Evidence for phenotypic plasticity in the Antarctic extremophile *Chlamydomonas raudensis* Ettl. UWO 241

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Abstract

Life in extreme environments poses unique challenges to photosynthetic organisms. The ability for an extremophilic green alga and its genetic and mesophilic equivalent to acclimate to changes in their environment was examined to determine the extent of their phenotypic plasticities. The Antarctic extremophile *Chlamydomonas raudensis* Ettl. UWO 241 (UWO) was isolated from an ice-covered lake in Antarctica, whereas its mesophilic counterpart *C. raudensis* Ettl. SAG 49.72 (SAG) was isolated from a meadow pool in the Czech Republic. The effects of changes in temperature and salinity on growth, morphology, and photochemistry were examined in the two strains. Differential acclimative responses were observed in UWO which include a wider salinity range for growth, and broader temperature- and salt-induced fluctuations in $F_v/F_m$, relative to SAG. Furthermore, the redox state of the photosynthetic electron transport chain, measured as $1-q_P$, was modulated in the extremophile whereas this was not observed in the mesophile. Interestingly, it is shown for the first time that SAG is similar to UWO in that it is unable to undergo state transitions. The different natural histories of these two strains exert different evolutionary pressures and, consequently, different abilities for acclimation, an important component of phenotypic plasticity. In contrast to SAG, UWO relied on a redox sensing and signalling system under the growth conditions used in this study. It is proposed that growth and adaptation of UWO under a stressful and extreme environment poises this extremophile for better success under changing environmental conditions.

Key words: Acclimation, Antarctica, *Chlamydomonas raudensis*, climate change, phenotypic plasticity, photostasis, PSII excitation pressure.

Introduction

Over 80% of the earth’s biosphere is permanently cold (Russel, 1990), and many photoautotrophic organisms such as green algae are able to withstand, and even flourish under, conditions that are considered non-optimal for life. Algae living in the Arctic and Antarctic have been studied for the last 170 years, but it is only in the last 20 years that great effort and success has been achieved in isolating these unique organisms and cultivating them in the laboratory for physiological studies (reviewed in Mock and Thomas, 2008).

The extremophilic green alga, *Chlamydomonas raudensis* Ettl. UWO 241 (hereafter referred to as UWO), was isolated from the deepest photobiotic zone 17 m below the permanent ice cover of Lake Bonney, Antarctica (Lizotte and Priscu, 1994). Photosynthetic organisms play a critical role in the permanently ice-covered lakes in West Antarctica as the primary productivity of the continent is largely provided by green algae and as algal symbionts with fungi. Temperature gradients are responsible for vertical stratification in aquatic systems; however, in Lake Bonney, stratification is...
the result of strong salinity gradients (Spigel and Priscu, 1996). UWO is adapted to high salt as the salinity of its natural habitat is ~700 mM which is above that of seawater (~545 mM) (Priscu, 1998). The depth at which UWO was collected consists of year-round and stable salinities of 700 mM and temperatures of 4–6 °C, while irradiance never exceeds 50 μmol quanta m⁻² s⁻¹ during austral summers (Spigel and Priscu, 1996).

The physiology of the low-light-, high-salt-, and low-temperature-adapted UWO has been researched for over a decade (reviewed in Morgan-Kiss et al., 2006). It has been established that UWO is an obligate psychrophile as its optimum growth temperature is 8 °C and it cannot grow above temperatures of 16 °C (Morgan et al., 1998). The organization, composition, and function of its photosynthetic apparatus are unique compared with the model C. reinhardtii, as observed by the large photosystem II (PSII) absorption cross-sections, small functional absorption cross-sections of PSI, high PSII:PSI ratios, and low chlorophyll (Chl) alb ratios (Morgan et al., 1998). In addition, UWO is unable to undergo the short-term acclimation response of state transitions as it is locked in State I (Morgan-Kiss et al., 2002). Neale and Priscu (1995) suggest that increased light harvesting has been favoured in the low-light-adapted UWO at the expense of photoprotection. However, as yet unidentified photo-protective mechanisms independent of the D1 repair cycle do exist in this organism (Pocock et al., 2007a). Lastly, it has been established that UWO is halotolerant rather than halophilic (Pocock et al., 2007b; Takizawa et al., 2009).

