environmental DNA (a,b) and mRNA (c,d) clone libraries.

**Table S1.** Physical and chemical characteristics for west and east basins of Lake Bonney.

**Table S2.** Polar night transition-dependent Pearson correlations (R) between *psbA* transcript vs. PAR.