Arctic *Micromonas* uses protein pools and non-photochemical quenching to cope with temperature restrictions on Photosystem II protein turnover

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Abstract *Micromonas* strains of small prasinophyte green algae are found throughout the world’s oceans, exploiting widely different niches. We grew arctic and temperate strains of *Micromonas* and compared their susceptibilities to photoinactivation of Photosystem II, their counteracting Photosystem II repair capacities, their Photosystem II content, and their induction and relaxation of non-photochemical quenching. In the arctic strain *Micromonas* NCMA 2099, the cellular content of active Photosystem II represents only about 50% of total Photosystem II protein, as a slow rate constant for clearance of PsbA protein limits instantaneous repair. In contrast, the temperate strain *Micromonas* NCMA 1646 shows a faster clearance of PsbA protein which allows it to maintain active Photosystem II content equivalent to total Photosystem II protein. Under growth at 2 °C, the arctic *Micromonas* maintains a constitutive induction of xanthophyll deepoxidation, shown by second-derivative whole-cell spectra, which supports strong induction of non-photochemical quenching under low to moderate light, even if xanthophyll cycling is blocked. This non-photochemical quenching, however, relaxes during subsequent darkness with kinetics nearly comparable to the temperate *Micromonas* NCMA 1646, thereby limiting the opportunity cost of sustained downregulation of PSII function after a decrease in light.

Keywords Prasinophyte · Photosystem II · Photoinactivation · Xanthophyll cycle

Abbreviations DTT Dithiothreitol

Introduction

*Micromonas* is a genera of small (1.5–3.0 μm) unicellular prasinophyte algae with a pear-shaped naked cell body, a single flagellum and a characteristic swimming behavior (Butcher 1952; Manton and Parke 1960). It belongs to the Mamiellales order and was the first described picoplanktonic species, initially characterized as *Chromulinapusilla* (Butcher 1952). *Micromonas* is a ubiquitous and cosmopolitan genera of picoeukaryote (Thomsen and Buck 1998), as strains occur in both near shore and oceanic environments and across a wide latitudinal temperature range (Butcher 1952; Foulon et al. 2008). In some locations, such as the coastal waters of the English Channel (Thomsen and Buck 1998), as strains occur in both near shore and oceanic environments and across a wide latitudinal temperature range (Butcher 1952; Foulon et al. 2008). In some locations, such as the coastal waters of the English Channel (Thomsen and Buck 1998), as strains occur in both near shore and oceanic environments and across a wide latitudinal temperature range (Butcher 1952; Foulon et al. 2008). In some locations, such as the coastal waters of the English Channel (Thomsen and Buck 1998), as strains occur in both near shore and oceanic environments and across a wide latitudinal temperature range (Butcher 1952; Foulon et al. 2008). In some locations, such as the coastal waters of the English Channel (Thomsen and Buck 1998), as strains occur in both near shore and oceanic environments and across a wide latitudinal temperature range (Butcher 1952; Foulon et al. 2008). In some locations, such as the coastal waters of the English Channel (Thomsen and Buck 1998), as strains occur in both near shore and oceanic environments and across a wide latitudinal temperature range (Butcher 1952; Foulon et al. 2008). In some locations, such as the coastal waters of the English Channel (Thomsen and Buck 1998), as strains occur in both near shore and oceanic environments and across a wide latitudinal temperature range (Butcher 1952; Foulon et al. 2008). In some locations, such as the coastal waters of the English Channel (Thomsen and Buck 1998), as strains occur in both near shore and oceanic environments and across a wide latitudinal temperature range (Butcher 1952; Foulon et al. 2008).
environmental adaptability, which could explain its broader global distribution (Archibald 2009; Worden et al. 2009). Phylogenetic analysis of several genes from worldwide Micromonas isolations revealed three (Guillou et al. 2004) to five (Slapeta et al. 2005) phylogenetically discrete clades, suggesting this taxon is a complex of cryptic species that started to diverge during the late Cretaceous (Slapeta et al. 2005). After detecting and quantifying the genetic clades in samples from tropical, temperate and arctic environments, Foulon et al. (2008) indicated three phylogenetic clades of Micromonas that occupy specific niches and confirmed the existence of cryptic species within the morphospecies Micromonas. Lovejoy et al. (2007) then isolated and characterized the growth of a psychrophilic arctic strain of Micromonas NCMA 2099.

The sensitivity of Arctic plankton to warming temperatures, in parallel with higher light, is important in view of current observations and model results that the arctic is becoming warmer at much faster rates than elsewhere (Stroeve et al. 2005). As part of our wider survey of phytoplankton susceptibilities to photo-inactivation of Photosystem II (Six et al. 2007, 2009; Key et al. 2010; Wu et al. 2011, 2012; Thomas and Campbell 2013; Campbell et al. 2013; Lavaud et al. 2016) we therefore sought to compare the responses of arctic and temperate strains of Micromonas to upward light challenges. Arctic Micromonas NCMA 2099 (Lovejoy et al. 2007) maintains growth at 0 °C, grows optimally at 6–8 °C, and is unable to grow above 12.5 °C. This psychrophilic strain shows light saturation of growth at or below 10 μmol photons m−2 s−1 and shows impairment of growth at higher irradiances. In contrast, temperate Micromonas NCMA 1646 grows optimally under warmer (18–22 °C), brighter conditions in the Mediterranean with growth-saturating light of 100 μmol photons m−2 s−1 or higher (McRose 2011). Our experiments showed that non-photochemical quenching is a major aspect of the differential responses of arctic and temperate Micromonas to light fluctuations.

