Photoadaptation to the polar night by phytoplankton in a permanently ice-covered Antarctic lake

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Abstract

Photosynthetic microorganisms are a primary source of new organic carbon production in polar ecosystems. Despite their importance, relatively little is known about how they adapt to the bimodal solar cycles that exist at high latitudes. To understand how phytoplankton adapt to the extreme seasonal change in photoperiod, we transplanted cultures of a well-studied laboratory model for photosynthetic cold adaptation, *Chlamydomonas raudensis* UWO241, back to the water column of Lake Bonney (McMurdo Dry Valleys, Antarctica) at the depth from which it was originally cultured. The organism was suspended at this depth in dialysis tubing to allow the microalgae to respond to the in situ light, temperature and dissolved ions. We then integrated in situ biological and chemical measurements with environmental molecular analyses and compared the responses of transplanted *C. raudensis* cultures with the natural phytoplankton community over the 6-week transition from Antarctic summer (24-h daylight) to polar night (24-h darkness). As solar radiation declined, natural communities exhibited a cessation of inorganic carbon fixation which was accompanied by a downregulation of expression of genes encoding for essential carbon fixation and photochemistry proteins. Transplanted *C. raudensis* cultures matched natural community trends in the regulation of photochemistry and carbon fixation gene expression, and shifted photochemical function to a shade adapted state in response to the polar night transition. We present a conceptual model for seasonal shifts in microbial community energy and carbon acquisition which integrates past cultivation-based studies in this model photosympthrophile with a body of recent work on adaptation of natural populations to polar night.

The ability to efficiently capture light energy to drive production of organic carbon compounds while avoiding the potential oxidative effects of absorbed but unused photons is critical for the survival of all photosynthetic life forms on Earth. In their natural light environment, photosynthetic organisms must respond to variability in photosynthetically available radiation (PAR) across broad time scales. A myriad of adaptive strategies exist to deal with PAR variability and ultimately the balance between energy capture by the photochemical apparatus with energy spent by downstream metabolic processes (Wilson et al. 2006; Demmig-Adams et al. 2012). The combination of light and low temperature is one of the most stressful environmental conditions for survival due to temperature-dependent imbalances between rates of light absorption and carbon fixation. Photosynthetic microplankton residing in permanently ice-covered Antarctic lakes provide an example of organisms that must deal with one of the most extreme examples of PAR variation under permanent low temperature stress (close to 0°C) as these systems undergo seasonal transitions from 24-h sunlight in the austral summer to complete darkness in the winter. To date, logistical considerations have never permitted in situ research to be conducted on the aquatic communities residing in these lakes during the summer-winter transition. As part of the International Polar Year, we received logistical support to study for the first time the physiological and metabolic shifts in extremely phytoplankton communities residing in the lakes of the McMurdo Dry Valleys, Antarctica, from 24 h sunlight to the polar night.

The water columns of the permanently ice-covered lakes in the McMurdo Dry Valleys undergo little vertical mixing and minimal exchange with the atmosphere or surrounding terrestrial environment, making them novel systems to study in situ phytoplankton responses to seasonal shifts in PAR (Priscu et al. 1988, 1997, 1999; Spigel and Priscu 1985). Permanently ice-covered and subglacial lakes contain the only
year-round liquid water environments on the Antarctic continent and, as such, support the only known metabolism to occur on the continent’s surface during the winter. As part of the ongoing NSF-funded McMurdo Dry Valleys Long Term Ecological Research program (http://www.mcmliter.org), intensive field studies have been performed for the past 20 yr during the austral fall and summer (October through January) and have provided a detailed view of how microorganisms residing during the period of continuous sunlight in these extreme ecosystems are not simply “surviving the extremes,” but are actively feeding, growing, and reproducing. Several recent papers from a project of international investigators characterized the shifts in diversity, environmental gene expression and metabolic activities of dry valley lake photosynthetic, heterotrophic and chemolithoautotrophic microbial communities during the transition from 24 h sunlight to the polar night (Bielewicz et al. 2011; Kong et al. 2012a,b; Thurman et al. 2012; Vick and Priscu 2012; Vick-Majors et al. 2014). Here we extended these efforts to understand adaptation to Antarctic polar night by integrating physiological and molecular responses of a well characterized green algal species originally isolated from Lake Bonney and reintroduced in dialysis chambers with that of the natural phytoplankton populations in the same lake.