Photosynthesis in UWO is compromised at high temperatures and there has been much interest in this in order to better understand the nature of its psychrophily (Morgan et al., 1998; Morgan-Kiss et al., 2002; Szyszka et al., 2007). Most studies have been performed on UWO cultures that were shifted to high measuring temperatures rather than grown and acclimated under high temperatures. However, Szyszka et al. (2007) examined the acclimative processes in UWO grown at high temperature (15 °C) and concluded that photostasis, or chloroplastic energy balance, was maintained through regulation of PSII/PSI stoichiometry and energy partitioning through the less understood constitutive non-regulatory energy quenching (Φₙₒ). Photosynthetic organisms in nature are exposed to potentially damaging energy imbalances within the chloroplast. This is particularly true for extremophiles that grow in environments that typically contain multiple stresses. It would be an obvious evolutionary advantage for extremophiles to possess acclimation mechanisms which would enable them to succeed in otherwise adverse environments. Acclimation to changes in the environment involves short-term, non-heritable adjustments to physiology, biochemistry, and/or morphology. Acclimatization potential is determined by the extent of an organism’s phenotypic plasticity and it allows an organism to cope with environmental change by altering its phenotype (reviewed in Valladares et al., 2007; Somero, 2010). More specifically, phenotypic plasticity refers to changes in behaviour, morphology, or physiology as a direct consequence of changes in the environment (Price et al., 2003).

Molecular phylogenetic analyses have revealed that UWO is a psychrophilic variant of the mesophile, C. raudensis Ettl. SAG 49.72 (hereafter referred to as SAG) (Pocock et al., 2004). The latter is the authentic C. raudensis strain that was isolated from a meadow pool in Nordmähren, Czech Republic (Pocock et al., 2004). Typically green algae are compared with the well characterized model organism C. reinhardtii. Comparison between the mesophilic and non-halotolerant SAG and the extremophilic UWO provides a novel model system for examining the characteristics and mechanisms unique to extremophilic organisms. To our knowledge, the only study to date that compares the two C. raudensis strains concludes that there is a differential physiological response to growth at high light and high temperature (Szyszka et al., 2007).

Over 97% of Antarctica is permanently ice covered. Recent changes in recorded ice-sheet surface temperatures suggest complex local climate trends in many Antarctic environments (Doran et al., 2002; Steig et al., 2009). Furthermore, it has been reported that the Antarctic Peninsula is one of the most rapidly warming regions on Earth (Doran et al., 2002; Turner et al., 2005). Climate field reconstruction studies have shown that the extent of warming in West Antarctica is as significant as that observed on the Antarctic Peninsula (Steig et al., 2009). Lake Bonney in West Antarctica is the natural habitat for UWO and it is reported that this lake as well as others in the valley system are undergoing climactic forcing that has resulted in a substantial rise in the lake level over a 30 year period (Bomblies et al., 2001). Thus, Lake Bonney is undergoing rapid changes, the air temperature is rising, and the subsequent ice-melt will dilute the lakes, making them less saline. Not only does this study examine physiological and photochemical effects of changes in growth temperature and salinity on the extremophile UWO and the mesophilic SAG but it compares their abilities to acclimate and, thus, their capacities for phenotypic plasticity. The extremophile UWO has adapted to an environment where multiple stresses exist, and the present data indicate that phenotypic plasticity in UWO involves acclimation processes that rely on redox sensing.

Materials and methods

Algal cultures

The extremophile algal strain, C. raudensis UWO 241 (UWO) was isolated from Lake Bonney, Antarctica by John Priscu and was donated by the Environmental Stress Biology Group at the University of Western Ontario (N. P. A. Huner). It is deposited at the Culture Collection of Algae and Protozoa, Scotland, UK as CCAP 11/131. The authentic strain C. raudensis Ettl SAG 49.72 (SAG) was isolated by Ettl, from a meadow pool near Rudná, Nordmähren, Czech Republic and is deposited at the Provasoli-Guillard Center.