Materials and methods

Culture growth and spectral measures

We cultured two strains of Micromonas, temperate origin NCMA 1646 at 20 °C under 36 and 185 μmol photons m−2 s−1 growth light, and arctic origin NCMA 2099 at 2 and 10 °C under 36 μmol photons m−2 s−1 growth light, in 6-well plates in a volume of 6.5 ml per well, in batch cultures in incubators. The strains were obtained from the Provasoli-Guillard National Center of Marine Phytoplankton and cultured in L1-Si media prepared using filtrated seawater according to (Keller et al. 1987; Guillard and Hargraves 1993). We used a 12:12 light/dark period and provided light from fluorescent tubes (Sylvania). The growth light was measured using a microspherical quantum sensor (US-SQS, Waltz, Germany). Cell growth was estimated using chlorophyll a fluorescence at 680 nm measured with a Molecular Devices Gemini EM spectrofluorometer. The growth rate (μ, d−1) was estimated as the slope of ln(Fluorescence680 nm) versus elapsed time.

Prior to each light treatment, the absorbance spectrum (a, m−1) from 400 to 750 nm of a culture sample was measured in a spectrophotometer (OLIS Cary 14) equipped with a DSPC integrating cavity sample chamber with an effective pathlength of ~20 cm, where near total internal reflectance within the cavity cancels light scattering resulting from suspended cells. We then extracted the cells into Mg-saturated 90 % acetone and measured chl a concentration (μg chl l−1) by absorbance (Porra 2002). The chl-specific absorption coefficient (a*, m−1 mg chl−1) was retrieved from whole-cell absorption spectra and chl a (Mitchell 1990; Ciotti et al. 2002; Cai et al. 2015). We then followed (Jesus et al. 2008; Mélèder et al. 2013) in the generation and interpretation of second-derivative spectra for detection of xanthophyll cycle pigments. Briefly, whole-cell spectra were normalized to the red chlorophyll a peak (673–675 nm) with 3–4 replicate spectra from independently grown cultures averaged for each species and treatment condition. The second derivatives of whole-cell spectra were computed with 2 nm interpolation using SpectralWorks software (OLIS). Second-derivative whole-cell spectra were normalized as in Mélèder et al. (2013), using the largest negative peak from 677 to 679 nm, and 3–4 replicates of these standardized second-derivative whole-cell spectra were then averaged for each species and treatment condition in order to detect changes in xanthophyll cycle pigment content.

Light treatments, flash yield determinations of PSII content and FRR measures

~30 ml of culture was harvested from 6 × 6-well plate cultures (6.5 ml per well), pooled and concentrated ~3 to 9 ml by centrifugation at 1800×g for 10 min followed by removal of 21 ml of media supernatant and resuspension of the cells into the remaining 9 ml. The concentrated cell suspension was then divided into three aliquots of 3 ml. A time zero (t0) sample was harvested by further centrifugation (5 min, 14,000×g) and then stored at −75 °C for subsequent chlorophyll analysis. A +inhibitor aliquot was created by adding either lincomycin to inhibit chloroplast protein synthesis and thereby block PSII repair (Tyystjärvi and Aro 1996) or dithiothreitol (DTT) (Bilger and Björkman 1990) to inhibit the xanthophyll deepoxidase enzyme and thereby...
prevent induction of xanthophyll-dependent non-photocchemical quenching (NPQ). The +inhibitor and −inhibitor aliquots were loaded into 1-cm spectrophotometer cuvettes. A micro-stir bar was placed into the sample which was then sealed in with a gas-tight resin plug that incorporates a temperature control loop immersed into a 2-ml sample volume, to maintain the culture sample at its growth temperature of 2, 10 or 20°C. The plug also incorporates a solid-state optode O2 sensor projecting into the sample volume with an accompanying solid-state temperature probe (FireSting system, Pyro Science GmbH, Aachen, Germany). The cuvette assembly was then placed in the Superhead optical unit of a Photon Systems Instruments FL3500 fluorometer (Brno, Czech Republic). Figures 1 and 2 and the associated legends outline the subsequent oxygen and Fast Repetition Rate fluorescence measurement and treatment protocol applied to samples. Cultures were shifted to a range of treatment light levels for (5–8) × 5 min measurement/treatment time courses. Temperate NCMA 1646 were treated at light levels from 189 to 797 μmol photons m−2 s−1, while arctic NCMA 2099 cultures were treated at light levels from 24 to 400 μmol photons m−2 s−1. Table 2 outlines the terms and definitions of photosynthetic parameters extracted directly or indirectly from the FRR induction traces using PSIWORKX-R (http://sourceforge.net/projects/psiworx/) and from the time course data. Parameters for photoinactivation, repair, induction and relaxation of non-photocchemical quenching were extracted from data pooled across the time course treatments at different light levels using data transform and curve fitting scripts implemented in R.