By virtue of the perennial ice-cover, low advective stream flow, and saline bottom waters, the lakes of the McMurdo Dry Valleys are permanently stratified and exhibit thermodynamic and geochemical conditions reflecting the climate evolution and watershed characteristics of each lake (Priscu 1997; Spigel and Priscu 1998). The food webs are relatively simple, consisting almost exclusively of microorganisms. The lakes support a variety of photosynthetic eukaryotes which reside in well-defined layers in the water column and form the base of a truncated food web dominated by the microbial loop (Priscu et al. 1999; Bielewicz et al. 2011; Kong et al. 2012b). The microalgae are adapted to permanent low temperatures and extreme shade as the ice-cover attenuates more than 90% of incident PAR and acts as a monochromator restricting light to a narrow spectral range in the blue-green region (Lizotte and Priscu 1990, 1992, 1994). These continuous shade conditions present an opportunity to study photosynthetic adaptation in organisms that have never been exposed to a saturating light environment.

One of the few microalgae isolated from the lakes of the McMurdo Dry Valleys is the psychrophilic chlorophyte, *Chlamydomonas raudensis* UWO241 (Neale and Priscu 1995, 1998). Two decades of intensive laboratory studies on axenic isolates of this organism have revealed that the photochemical apparatus is finely adjusted to the extremely stable low light and low temperature environment from which it was isolated (Morgan-Kiss et al. 2002a,b, 2005, 2006; Morgan-Kiss and Dolhi 2011). Evolution of a highly efficient photochemical apparatus in this species has been as a consequence of a loss of multiple acclimatory responses to environmental variability (Morgan-Kiss et al. 2002a,b; Pocock et al. 2007; Szyszka et al. 2007). Experiments on acclimation to light quality showed that *C. raudensis* exhibits a novel requirement for blue light and reverts to a downregulated photochemical state under dark incubation or in the presence of red light (Morgan-Kiss et al. 2005). These early studies led to a working model for seasonal modulation of the energy conversion efficiency between absorbed light and photochemical energy generation. This model is based on the premise that the photochemical apparatus of this photosynthetic chlorella remains intact but shuts down during the long, dark Antarctic winter (Morgan-Kiss et al. 2005, 2006). This strategy is similar to that used by overwintering evergreens which modulate photochemical efficiency on a seasonal level, exhibiting a prolonged state of lowered energy conversion efficiency for the entire winter season and quickly converting to efficient energy capture during the short growing season (Demmig-Adams et al. 2012). Retaining the photochemical apparatus rather than undergoing de novo synthesis of the large numbers of proteins, pigments and photosynthetic membranes necessary for light energy capture provides an adaptive advantage for dry valley lake phytoplankton, which must grow and reproduce under the cold and low light conditions that exist during a short summer season.

In one of the few studies to examine the survival mechanisms of polar microalgae to the prolonged darkness of winter, Palmsano and Sullivan (1982, 1983) proposed that mobilization of stored carbon reserves is an important adaptive winter survival strategy in sea ice diatoms. Pancaldi and colleagues (Baldisserotto et al. 2005; Ferroni et al. 2007) also provided evidence that the polar marine microalgae, *Koliella antarctica,* undergoes substantial structural and functional reorganization of the photochemistry in response to simulated polar winter in the laboratory. Our study represents the culmination of intensive field activities during the 2007-2008 season and is the first to link the physiological and metabolic responses of a model “psychrophile” reintroduced into its native polar environment during the transition to polar night.