Culture conditions

Cultures were grown in 250 ml pyrex culture tubes suspended in thermo-regulated aquaria at temperatures of 8 °C and 15 °C for UWO and 24 °C and 15 °C for SAG. Irradiance was provided as
Continental light at a photon flux density of 20 μmol photon m⁻² s⁻¹. Light was supplied by fluorescent tubes (Sylvania CW-40) and was measured with a quantum sensor (Model LI-189, Licor Inc., Lincoln, NE, USA). All cultures were continuously aerated under ambient CO₂ conditions. Cultures were grown in a modified Bold’s Basal Medium and salinity was adjusted using NaCl (Nichols and Bold, 1965). The salinity range for UWO was 10 mM to 1.3 M NaCl and for SAG it was 10–100 mM NaCl. The individual strains were grown up to their maximum salinity capacities above which the cultures were unable to grow.

Specific growth rates

Growth was monitored as changes in the optical density at 750 nm. The specific growth rates were calculated as the slope of the ln-transformed exponential portions of the growth curves. All experiments were performed on cells harvested during their respective mid-exponential growth phases.

Modulated room temperature Chl a fluorescence

In vivo room temperature Chl a fluorescence measurements were made using a pulse amplitude modulated fluorometer (WATER-PAM; Heinz Walz, Effeltrich, Germany). Aliquots of 3 ml containing a total of 5–6 μg of Chl were placed in a cuvette maintained at the individual growth temperatures of either 8, 15, or 24 °C using a home-built thermo-regulator. Prior to measurement, cells were dark adapted for 10 min to visualize Qₓ fully. Minimum fluorescence (Fₒ) was measured using a weak modulated light (<0.05 μmol m⁻² s⁻¹), and maximum fluorescence (Fₘ) was determined by applying a 5500 μmol m⁻² s⁻¹ saturating flash for 800 ms. Variable fluorescence (Fᵥ) was calculated as Fₘ – Fₒ, and maximum PSII photochemical efficiency was calculated as Fᵥ/Fₘ (Krause and Weis, 1991). PSII excitation pressure was calculated as 1 – qp [(Fₘ – Fₒ)/(Fₘ – Fₒ)], with qp being the coefficient for photochemical quenching (van Kooten and Snel, 1990).

Low temperature fluorescence

Low temperature (77 K) Chl fluorescence emission spectra were collected using a FluoroMax 2 fluorometer with the integrated software package DataMax ver. 2.20 (Jobin Yvon SPEX Instruments S. A. Inc., Edison, NJ, USA). The Chl fluorescence emission spectra of whole cells of C. raudensis were excited at 436 nm and recorded between 650 nm and 750 nm using a slit width of 4 nm for excitation and 2 nm for emission. The Chl concentration was 6.3–10.6 μg ml⁻¹. State transitions were induced as described in Turpin and Bruce (1990). Fresh samples of exponentially growing cultures were harvested and frozen immediately in the light to induced a state 1 condition, whereas samples were incubated for 20 min in the dark before freezing in liquid nitrogen to induce state 1 to state 2 shift. All spectra represent an average of three scans from each experiment and were normalized to their respective peaks.

Results

The effects of temperature and salinity on growth and cell ultrastructure

It is shown for the first time that the upper critical salinity limit for the psychrophilic algal strain UWO is temperature dependent. The upper salinity limit for near-maximum growth at 8 °C was 1000 mM whereas at 15 °C it was 700 mM (Fig. 1A). Beyond these salinity limits, the specific growth rates decreased sharply (Fig. 1A). Furthermore, despite the fact that UWO has adapted to a natural salinity of 700 mM, the specific growth rates were 35% and 45% greater at 10 mM relative to 700 mM when grown at 8 °C and 15 °C, respectively (Fig. 1A). In order to determine if cultures of UWO could grow above 15 °C at low salinities, cultures were grown at 20 °C and 10 mM and 700 mM, but growth was completely inhibited (data not shown). In contrast to UWO, the mesophilic counterpart SAG was not halotolerant (Fig. 1B). The salinity limit for SAG beyond which growth could occur was between 100 mM and 120 mM at both growth temperatures (Fig. 1B).