**Protein analyses**

Total protein extractions were performed upon frozen cell pellets that were resuspended into 500 μl of 1× low TRIS-protein extraction buffer (50 mM TRIS buffer, 2% lithium dodecyl sulfate, 10% glycerol, 0.5 mM EDTA, 0.1 mg/ml...
4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (protease inhibitor). Cells were lysed in Sigma-Aldrich bead beater tubes three times for 60 s at 4.5 m/s (MP Biomedicals Fastprep 24), with 60 s on ice between homogenization periods. The bead tubes were then centrifuged in a desktop centrifuge for 5 min at 14,800 x g, divided into 5 aliquots of 40–50 μl and one aliquot of approximately 100 μl. These were stored at −80 °C.

Total protein concentration was determined using the BCA assay with bovine gamma globulin standards as per the manufacturer’s recommendations. Absorbance at 562 nm was measured using a Spectramax plate reader. Protein concentration of samples was quantified using the linear regression of the standard curve on triplicate samples.

For immunoquantititation (Brown et al. 2008) of PsbA, protein extracts were prepared with 0.5 μg total protein per 10 μl load. For FtsH immunoquantititations, protein extracts were prepared with 5 μg total protein in 25 or 35 μl loads. Dithiothreitol was added to a final concentration of 50 mM with the remaining sample volume made up with 1 x Bolt sample buffer from Life Technologies. Standards were made by diluting stock PsbA or FtsH protein standard in the same Bolt sample buffer with a final concentration of 50 mM dithiothreitol. Samples and standards were heated at 70°C for 5 min and centrifuged to collect condensation. Prepared samples were stored at −20 °C if not used immediately.

Proteins were separated by SDS-PAGE electrophoresis in 4–12 % Bis Tris Plus 17 well polyacrylamide gels from Life Technologies. Novex Sharp Pre-stained protein standard was loaded in one lane along with MagicMark XP Immunoblot standard, both from Life Technologies. Gels were run for 30–35 min at 200 V using a Bio-Rad powerpack in 1x MES running buffer from Life Technologies. Protein was transferred from the gel to Biorad PVDF over 60 min at 20 V in 1x Bolt Transfer Buffer with Bolt Antioxidant and methanol. Following the transfer, the PVDF was blocked in 2 % ECL blocking solution made with 1x TBS-T, protein side up, on an orbital shaker, for 60 min. For PsbA, the primary antibody AS05-084 lot 1207 (AgriSera) was added in 2 % ECL blocking solution in a 1/20,000 dilution. The secondary antibody was an α-rabbit antibody, AbCam6721 in 2 % ECL blocking...
solution in a 1/20,000 dilution. FtsH blots used the primary antibody AS11-1789 lot 1304 (AgriSera), at a 1/5000 dilution. Secondary antibody was the same as for PsbA blots, but at a 1/5000 dilution. Each antibody incubation was 60 min, followed by a rinse cycle with 1× TBS-T: two brief rinses, one 15-min rinse, and three 5-min rinses. Immunoblots were imaged using a Biorad Versadoc with 800 μl of ECL Select detection agent, consisting of Luminol Select and Peroxide in a 1:1 ratio. Quantitation was done using Bio-Rad Image Lab 4 for Windows. The concentration of the protein of interest was determined based on adjusted volume with a global background subtraction, and a linear or polynomial regression of the standards. A representative immunoblot image for FtsH is shown in Fig. 3.

**Results**

Under low light and at 20 °C, the temperate *Micromonas* NCMA 1646 had a growth rate of 0.42 d⁻¹, increasing to 1.12 d⁻¹ under higher light (Table 1). The psychrophilic arctic *Micromonas* NCMA 2099 growing at 2 °C and low light grew at only 0.23 d⁻¹ but achieved 0.34 d⁻¹ at 10 °C, near its upper temperature limit for growth (Lovejoy et al. 2007). Thus, our arctic NCMA 2099 grew almost as fast as our temperate NCMA 1646 under comparable low light levels, but the temperate NCMA 1646 could achieve much faster growth at higher light, well above the light tolerance range of the arctic strain (data not shown). Interestingly maximum photochemical yield of PSII (Fv/FM) was comparable across strains and growth conditions except for the arctic strain growing near its upper limit of 10 °C, which showed a lower Fv/FM, consistent with sustained photoinhibition. The chl-specific absorption coefficient for 400 to 700 nm; m² (mg chla)⁻¹ (a*) (Ciotti et al. 2002) was similar across the strains and growth conditions (Table 1).

Figures 1 and 2 and the associated legends outline the oxygen and fast repetition rate fluorescence measurement and treatment protocol applied to analyze the photophysiological responses of the *Micromonas* strains to changing light.

The temperate NCMA 1646 cells (Fig. 2a) show a significant drop from Fₘ measured after 300 s of dark acclimation, to Fₘ₂₅ taken from the subsequent FRR induction.