**Methods**

**Sampling site**

Lake Bonney is a saline lake located in the Taylor Valley, McMurdo Dry Valleys, Antarctica (77°00’S, 162°52’E), and is one of several lakes in the region with year-round liquid water and permanent ice cover of 3-6 m in thickness. The McMurdo Dry Valleys represents the largest ice-free expanse on the Antarctic continent (∼ 4000 km²) with a mean annual air temperature of ∼ −20°C. Sunlight does not reach the water column of Lake Bonney from mid-April to mid-August. Lake Bonney has two ~ 40 m deep lobes (east lobe, ELB; west lobe, WLB), which are separated by a 50-m-wide
bedrock sill at ~13 m below the ice surface. The sill allows exchange of surface waters between the lobes, but prevents direct exchange of the deep saline waters between the two basins. Both lobes are chemically stratified with photic zones that extend to the depth of the chemoclines (Spigel and Priscu 1998). The near surface phytoplankton in the lake have been shown to be P-deficient whereas upward diffusion of nutrients across the chemocline reduces the degree of nutrient stress to the organisms forming the deep chlorophyll layers (Priscu 1995; Dore and Priscu 2001).

**Limnological measurements and lake water sampling**

Limnological measurements and lake water sampling in the east lobe of Lake Bonney were part of a larger project on phytoplankton responses to polar night and have been described in detail elsewhere (Bielewicz et al. 2011; Kong et al. 2012b). PAR, light-mediated primary productivity (PPR), and chlorophyll a (Chl a) were collected and measured using previously described methods (Kong et al. 2012b). PAR depth profiles were measured with a LICOR LI-193 spherical quantum sensor (LI-COR Biosciences, Nebraska). Chl a was estimated with a bbe Moldaenke profiling spectrophotometer (Beutler et al. 2002). PPR was measured using 14C-bicarbonate incorporation into particulate matter over a 24 hr incubation of two light and one dark bottle at selected depths (Kong et al. 2002b). For nucleic acid analyses, water was collected with a 5-L Niskin bottle at 18 m, and 1-L was filtered onto sterile 47 mm 0.45 μm Durapore polyvinylidene fluoride membrane filters (Millipore, Massachusetts). Filters were immediately frozen in liquid nitrogen, transported to McMurdo Station in a dewar, and shipped in the dark on dry ice to U.S. laboratories. All depths are referenced to the hydrostatic water level in the borehole unless noted otherwise.

**Transplant experiment**

Cultures of *C. raudensis* UWO241 grown to a cell density of 2 × 10^7 cells mL^-1 in Bold’s basal medium supplemented with 0.7 M NaCl (BBM+ medium) under laboratory controlled conditions (8°C/20 μmol photons m^-2 s^-1/blue filtered-light) were collected in 50 mL falcon tubes by centrifuging at 3000 rpm for 15 min. All cultures were resuspended in BBM+ medium for shipment to Antarctica. Cultures were kept on ice in the dark during transport from the U.S. to McMurdo Station, Antarctica. Upon arrival, cultures were diluted in 0.2-μm filtered East Lobe Bonney lake water from a sampling depth of 17 m (i.e., the original depth from which *C. raudensis* was isolated). Diluted cultures (approximately 10^6 cells L^-1) were acclimated to the lake water for 2 weeks in a temperature-controlled environmental room in McMurdo Station at 4°C/15 μmol photons m^-2 s^-1.

For deployment in the lake, 400 mL of the adapted cultures were transferred into a series of ~ 1 m long 12 Spectra/Par2 dialysis membranes (Supplier No. 132678, 25 mm flat width, 12-14 kDa MWCO, Spectrum Laboratories), which were then attached to a stainless steel frame (Fig. 1). The frame with bags was deployed on 23 February 2008 (T0) at 17 m in ELB, the depth where *C. raudensis* UWO241 was originally isolated. Samples were harvested once a week beginning on 01 March 2008 (T1). The last sample was collected on 30 March 2008 (T5).