Electron micrographs of UWO showed no salt-induced changes in chloroplast ultrastructure in cultures grown at 8 °C (Fig. 2A, B). However, UWO grown at 15 °C resulted in more appressed thylakoid membranes at 10 mM (Fig. 2C) while at 700 mM starch accumulations were interspersed throughout the thylakoid membranes (Fig. 2D, arrow). Any accumulated starch in UWO grown at 8 °C at all salinities as well as at 15 °C and 10 mM was at the periphery of the pyrenoids (Fig. 2A–D). No salt or temperature effects were observed on thylakoid membrane organization or starch accumulation in SAG (Fig. 2E–H).

The effects of temperature and salinity on room-temperature and low-temperature chlorophyll a fluorescence

Maximum PSII photochemical efficiency (Fᵥ/Fₘ) was measured to determine if the various temperature and salinity

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Fig. 1. Growth kinetics for the extremophile C. raudensis UWO 241 (A) and the mesophile C. raudensis SAG 49.72 (B) measured as changes in the optical density at 750 nm. UWO was grown at 8 °C (open circles) and 15 °C (filled circles) at salinities ranging from 10 mM to its upper critical salinity limit of 1300 mM. SAG was grown at 24 °C (filled circles) and 15 °C (open squares) at salinities ranging from 10 mM to its upper critical salinity limit of 100 mM. Data represent means ± SE from 3–5 independent experiments.
Fig. 2. Electron micrographs of whole cells of *C. raudensis* UWO 241 grown at 8 °C and 10 mM (A), at 8 °C and 700 mM (B), at 15 °C and 10 mM (C), and at 15 °C and 700 mM (D), and of *C. raudensis* SAG 49.74 grown at 24 °C and 10 mM (E), 24 °C at 100 mM (F), at 15 °C and 10 mM (G), and at 15 °C and 100 mM (H) NaCl. N, nucleus; P, pyrenoid; TM, thylakoid membrane; S, starch. Scale bars represent 1.0 μm.
regimes induced a stress response. The data showed that UWO was not stressed by an increase in growth temperature at the low salinity of 10 mM, while at 700 mM and 850 mM the lower $F_v/F_m$ values indicated a temperature-induced stress (Table 1). Salt-induced stress, as observed by lower $F_v/F_m$ values, occurred at both temperatures, and this was more pronounced at 15 °C (Table 1). Maximum PSII photochemical efficiency ($F_v/F_m$) for SAG was less affected by temperature or salinity (Table 1). However, it is important to note that the upper critical salt concentration for SAG was 100 mM. PSII excitation pressure is estimated by the Chl a fluorescence parameter 1–qp. This parameter reflects the reduction state of PSII (Q A) and is used as a relative measure for the reduction state of the plastoquinone pool (Huner et al., 1998). The salt- and temperature-induced increases in 1–qp in UWO mirrored the decreases in $F_v/F_m$

Table 1. Maximum PSII photochemical efficiency ($F_v/F_m$) and PSII excitation pressure (1–qp) for UWO grown at 8 °C and 15 °C at salinities ranging from 10 mM to 850 mM NaCl and SAG grown at 24 °C and 15 °C at salinities ranging from 10 mM to 100 mM NaCl. Values represent means ±SE from three independent experiments.

|          | $F_v/F_m$ | 1–qp  |
|----------|-----------|-------|
| UWO      |           |       |
| 10 mM    | 0.80±0.00 | 0.11±0.01 |
| 700 mM   | 0.71±0.01 | 0.21±0.03 |
| 850 mM   | 0.69±0.02 | 0.25±0.03 |
| SAG      |           |       |
| 10 mM    | 0.80±0.01 | 0.09±0.01 |
| 60 mM    | 0.82±0.02 | 0.09±0.01 |
| 80 mM    | 0.82±0.01 | 0.08±0.01 |
| 100 mM   | 0.80±0.01 | 0.12±0.00 |