**Table 1** Strain information, growth and photophysiological properties

| Strain | Property | Value |
|--------|----------|-------|
| NCMA 1646 | Origin | Mediterranean |
| NCMA 2099 | Origin | Baffin Bay |
| | Growth temperature (°C) | 20 | 2 |
| | Growth light (μmol photons m⁻² s⁻¹) | 20–36 | 185 |
| | Cell diameter (μm) | 2–3 | 2–3 |
| | chl b/a | 0.98 (0.04) | 0.77 (0.06) |
| | Growth rate (d⁻¹) | 0.42 (0.23) | 0.79 (0.01) |
| | Fv/FM | 0.57 (0.03) | 0.59 (0.01) |
| | σₚₛₛ (Å² quanta⁻¹) | 934 (66) | 747 (52) |
| | a*, m² (mg chla)⁻¹ | 0.009 (0.001) | 0.0092 (0.0004) |
| | fmo FtsH (μg protein⁻¹) | 6.0 (0.7) | 9.6 (1.8) |
| | fmo PsbA (μg protein⁻¹) | 116 (29) | 79 (20) |
| | fmo [PSII]active (μg protein⁻¹) | 95 (9) | 42 (8.6) |

n = 3–35, (SD)
| Parameter | Equation | Definition, units | Reference |
|-----------|----------|-------------------|-----------|
| $F_0$     | Minimal fluorescence with PSII open | van Kooten and Snel (1990) |
| $F_M$     | Maximal fluorescence with PSII closed | van Kooten and Snel (1990) |
| $F_S$     | Fluorescence at an excitation level | van Kooten and Snel (1990) |
| $F'_M$    | Maximal fluorescence with PSII closed in at an excitation level | van Kooten and Snel (1990) |
| $F_{M,2s}$| Maximal fluorescence with PSII closed 2 s after excitation | Fig. 2 |
| $F'_0$    | Minimal fluorescence with PSII open, estimated for cells under excitation, including influence of photoinactivation | Oxborough and Baker (1997) and Ware et al. (2015a, b) |
| $F'_0$\text{Oxborough} | $1/(1/F_0 - 1/F_M + 1/F'_M)$ | \begin{align*} \text{Minimal fluorescence with PSII open, estimated for cells under excitation, excluding influence of photoinactivation} \\ \text{Non-photochemical quenching} \end{align*} | Oxborough and Baker (1997) and Ware et al. (2015a, b) |
| $\rho$    | Excitation connectivity among PSII centers | Kolber et al. (1998) |
| $\sigma_{PSII}$ | Functional absorbance cross section for PSII photochemistry | Kolber et al. (1998) |
| $\sigma_{PSII}'$ | Functional absorbance cross section for PSII photochemistry under excitation | Kolber et al. (1998) |
| $\sigma_{PSII,2s}$ | Functional absorbance cross section for PSII photochemistry 2 s after excitation | Fig. 2 |
| $\tau_1$  | Slow lifetime for PSII reopening after saturating flash, s | Kolber et al. (1998) |
| $\tau_2$  | Fast lifetime for PSII reopening after saturating flash, s | Kolber et al. (1998) |
| NPQ       | $F_M - F_M'/F_M'$ | Quantum yield for non-photochemical excitation dissipation | Genty et al. (1989), Kramer et al. (2004) and Klughammer and Schreiber (2008) |
| $Y(PSII)$ | $(F'_M - F_M)/F_M'$ | Quantum yield for regulated non-photochemical excitation dissipation | Genty et al. (1989), Kramer et al. (2004) and Klughammer and Schreiber (2008) |
| $Y(NO)$   | $Y(NO) = F_M/F_M'$ | | |
| $Y(NPQ)$  | $F_M/F_M' - F_M/F_M'$ | | |
| $q_P$     | $(F'_M - F_M)/F_M'/F'_M$ | Photochemical quenching of fluorescence ~ fraction of open PSII | van Kooten and Snel (1990) |
| $k_{pi}$  | $[\text{PSII}]_{active,t} = [\text{PSII}]_{active,0} \cdot e^{(-k_{pi} t)}$ | First-order rate constant for photoinactivation of PSII, s$^{-1}$ | Kok (1956) |
| $\sigma_i$| $[\text{PSII}]_{active,t} = [\text{PSII}]_{active,0} \cdot e^{(-\sigma_i t)}$ | Target size for photoinactivation of PSII across multiple excitation levels I, m$^2$ photon$^{-1}$ | Oliver et al. (2003), Key et al. (2010) and Campbell and Tyystjärvi (2012) |
| $k_{rec}$ | $[\text{PSII}]_{active,t} = [\text{PSII}]_{active,0} \cdot (k_{rec} + (k_{rec} + k_{rec})/k_{rec})$ | First-order rate constant for recovery of photoinactivated PSII, s$^{-1}$ | Kok (1956) |
| $k_{rec,\text{inact}}$ | $[\text{PSII}]_{active,t} = (([\text{PSII}]_{active,0} + k_{rec,\text{inact}})/k_{rec}) + ((k_{rec} + (k_{rec} + k_{rec})/k_{rec}) + e^{-[PSII]_{inact}/k_{rec}})$ | First-order rate constant for recovery of photoinactivated PSII, allowing for initial pool of $[PSII]_{inact}$, s$^{-1}$ | |
Table 2 continued