**Sampling**

Two bags were harvested once a week for each sampling timepoint. A 200 mL subsample was removed for RNA isolation and treated with 10% diethyldiprocarbonate-treated deionized water and concentrated onto 47-mm 0.45-μm Durapore polyvinylidene fluoride membrane filters (Millipore) at 5°C using a vacuum of 25 kPa. A replicate filter was collected for DNA analyses. The filters were frozen immediately in liquid nitrogen for 5 min and were transported to McMurdo station, where they were stored at -80°C for shipment to our U.S.-based laboratories.

**Photosynthesis-irradiance curves**

Samples were collected for photosynthesis-irradiance (PI) curves on 24 February 2008 and 30 March 2008. PI experiments were conducted in an incubator described by Lizotte and Priscu (1992). Briefly, the system consisted of a circulation bath (10% ethanol) illuminated from below by two 300-W tungsten-halogen lamps. Light was adjusted with neutral density screens to produce irradiance levels ranging from 3 μmol photons m^-2 s^-1 to 240 μmol photons m^-2 s^-1. Samples were inoculated with 14C-bicarbonate to a final activity of ~ 1 μCi mL^-1. Two-milliliters aliquots were dispensed into a series of prewashed 20 mL glass scintillation vials.
0.5 mL of 3N HCl was immediately added to three vials to correct for any potential background radioactivity; this activity was subsequently subtracted from all samples. Following 4-4.6 h incubation at 5°C, the reactions were terminated by adding 0.5 mL 3N HCl to all remaining vials. The acidified samples were dried at 70°C, rehydrated with 2 mL deionized water and 17 mL of Cytoscent scintillation cocktail (ICN Pharmaceuticals) was added to each vial. The radioactivity in all samples was determined by standard scintillation counting. Dissolved inorganic carbon, required for rate determination, was determined by infrared gas spectrometry of acid-sparged samples (Priscu 1995). Rates of carbon fixation were normalized to Chl a concentration and plots of normalized photosynthesis against irradiance were fitted with a hyperbolic tangent function using Marquardt’s Algorithm, which allowed the initial linear slope of the curve (alpha) and the maximum specific photosynthetic rate (μmol m−2 s−1) to be calculated (Lizotte and Priscu 1992).

RNA isolation
Total RNA was isolated from filtered transplant cultures or lake water using a combination of the RNeasy Mini Kit (Qia-gen, California) and FastRNA Pro Soil-Direct Kit (MP Biomedica, Ohio) according to Kong et al. (2002a). Steel tweezers and scissors treated with RNAZap (Ambion, Texas) were used to cut the filter into small pieces in a Petri dish pretreated with RNAZap. The pieces were transferred into an RNase-free lysing matrix tube E (MP Biomedical, California) that contained 700 μL of RLT solution (QIAGEN, California). To maximize cell lysis and minimize nucleic acid shearing, cells were treated for 30 s using a Mini-beadbeater (Biospec, Oklahoma) and immediately incubated on ice for 1 min and repeated. An equal volume of 70% ethanol (800 μL) was added to the homogenized lysate, and mixed well by vortexing. The remainder of the nucleic acid extraction protocol was performed in accordance with the manufacturer’s instructions (Qiagen, California). Contaminating DNA was removed according to the manufacturer’s instruction by incubating 33 μL of the eluted nucleic acid with three unit of RNase-free DNase (Ambion, Texas). To determine the integrity and purity of nucleic acids, 5 μL of each fraction was run in 1.0% agarose gel containing 0.0001% ethidium bromide. RNA concentrations were obtained with a Nanodrop® ND-1000 Spectrophotometer (Nanodrop Technologies, Delaware). One hundred ng of total RNA was reverse transcribed to single stranded cDNA using an iScript cDNA synthesis kit (Bio-Rad, California), and additional reactions for each sample were set up without reverse transcriptase (RT) to ensure the absence of genomic DNA in no-RT controls.