Table 2. Ratios of Chl a fluorescence emission band maxima as estimated by fluorescence spectra at 77 K

UWO was grown at 8 °C and 15 °C at salinities that ranged from 10 mM to 850 mM NaCl, and SAG was grown at 24 °C and 15 °C at salinities that ranged from 10 mM to 100 mM NaCl. Values represent means of three discrete samples taken from one experimental culture.

|          | PSII:PSI, $F_{684}/F_{710}$ | PSII:PSI, $F_{684}/F_{710}$ | LHCII:PSII, $F_{684}/F_{694}$ |
|----------|-----------------------------|-----------------------------|-----------------------------|
| UWO      |                             |                             |                             |
| 10 mM    | 2.0                         | 1.8                         | 1.1                         |
| 700 mM   | 2.5                         | 2.1                         | 1.2                         |
| 850 mM   | 2.6                         | 2.2                         | 1.2                         |
| SAG      |                             |                             |                             |
| 10 mM    | 0.9                         | 0.7                         | 1.2                         |
| 80 mM    | 1.4                         | 1.3                         | 1.1                         |

Fig. 3. Chlorophyll a fluorescence emission spectra at 77 K of whole cells of C. raudensis UWO 241 grown at 8 °C (A) and 15 °C (B) and C. raudensis SAG 49.72 grown at 24 °C (C) and 15 °C (D). UWO was grown at 10 mM (solid line), 700 mM (medium dashed line), and 850 mM (long dashed line) NaCl, and SAG was grown at 10 mM (solid line) and 80 mM (medium dashed line) NaCl. Spectra represent an average of three corrected scans and were normalized to their respective maximum peaks.
ratios, whether measured as $F_{684}/F_{710}$ or $F_{694}/F_{710}$, were consistently higher in UWO relative to SAG (Table 2). Growth temperature had little effect on PSII:PSI ratios in both strains, but increasing the salinity resulted in increased PSII:PSI ratios in both strains (Table 2). The size of the peripheral antennae relative to PSII core units can be estimated by the LHCII:PSII ratios. The LHCII:PSII ratios were similar for UWO and SAG irrespective of salt or temperature growth conditions (Table 2).

The salt-induced decrease in fluorescence emission in PSI ($F_{710}$) in SAG prompted examination of the capacity for state transitions in the two strains. State transitions are a rapid way to maintain an energy balance between the two photosystems. As previously observed by Morgan et al. (2002), UWO was unable to undergo state transitions when grown under its natural temperature and salinity of 8 °C and 700 mM and at 15 °C and 700 mM (Fig. 4A, B). As reported by Takizawa et al. (2009), minor increases in PSI 77 K fluorescence emission ($F_{710}$) were observed in UWO at the low salinity of 10 mM at both temperatures (data not shown). However, here it is shown for the first time that UWO grown at 15 °C and 850 mM undergoes a pronounced increase in PSI fluorescence ($F_{710}$) after a 20 min dark incubation to induce state 2 (Fig. 4D). To date, there have not been any studies on the ability of SAG to undergo state transitions. It is clear from the present data that SAG is similar to UWO in that 77 K fluorescence emission spectra do not change when cultures are pre-treated with state 1- and state 2-inducing protocols (Fig. 5A, B).

**Discussion**

Here it is shown for the first time that the optimal growth temperature for the extremophile, UWO, is salt dependent. Up to a salinity of 700 mM which is that found in UWO’s natural environment, growth was marginally affected by temperature (Fig. 1A). Above this salinity level, the specific growth rates decreased at the higher growth temperature of 15 °C in contrast to growth at 8 °C. Thus, 700 mM is the upper critical salinity limit for UWO grown at 15 °C, whereas at 8 °C the upper critical salinity limit was 1000 mM. Clearly the stress imposed on this extremophile by higher growth temperatures up to 15 °C can be mitigated by reducing the salinity. This suggests that UWO could maintain growth if lake temperatures increased and salinity decreased simultaneously. In contrast to its genetic counterpart, salinity had a minimal effect on the temperature range for growth in the mesophilic strain, SAG (Fig. 1B). It cannot be assumed that salinity stress is equal for the two strains nor can it be suggested that the physiological impact of high temperature stress in the psychrophile is equal to low temperature stress in the mesophile. However, in this study each strain was grown and characterized at temperature and salinity combinations that push them to their limits for growth.