| Parameter     | Equation                                                                 | Definition, units                                                                 | Reference |
|---------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-----------|
| $k_{eqp}$     | $1 - (1/(k_{eqp} + I))$                                                   | Half-saturation light level for photochemical quenching, mol photons m$^{-2}$ s$^{-1}$ |           |
| $k_{npq}$     | $1 - Y(NPQ) = (1 - (1/(k_{npq} + I))) + (1 - (1/(k_{npqlow} + I)))$        | Half-saturation light level for induction of non-photochemical quenching, mol photons m$^{-2}$ s$^{-1}$ |           |
| $k_{npqlow}$  | $1 - Y(NPQ) = (1 - (1/(k_{npqlow} + I)))$                                 | First-order rate constant for induction of non-photochemical quenching, s$^{-1}$   |           |
| $k_{npqslow}$ | $1 - Y(NPQ) = (1 - (1/(k_{npqslow} + I)))$                                | Zero-order rate constant for time dependent induction of slow phase of non-photochemical quenching, s $^{-1}$ |           |
| $k_{rnpq}$    | $1 - Y(NPQ) = (1 - (1/(k_{rnpq} + I)))$                                  | First-order rate constant for relaxation of non-photochemical quenching, s$^{-1}$   |           |
| $a\bar{\alpha}$ | $\sigma_{PSII} = \frac{F_0}{F_{PSII}} * Y(PSII) * \frac{PSII}{V} * \frac{V}{r}$ | The chl-specific absorption coefficient for 400 to 700 nm: m$^{-2}$ (mg chla)$^{-1}$ |           |

repeated after a further 2-s dark period. This shows a rapid induction of some non-photochemical quenching (NPQ) in response to the initial FRR flashlet induction train. Over 300-s incubation at 400 μmol photons m$^{-2}$ s$^{-1}$, the level of $F_0'$ increased significantly in temperate NCMA 1646 (Fig. 2a, open and closed symbols), showing significant inactivation of PSII (Ware et al. 2015a), and the level of $F_M'$ declined significantly from the initial level of $F_M$ showing induction of NPQ. Most of this induced NPQ1 relaxed after only a 2-s dark period. The presence of DTT (closed symbol trace) partially blocked the induction of NPQ, since the level of $F_M'$ remained close to the initial $F_M$ level, although there was still further relaxation after a 2-s dark period.

In the arctic NCMA 2099 grown at 2 °C and 36 μmol photons m$^{-2}$ s$^{-1}$ (Fig. 2b), the initial FRR induction protocol had limited effect upon the response to the subsequent flash after 2-s darkness, so a single induction flashlet train was not sufficient to provoke rapid induction of NPQ. The 300-s light treatment provoked a significant drop from $F_0$ to $F_S$, and from $F_M$ to $F_M'$, showing much larger induction of NPQ than in temperate NCMA 1646 treated at 400 μmol photons m$^{-2}$ s$^{-1}$ (Fig. 2a). There was only slight relaxation of NPQ during a 2-s dark period. In the presence of DTT to inhibit xanthophyll deepoxidation, the overall level of fluorescence increased, reflecting a partial inhibition of NPQ. Also note the larger drop from $F_S$ to $F_0'2s$ in the presence of DTT, reflecting more PSII reaction center closure under illumination when NPQ is inhibited.

We sought to measure and compare susceptibility to photoinactivation of PSII, capacity for repair of PSII and the light and time dependencies of induction of non-photochemical quenching. In our related previous studies (Lavaud et al. 2004; Six et al. 2007, 2009; Key et al. 2010; Wu et al. 2011, 2012; Thomas and Campbell 2013; Li and Campbell 2013; Li et al. 2015; Lavaud et al. 2016), the amplitudes of induction of non-photochemical quenching were moderate and we were able to correct for any residual influence of non-photochemical quenching on the time courses of $F_{PSII}/F_M$ or $F_{PSII}/F_M'$ which we used as proxies for changes in the function of PSII (Wu et al. 2012). In the arctic NCMA 2099, however, large and rapid induction of non-photochemical quenching, which did not fully relax within 2 s (Fig. 2b), was clearly a dominant influence on $F_{PSII}/F_M'$, and the relaxation of non-photochemical quenching in the arctic NCMA 2099 was slow enough (20 s or greater, data not presented) as to prevent the use of $F_{PSII}/F_M$ to kinetically track changes in [PSII]$_{active}$.