DNA isolation
Filters were cut into small pieces by sterile steel tweezers and scissors and total DNA was isolated from transplant samples using Fastprep DNA kit for soil (MP Biomedicals, California), according to the manufacturer’s instruction. Real-time PCR
According to known sequences in C. raudensis UWO241 (GenBank accession number DQ196177 for rbcL and AY325305 for psbA), specific primers were designed for rbcL and psbA using DNA software 5.2.2. Target fragments were amplified in an iCycler PCR (Bio-Rad, California) with the primers specific for rbcL (rbcLFS95: 5’-GAC TTC ACG AAA GAC GAC GA-3’ and rbcCR: 5’-GGAGTGGATACCCTGGTTT-3’) and psbA (psbAKR: 5’-TTATGCACCATCCACATG-3’ and psbAKR: 5’-GATTAACGTCAAAATGTAACG-3’). Environmental primer sets targeting either form IA/B rbcL or psbA were used to amplify these photosynthetic genes in the natural phytoplankton community from lake water as previously described by Kong et al. (2012b, 2014). Real-time PCR was performed in duplicates in a 25-μL reaction mixture containing 1 μL of cDNA, 1.5 μL of 10 pmol μL−1 and 12.5 μL iQ SYBR Green Supermix (Bio-Rad, California). Amplification conditions for both genes were 5 min at 95°C followed by 35 cycles of 1 min at 94°C, 20 s at 52°C, and 30 s at 72°C and fluorescence intensity was acquired at 83°C (above the melting point of primer dimers). To determine the melting temperature and PCR product specificity, a melting curve was obtained by heating from 50°C to 95°C. Data analysis was carried out using iCycler software version 3.01 (Bio-Rad, California). 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 according to Kong et al. (2012b) from plasmids containing the target inserts. RbcL and psbA amplicons were amplified from either C. raudensis DNA or environmental DNA with the four sets of primer pairs mentioned above and the PCR products were cloned into the pGEM-T Easy Vector (Promega). High-purity plasmids were isolated from the correct insert clones of each target gene using Plasmid Miniprep Kit (Qiagen, California). The plasmid DNA concentration was determined on a Nanodrop® ND-1000 Spectrophotometer (Nanodrop Technologies, Delaware) and the copy numbers of target genes were calculated directly from the concentration of the isolated plasmid DNA assuming 1.096 × 10−12 g/bp. All standard curves were generated from 10-fold serial dilutions of DNA with known copy numbers and were subjected to real-time PCR assay in duplicate.

Results and discussion
Natural communities
Sub-ice PAR during summer (December to February) was typically between 15 μmol photons m−2 s−1 to 20 μmol photons m−2 s−1 down to 10 m below the top of the ice cover in the east lobe of Lake Bonney and never exceeded 11 μmol photons m−2 s−1 below 15 m (Fig. 2A). Water column PAR decreased precipitously beginning in mid-February and was below measurable levels throughout the water column by
adaptation of photosynthetic processes (Morgan-Kiss et al. 2002a, 2002b, 2005, 2006; Pocock et al. 2004, 2007; Gudynaitė-Savitch et al. 2006; Szyszkia et al. 2007; Takizawa et al. 2009; Possmayer et al. 2011; Dolhi et al. 2013). From this body of work, several working models have emerged regarding environmental adaptation to extreme environmental conditions which would be prohibitive for growth for many photosynthetic organisms (Morgan-Kiss et al. 2006; Dolhi et al. 2013). One model postulated that as a survival strategy to permanent darkness during the Antarctic winter and a short growing season in the summer, dry valley lake phytoplankton retain their photosynthetic apparatus in a downregulated state in a mechanism similar to overwintering evergreens (Morgan-Kiss et al. 2005, 2006). Retaining the photosynthetic apparatus would be an adaptive advantage and an important survival mechanism for an obligate phototroph to compete with other organisms capable of mixotrophy (Laybourn-Parry 2002) during the short summer season. However, this model represents a prediction based on laboratory manipulations and does not fully account for the complexity of oscillations in environmental conditions to which natural dry valley lake phytoplankton communities are exposed.