![Fig. 4. Chlorophyll a fluorescence emission spectra at 77 K of whole cells of the extremophile C. raudensis UWO 241 grown at 8 °C and 700 mM (A), at 8 °C and 850 mM (B), at 15 °C and 700 mM (C), and at 15 °C and 850 mM (D) that were frozen immediately after harvest in the light (solid lines) or after a 20 min incubation in the dark (dashed lines). Insets represent the difference between the spectra generated from the light and dark incubations. Spectra represent an average of three corrected scans and were normalized to their respective maximum peaks.](#)

![Fig. 5. Chlorophyll a fluorescence emission spectra at 77 K of whole cells of the mesophile, C. raudensis SAG 49.72 grown at 24 °C and 10 mM (A) and 24 °C and 80 mM (B) that were frozen immediately after harvest in the light (solid lines) or after a 20 min incubation in the dark (dashed lines). Insets represent the difference between the spectra generated from the light and dark incubations. Spectra represent an average of three corrected scans and were normalized to their respective maximum peaks.](#)
Similar to the findings of Nadeau and Castenholz (2000), it is suggested that the phenotypic plasticity observed in UWO could be considered partially responsible for the success of this photosynthetic organism in the extreme environment of low temperature, high salinity, and low light. Populations living in environmentally different habitats are exposed to contrasting selective pressures, and this may result in a differential extent of plastic responses to comparable and potentially stressful changes in the environment (reviewed in Valladares et al., 2007). The driving selective forces in extreme environments are abiotic (chemical and physical), rather than biotic (resource competition), and here it is shown that UWO responds to abiotic stress by increasing its tolerance to stressful temperatures and salinities (Fig. 1A).

The maintenance of photostasis allows photosynthetic organisms to maintain growth despite changes in their environments, and this is achieved through different acclimation processes (Durnford and Falkowski, 1997; Huner et al., 1998; Ensminger et al., 2006). Shifts in photostasis can be observed as changes in PSII excitation pressure which is measured by the Chl fluorescence parameter, 1–qP (Huner et al., 2002). The modulation of PSII excitation pressure has been described as an important chloroplastic redox signal in acclimation processes (Huner et al., 1998).

The maintenance of growth, the gradual decreases in maximum photochemical efficiency (Fv/Fm), and the corresponding increases in PSII excitation pressure (1–qP) indicate that UWO can acclimate through redox sensing (Table 2). Huner et al. (2002) discuss the activation of redox sensing as the result of decreasing growth temperature or increasing irradiance. Here it is shown for the first time that growth under either high salinity or high temperature in UWO resulted in high PSII excitation pressure (Table 1). Moreover, the combination of high temperature and high salinity had a cumulative effect on 1–qP in UWO (Table 1).

In contrast to UWO, SAG did not modulate 1–qP beyond the upper control level of ~0.20 (Maxwell et al., 1995) (Table 1). Under the temperature and salinity regimes used in this study, it was observed that UWO relied on acclimation through redox sensing whereas SAG relied on different acclimation processes (Table 1). These results lead to the hypothesis that UWO and perhaps other extremophiles have increased phenotypic plasticity and, thus, can survive multi-stress environments due to the capacity for redox sensing and signalling.

One acclimation mechanism involved in the maintenance of photostasis is the ability to regulate the distribution of absorbed light energy between the two photosystems, and this is determined using 77 K fluorescence emission spectra (Bonaventura and Myers, 1969; Allen, 1992; Wollman, 2001). Previous studies show that UWO is deficient in PSI fluorescence emission compared with SAG (Szszyka et al., 2007) and the present data support this and show that temperature or salinity had a minimal effect on 77 K in UWO (Fig. 3A, B). Interestingly, a pronounced salt-related decrease in PSI fluorescence emission for SAG grown at both temperatures was observed (Fig. 3C, D). Here it is shown that SAG, in contrast to UWO, responds to high salinity by adjusting the energy distribution between the two photosystems (Table 2, Fig. 3C, D).