We therefore sought an alternate, rapid, noninvasive measure of [PSII]$_{active}$ to track photoinactivation and repair. Oxborough et al. (2012) and Silsbe et al. (2015) introduced $F_0'/' \sigma_{PSII}$ as a rapid measure of [PSII]$_{active}$ with
calibration against slower oxygen flash yield measures (Chow et al. 1989; Suggett et al. 2009) of \([\text{PSII}]_{\text{active}}\). We found good correlation between \(F_0'/\sigma_{\text{PSII}}'\) and oxygen flash yield measures of \([\text{PSII}]_{\text{active}}\) for culture samples taken direct from growth conditions. This useful correlation, however, diverged after photoinhibition time courses, because photoinhibition causes a rise in \(F_0'\) (Ware et al. 2015a, b) through an increase in the fluorescence yield of photoinactivated PSII, unrelated to any increase in \([\text{PSII}]_{\text{active}}\). Oxborough and Baker (1997) derived an estimator of \(F_0'\) (Table 2) that corrects for the influence of non-photochemical quenching on \(F_0\), but which excludes the cumulative influence of photoinactivation. We therefore plotted \(F_0''\text{Oxborough}/\sigma_{\text{PSII}}''\) versus oxygen flash yield measures of \([\text{PSII}]_{\text{active}}\) measured on the same samples, and found a correlation that was robust in the face of accumulated photoinactivation of PSII (Fig. 4); compare the open symbols showing measurements of samples taken directly from growth conditions, with the closed symbols showing measurements of samples after a high-light treatment to induce some photoinhibition, with or without the presence of lincomycin to block PSII repair. The data were fit with a pooled regression of slope 0.9088, intercept \(1.317 \times 10^{-6}\) and \(R^2\) of 0.7139. In other work (Murphy et al. 2016) we are now extending this proxy for \([\text{PSII}]_{\text{active}}\) to other species and growth conditions.

In the current study, we measured time courses (representative data in Fig. 5a–l) of PSII function measured under different treatment light levels, in the presence or absence of lincomycin to block PSII repair or DTT to block xanthophyll cycling. We used the parameter \(F_0''\text{Oxborough}/\sigma_{\text{PSII}}''\) (Fig. 5g, h) as a proxy for the content of \([\text{PSII}]_{\text{active}}\) (Fig. 4) to fit estimates of \(\sigma_i\), a target size parameterization of the susceptibility of Micromonas to photoinactivation of PSII (Tables 2, 3). \(\sigma_i\) is based upon the assumption that, at least up to moderately high light, photoinactivation is a linear product of cumulative photon dose (Oliver et al. 2003; Campbell and Tyystjärvi 2012). For this data set, for each combination of growth condition and strain we fit the set of time course measurements of \(F_0''\text{Oxborough}/\sigma_{\text{PSII}}''\) measured under different treatment lights with a pooled \(\sigma_i\) (Table 3). \(\sigma_i\) was comparable within confidence intervals at 1 to \(1.4 \times 10^{-24}\) \(\text{m}^2\) \(\text{photon}^{-1}\) across the strains and growth conditions, with the intriguing exception of arctic NCMA 2099 grown at 2 °C, which showed a significantly higher \(\sigma_i\) of \(3.6 \times 10^{-24}\) \(\text{m}^2\) \(\text{photon}^{-1}\) (Table 3) indicating

![Fig. 4 Fluorescence metric of \([\text{PSII}]_{\text{active}}\). \(F_0''\text{Oxborough}/\sigma_{\text{PSII}}''\) plotted versus \([\text{PSII}]_{\text{active}}\) \(\mu\text{mol} \cdot \text{m}^{-1}\) determined from oxygen flash yields (Fig. 1). Open symbols show samples measured directly from growth conditions. Closed symbols show samples measured after a high-light treatment to induce some photoinhibition, with or without the presence of lincomycin to block PSII repair. Pooled linear regression with a slope 0.9088, intercept \(1.317 \times 10^{-6}\) and \(R^2\) of 0.7139](chart)
a higher susceptibility to photoinactivation under that growth condition. We speculate that at low growth temperature 
*Micromonas* suffers increased ROS toxicity under excess light, leading to increased susceptibility to photo-
inactivation at a given photon dose (Vass 2011, 2012).

Using $\sigma_t$ estimated in the presence of lincomycin as an input, we then estimated $k_{rec}$, s$^{-1}$, a first-order rate constant for functional recovery of photoactivated PSII (Kok 1956) using the time/light courses of $F_{O^{'}}/O$ and $\sigma_{PSII}'$ measured in the absence of lincomycin (Fig. 5g, h), with PSII repair active (Table 3). Temperate NCMA 1646 showed an upregulation of $k_{rec}$ with an increase in growth light at 20 °C (Table 3). To our surprise under comparable growth lights of 20–36 µmol photons m$^{-2}$ s$^{-1}$, the fitted $k_{rec}$ in arctic NCMA 2099 at 2 °C was comparable to the fitted $k_{rec}$ in temperate NCMA 1646 at 20 °C, implying that at least under low growth light and moderate treatment lights the arctic NCMA 2099 was able to maintain an active membrane-based PSII repair cycle (Nixon et al. 2010; Komenda et al. 2012), which is blocked by addition of lincomycin to inhibit PsbA translation.