We returned *C. raudensis* to its original isolation depth of 17 m in east lobe Lake Bonney in dialysis tubing during the polar night transition from late February to early April (Fig. 1). Cultures of *C. raudensis* were hand-carried to Antarctica, transferred to 17 m filtered lake water and allowed to acclimate for 2 weeks in the laboratory at 5 μmol photons m⁻² s⁻¹ and 4°C. Following acclimation, cultures were transferred to dialysis tubes and suspended in the lake at 17 m from late February (full sun) to early April (complete darkness; Fig. 1A).

Photosynthesis vs. irradiance experiments conducted on dialysis tube subsamples on 24 February 2008 and on 30 March 2008 revealed that photosynthetic efficiency increased from 0.0062 mg C mg Chl⁻¹ h⁻¹/μmol photons m⁻² s⁻¹ to 0.0074 mg C mg Chl⁻¹ h⁻¹/μmol photons m⁻² s⁻¹ (average standard errors 0.0004 and 0.0003, respectively). Biomass specific maximum rate of photosynthesis decreased from 0.42 mg C mg Chl⁻¹ h⁻¹ to 0.30 mg C mg Chl⁻¹ h⁻¹ (Fig. 3A), resulting in a drop in the photoacclimation index (Ii) from 68 μmol photons m⁻² s⁻¹ to 41 μmol photons m⁻² s⁻¹. These results showed that the phytoplankton became more shade acclimated as light decreased during the summer-winter transition. Changes in photochemistry and inorganic carbon fixation potential were determined over this same period by measuring protein and gene transcript (mRNA) abundance of *psbA*, encoding the major photosystem II reaction center protein D1, and *rbCL*, encoding the large subunit (LSU) of Rubisco. Both D1 and LSU abundance increased transiently following reintroduction to the lake (Fig. 3B), which was presumably an artifact of re-acclimation to the natural environment. Once acclimated,
LSU declined steadily during the transition to polar night, while D1 levels remained relatively constant. These results are in agreement with an earlier winter adaptation model developed for *C. raudensis* (Morgan-Kiss et al. 2005, 2006) which predicted that CO₂ fixation would decline with decreasing PAR, but the cells would maintain stable levels of major photochemistry proteins during the polar winter. In contrast with photosynthetic protein levels, expression of both *rbcl* and *psbA* declined during the polar night transition and exhibited a positive correlation with PAR levels ($r = 0.90$, and $r = 0.92$, for *rbcl* and *psbA*, respectively, $p < 0.05$; Fig. 3C). Transcriptional activity (i.e., the ratio of *rbcl* or *psbA* mRNA/DNA) varied dramatically between the two photosynthetic genes. The ratio of *psbA* mRNA/DNA was significantly higher than that of *rbcl* at all time points (Fig. 3D), which supports past observations that *C. raudensis* maintains very high levels of PS II as a consequence of adaptation to extreme shade (Morgan et al. 1998). Transcriptional activity of *psbA* declined during the summer-winter transition, and exhibited a transient rise late in the season (Fig. 3D). In contrast, transcriptional activity of *rbcl* remained relatively low throughout the polar night transition, which was reflected in low rates of primary production in the deep photic zone (Figs. 2C, 3D).