Increasing the growth salinity for SAG resulted in a relative decrease in PSI fluorescence emission; thus the potential for SAG to undergo state transitions was examined. State transitions offer short-term acclimation to conditions that result in an energy imbalance between the two photosystems (Allen, 1992; Mullineux and Emlyn-Jones, 2005). Earlier studies have shown that UWO is unable to undergo state transitions when grown at 8 °C and 700 mM NaCl (Morgan et al., 2002). This was confirmed by the present data for UWO at both growth temperatures and all salinities (Fig. 4A, C). Interestingly, it is shown for the first time that the authentic C. raudensis strain, SAG, is similar to its extremophilic counterpart in that it is also locked in state 1 (Fig. 4). Therefore, SAG does not possess the state transition acclimation response.

Despite not possessing the ability for state transitions, short-term shifts in energy distribution between the photosystems have been reported by Takizawa et al. (2009) for UWO grown under low salinity and low temperature. They concluded that spillover is the most likely candidate to explain the change in energy redistribution. Spillover in itself does not require migration of antenna components but can be linked to, among other things, change in appression of thylakoid membranes (see, for example, Guenther and Melis, 1990). The same low salt effect is observed on UWO cultures grown at 8 °C and 15 °C (data not shown).

However, UWO grown at high salinity (850 mM) and high temperature (15 °C) resulted in an even higher 77 K fluorescence emission in PSI when incubated in dark, aerobic conditions (Fig. 4D). This indicates a regulatory ‘spillover’ effect in UWO cultures grown at low salinity at either low or high temperatures and in cultures grown under the stress combination of high temperature and high salinity (Fig. 4). Potentially, a change in capacity for spillover would be demonstrated as a change in appression of stroma lamellae in the ultrastructure of the thylakoid membranes (Vacha et al., 2007). Changes in the appression of the thylakoid membrane have been observed in UWO grown at 15 °C and 700 mM, but there was not a corresponding change in the capacity for spillover (Figs 2D, 4C). Therefore, a clear correlation between thylakoid membrane ultrastructure and 77 K emission spectra cannot be found in this study.

The ability of photosynthetic organisms to maintain photostasis confers an advantage through an increase in the extent of phenotypic plasticity. Despite the fact that UWO has adapted to a very stable yet extreme environment, it is able to withstand multiple stresses while maintaining a high capacity for growth. It is postulated from this that part of what makes an organism extremophilic is a large capacity for phenotypic plasticity through acclimation processes. There is some evidence for endemicity in Antarctic lakes, but Laybourn-Perry and Pearce (2010) provide some support for the ‘everything is everywhere’ hypothesis as distinct ecotypes are evolving in these lake systems. It is
believed that UWO is an example of the latter hypothesis. The lack of acclimation in SAG relative to UWO resulted in a sharp transition point between salt concentrations that enable or limit growth. Unlike UWO, SAG was unable to gradually adjust its physiological responses to changes in temperature and salinity (Fig. 1). SAG responded to growth at high salinity by adjusting energy distribution between the photosystems as observed by increased PSII:PSI stoichiometry (Table 2). It is proposed that phenotypic plasticity in the extremophilic and mesophilic strains involves different acclimation processes. It is suggested that UWO possesses an active redox sensing–signalling mechanism as a consequence of its adaptation to a multiple stress environment. Further studies are needed to determine if this is the case in other extremophilic green algae. Current evidence points to rapid local climactic changes in Lake Bonney (Spigel and Priscu, 1996; Lyons et al., 2007). The present data indicate that simultaneous increases in temperature and decreases in salinity may not be problematic for the extremophilic UWO. Lastly, it is suggested that extremophiles must possess the capacity for phenotypic plasticity which in itself will provide a greater resilience to environmental fluctuations linked to global climate change.

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