The $k_{rec}$ formulation assumes that at $t_0$ of the time course all PSII is in the form [PSII]$_{active}$ and that the subsequent accumulation of [PSII]$_{inactive}$ during the light treatment generates the key substrate for PSII repair. We know that the PSII repair cycle involves multiple intermediates (Tyystjärvä et al. 2005; Nixon et al. 2010; Komenda et al. 2012). If growing cells contain an initial pool of [PSII]$_{inactive}$ $t_0$, this leads to an overestimation of $k_{rec}$ under the simple Kok model (Kok 1956).

In Fig. 6a, we compare the content of [PSII]$_{active}$ measured using oxygen flash yields with the content of the PsbA protein subunit from Photosystem II determined by quantitative immunoblotting from the same culture samples (Fig. 6a). The arctic strain NCMA 2099 growing at 2 °C (closed triangle) contained only 42 fmol [PSII]$_{active}$ ($\mu$g protein$^{-1}$), compared to 79 fmol PsbA ($\mu$g protein$^{-1}$). Therefore, ~37 fmol PsbA ($\mu$g protein$^{-1}$) were in the form of [PSII]$_{inactive}$ before the start of any light treatment (Fig. 5). In marked contrast, the temperate strain NCMA 1646 growing at 20 °C and 185 µmol photons m$^{-2}$ s$^{-1}$ (open circle) contained 133 fmol [PSII]$_{active}$

### Table 3 Photosystem II functional parameters from curve fitting (95 % CI)

| Parameter | Temperate NCMA 1646 | Arctic NCMA 2099 |
|-----------|---------------------|------------------|
| Growth temperature (°C) | 20 | 20 | 2 | 10 |
| Growth light (µmol photons m$^{-2}$ s$^{-1}$) | 20–36 | 185 | 20–36 | 20–36 |
| $n$ | 12 | 16 | 35 | 14 |
| Dark $\tau_1$ (s) | $1.3 \times 10^{-2}$ (1.3 $\times 10^{-3}$) | $1.0 \times 10^{-2}$ (0.0014) | $8.4 \times 10^{-3}$ (1 $\times 10^{-3}$) | $1.3 \times 10^{-2}$ (1.4 $\times 10^{-3}$) |
| Dark $\tau_2$ (s) | $3 \times 10^{-4}$ (1 $\times 10^{-5}$) | $3 \times 10^{-4}$ (1 $\times 10^{-5}$) | $5.8 \times 10^{-4}$ (7.3 $\times 10^{-5}$) | $5 \times 10^{-4}$ (1.7 $\times 10^{-5}$) |
| Growth light $\tau_1$ (s) | $8.6 \times 10^{-3}$ (4.6 $\times 10^{-4}$) | $6 \times 10^{-3}$ (1 $\times 10^{-3}$) | $1.1 \times 10^{-2}$ (2.9 $\times 10^{-3}$) | $9.5 \times 10^{-3}$ (2.4 $\times 10^{-3}$) |
| Growth light $\tau_2$ (s) | $5.5 \times 10^{-4}$ (2.3 $\times 10^{-5}$) | $4.3 \times 10^{-4}$ (2.8 $\times 10^{-5}$) | $1.2 \times 10^{-3}$ (7.3 $\times 10^{-5}$) | $9 \times 10^{-4}$ (8.2 $\times 10^{-5}$) |
| $\sigma_t$ (m$^2$ photon$^{-1}$) | $1.4 \times 10^{-24}$ (4 $\times 10^{-25}$) | $1.3 \times 10^{-24}$ (1.4 $\times 10^{-25}$) | $3.6 \times 10^{-24}$ (5 $\times 10^{-25}$) | $1 \times 10^{-24}$ (4.3 $\times 10^{-25}$) |
| $k_{rec}$ (s$^{-1}$) | $2 \times 10^{-4}$ (2 $\times 10^{-4}$) | $5 \times 10^{-4}$ (9 $\times 10^{-5}$) | $2.7 \times 10^{-4}$ (9 $\times 10^{-5}$) | $9.2 \times 10^{-5}$ (2.5 $\times 10^{-4}$) |
| $k_{rec inactive}$ (s$^{-1}$) | $2 \times 10^{-4}$ (9 $\times 10^{-5}$) | $5 \times 10^{-4}$ (9 $\times 10^{-5}$) | $7 \times 10^{-5}$ (1.5 $\times 10^{-5}$) | $2 \times 10^{-5}$ (4.8 $\times 10^{-5}$) |
| $k_{ep}$ (µmol photons m$^{-2}$ s$^{-1}$) | 145 (8) | 228 (11) | 64 (4) | 159 (13) |
| DTT $k_{ep}$ (µmol photons m$^{-2}$ s$^{-1}$) | 56 (11) | 103 (12) | 59 (6) | 97 (5) |
| $k_{epq}$ (µmol photons m$^{-2}$ s$^{-1}$) | 2616 (6500) | 166 (70) | 49 (8) | 159 (53) |
| $k_{epqq}$ (s$^{-1}$) | 0.02 (3.1) | 0.0015 (0.0003) | 0.003 (0.001) | 0.003 (0.0007) |
| $k_{epqq dow}$ | $<1 \times 10^{-5}$ | $4.1 \times 10^{-5}$ (1.5 $\times 10^{-5}$) | $2 \times 10^{-6}$ (9 $\times 10^{-6}$) | $2 \times 10^{-6}$ (8 $\times 10^{-6}$) |
| $k_{epqq s}$ (s$^{-1}$) | $1 \times 10^{-7}$ (1 $\times 10^{-4}$) | $4.1 \times 10^{-4}$ (6 $\times 10^{-4}$) | $3 \times 10^{-4}$ (5 $\times 10^{-4}$) | $5.6 \times 10^{-4}$ (2 $\times 10^{-4}$) |
| DTT $k_{epqq}$ (µmol photons m$^{-2}$ s$^{-1}$) | n.d. | n.d. | 39 (14) | 1495 (575) |
| DTT $k_{epqq}$ (s$^{-1}$) | n.d. | n.d. | 0.002 (0.0003) | $>0.07$ (214,815) |
| DTT $k_{epqq}$ (s$^{-1}$) | n.d. | n.d. | $<2 \times 10^{-5}$ | $1.1 \times 10^{-5}$ (2.2 $\times 10^{-5}$) |