**Expression of functional genes in transplanted vs. natural phytoplankton communities**

Transcript levels of *psbA* and *rbcl* were measured in natural 17 m populations together with transplanted cells to determine how biochemical changes in the natural phytoplankton community residing in the deep photic zone of East Bonney reflected the biochemistry of *C. raudensis* monocolonies within the dialysis tubes (Fig. 4). Phylogenetic analysis of the natural phytoplankton residing at 17 m ELB revealed that the deeper waters were dominated by *Chlamydomonas* sp. and other polar chlorophyte species (Bielewicz...
Expression of *rbcL* declined in dialysis tube monocultures and natural populations, and exhibited a statistically significant positive correlation with PAR ($r = 0.73$ and $0.51$, for *rbcL* in transplant and natural samples, respectively, $p < 0.05$; Fig. 4A, inset). Comparison of *psbA* expression levels between the transplant and the natural samples indicated that both exhibited similar temporal trends over the summer-winter transition; however, *psbA* expression in the natural community exhibited no significant correlation with PAR ($r = 0.27$, $p > 0.10$; Fig. 4B, inset). The low level of association between *psbA* expression and PAR was a consequence of a transient increase in *psbA* transcript abundance in late March in both transplant and natural samples (Fig. 4B). This transient rise in expression of the PSII photochemistry gene late in the polar night transition also correlated with trends in Chl a (Fig. 2B) which indicates that the phytoplankton responded to reduced light levels by synthesizing more pigment within the photochemical apparatus. These results also agreed well with evidence for shade adaptation in *C. raudensis* cultures collected late in the seasonal transition (Fig. 3A) and the late-season stimulation of *psbA* transcriptional activity (Fig. 3D).

**A model for polar night adaptation**

Our work presented here represents the culmination of an intensive study to understand how planktonic microbial communities residing in the McMurdo Dry Valley lakes respond to the extreme seasonal oscillations during the transition from Antarctic summer (full sunlight) to winter (complete darkness). Earlier papers focused on the natural communities and reported a coupling between light availability, primary production, and expression of major photosynthetic genes (*rbcL*, *psbA*), indicating downregulation of the light dependent primary production during the polar night transition (Kong et al. 2012b, 2014). With cessation of photosynthesis, abundance of mixotrophic organisms such as the Chrysophyceae *Ochromonas* as well as heterotrophic nanoflagellates and ciliates increased in the autumn (Vick-Majors et al. 2014). Phototrophic nanoflagellates exhibited higher grazing rates in WLB during the month of March (Thurman et al. 2012). Potential shifts in bacterial community function were also observed, including detection of the *cbbM* gene throughout the polar night transition, encoding for the major RubisCO of chemolithoautotrophic bacteria (Kong et al. 2012a). Vick-Majors et al. (2014) also found evidence of several bacteria taxa (*Pelagibacter*, Flavobacteriaceae, *Oceanospirillales*) which can produce proteorhodopsins, indicating that photoheterotrophic metabolism is likely part of carbon and energy cycling in the dry valley lakes.

This study represents an extension in our understanding of environmental adaptation of a model photopsychrophile from models developed under laboratory-controlled conditions to ecologically relevant physiological responses to the organism’s natural environment. Our data show that expression of genes involved in photochemically derived energy capture and carbon acquisition (*psbA*, *rbcL*) are downregulated in both transplanted monocultures of *C. raudensis* exposed to its natural environment and in the natural phytoplankton population (Fig. 4). This downregulation is accompanied by the loss of the key carbon fixation enzyme RubisCO (Figs. 3, 5) which agreed with original model which predicted downregulation of photosynthesis in favor increased respiration rates (Morgan-Kiss et al. 2006). In addition, maintenace of PsbA protein abundance throughout the polar transition supports our earlier prediction that photochemical complexes are structurally maintained (Figs. 3, 5; Morgan-Kiss et al. 2006). Maintenance of major
photochemical machinery in a downregulated state throughout the winter is likely a critical adaptive strategy for obligate photosynthetic microalgae to compete with mixotrophic protists which can continue to acquire carbon and energy in the absence of light (Fig. 5). Expression of both photosynthetic genes in transplant and natural samples, transcriptional activity of psbA, as well as Chl a abundance also increased as solar radiation diminished later in the season (Figs. 2B, 3, and 4). This transient stimulation in the photosynthetic community was not predicted in our earlier models and highlights the importance of field-based studies to complement laboratory manipulated experiments.

Priscu and colleagues used a large body of data on PAR and carbon pools in the McMurdo Dry Valley lakes to compile a PAR-driven model of carbon flux and transformation in Lake Bonney (Priscu et al. 1999). The model revealed several important points, including the importance of mixotrophic activity on heterotrophic production as well as carbon incorporation into biomass. The model also predicted several examples of disequilibrium in the carbon balance of these truncated aquatic food webs. For example, all values for net primary production to respiration ratios (P:R) were predicted to be <1, suggesting that growth of Lake Bonney bacterio-plankton would ultimately be limited if phytoplankton excretion is the only available source of organic carbon. Grazing rates for mixotrophic and heterotrophic flagellates were also predicted to exceed pool sizes of prey. One major caveat from this seminal work was that their predictions were largely from data collected during annual field activities restricted to a 4-month period (October–January).

We combined primary productivity and Chl a data generated in our summer-to-winter study with the single complementary study by Lizotte et al. (1996) that focused on the winter-spring transition to examine annual trends in water column productivity and phytoplankton biomass (Fig. 6). Photosynthetic activity began with the onset of light availability in mid-September and reached maximum levels in early November (Fig. 6A), while Chl a levels remained relatively constant during the winter-spring transition (Fig. 6B). Carbon production remained high until early March when it declined rapidly as light availability was reduced to 10% of summer values. Seasonal Chl a trends were uncoupled from light availability and photosynthesis during the polar night transition and continued to increase until the last measurement in early April (Fig. 6B). The accumulation of Chl a at the end of the growing season may be indicative of photoacclimation to extreme shade (i.e., increase in Chl a per cell).
or may reflect biomass accumulation of mixotrophic algae which supplement carbon and energy acquisition with phagotrophy (Thurman et al. 2012) (Figs. 5, 6). Losses in Chl a between fall (April) and spring (September) represent phytoplankton sinking, death/lysis, viral activity and/or grazing, all of which would be important processes in lake carbon and nutrient redistribution.

We estimated total annual carbon production in the east lobe of Lake Bonney to be 1663 kg carbon produced from photosynthetic activity. This estimate is lower than the values predicted by the model by Priscu et al. (2996-5370 kg) (1999) corroborating past reports that that new carbon produced by photosynthetic activity in Bonney is insufficient to support contemporary heterotrophic bacterioplankton production (Takacs and Priscu 1998; Priscu et al. 1999). By comparing bacterioplankton incorporation rates of leucine vs. thymidine, Vick and Priscu (2012) predicted that Lake Bonney bacterioplankton switch from utilizing phytoplankton-derived carbon to other sources of organic carbon once photosynthetic activity ceases in the fall. Utilization of a bulk organic carbon pool which is available to bacterioplankton through upward diffusion of ancient pools of relict dissolved organic matter is likely important for supplementing energy and carbon for heterotrophic organisms (Priscu et al. 1999; Vick and Priscu 2012). While there is genetic evidence for enrichment of bacteria potentially capable of chemolithoautotrophy or photoheterotrophy during the polar night transition (Kong et al. 2012a,b; Vick-Majors et al. 2014), the current lack of rate measurements for these groups makes it difficult to assess their contributions to carbon and energy in the dry valley food web.

We now have continuous data on the dry valley lake plankton dynamics covering the entire period when adequate PAR exists to drive photosynthesis. However, gaps remain in understanding the survival and activity of the microbial community across 6 months of complete darkness during the winter. The extreme logistical constraints during the Antarctic winter make field activities during this time virtually impossible. Autonomous year-round sampling and sensing instrumentation have been recently deployed in Lake Bonney (Winslow et al. 2014) which will help define the habitability of these extreme aquatic systems during the polar night. Incorporation of real-time measurements of microbial community dynamics during the winter as well as an improved appreciation for alternative autotrophic contributions to energy and carbon inputs will be critical in assessing climate-associated shifts in microbial community functioning of these unique and sensitive ecosystems.

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