Italic values indicate poorly constrained value

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[PSII]active, with only a negligible pool of [PSII]inactive under growth conditions.

We therefore fit our data with an alternate formulation of $k_{\text{recinact}}$ (Tables 2, 3; Fig. 6b) that estimates the rate constant for repair of Photosystem II after allowing for an initial pool of [PSII]inactive $t_0$. We used the difference between growth [PSII]active and growth PsbA content as our estimator for the initial content of [PSII]inactive. For the arctic NCMA 2099, these fitted $k_{\text{recinact}}$ (Table 3) were indeed three- to fourfold smaller than the simpler (Kok 1956) $k_{\text{rec}}$ fits (Table 3), while for the temperate strains $k_{\text{rec}}$ and $k_{\text{recinact}}$ were similar because those cultures contained little [PSII]inactive $t_0$ under growth conditions. Furthermore, the estimates of $k_{\text{recinact}}$ accord well with experimentally independent estimates of the rate constant for clearance of PsbA protein, $k_{\text{psbA}}$ (Fig. 6b). In parallel with our analyses of PSII function and content, we measured the abundance of the total pool of FtsH protease subunits (Table 1), some of which mediate progressive proteolytic degradation of the PsbA protein, catalyzing this rate limiting step (Nixon et al. 2010; Komenda et al. 2012; Campbell et al. 2013).

The arctic cells at 2 °C had both the highest protein allocation to FtsH, and the highest ratio of FtsH/PsbA, suggesting regulation of a key enzyme content to partially counter the strong kinetic restriction on PsbA clearance at low temperature (Fig. 6b; triangles).

To summarize the time and light response of PSII closure ($q_P$) (Fig. 5k, l), we used a Michaelis–Menten formulation to define a half-saturation light level for $q_P$, $k_{\text{eqp}}$, fit across time courses measured at different treatment light levels for each combination of strain and growth condition. As expected, the temperate NCMA 1646 at 20 °C responded to increased growth light with an increase in $k_{\text{eqp}}$ from 145 to 228 μmol photons m$^{-2}$ s$^{-1}$ as the cells acclimated with decreased light capture through a smaller $\sigma_{\text{PSII}}$ (A$^2$ quanta$^{-1}$) (Table 2). The arctic NCMA 2099 at 2 °C showed a low $k_{\text{eqp}}$ of 64 μmol photons m$^{-2}$ s$^{-1}$, reflecting low temperature restrictions on metabolic consumption of reductant, as shown by long life times $\tau_1$ and $\tau_2$ (s) for removal of electrons from PSII under growth light (Table 3). Arctic NCMA 2099 at 10 °C largely escaped from this temperature restriction on metabolism as $\tau_1$ and $\tau_2$ under growth light decreased to ranges comparable to the temperate NCMA 1646 at 20 °C and $k_{\text{eqp}}$ increased to 159 μmol photons m$^{-2}$ s$^{-1}$, comparable to the temperate NCMA 1646 at 20 °C and the same growth light level.

To test the importance of xanthophyll pigment cycling to mediate excitation dissipation, we used the inhibitor dithiothreitol (DTT) (Bilger and Björkman 1990). Addition of DTT sharply decreased $k_{\text{eqp}}$ for the temperate NCMA 1646 and for the arctic NCMA 2099 at 10 °C, showing that loss of ongoing xanthophyll cycling lowered the flux of excitation into non-photochemical paths and increased
closure of PSII. Arctic NCMA 2099 at 2 °C in contrast showed no change in ke qp in response to DTT so that blockage of ongoing xanthophyll cycling did not measurably affect immediate excitation pressure upon PSII. These distinctions are illustrated qualitatively in Fig. 2, where addition of DTT to temperate NCMA 1646 results in a large closure of PSII under illumination that relaxes upon 2 s of darkness (compare dark + lincomycin trace to open –lincomycin trace, Fig. 2a). In contrast, addition of DTT to arctic NCMA 2099 provokes only moderate additional PSII closure (compare dark + lincomycin trace to open –lincomycin trace, Fig. 2b). In both the arctic and temperate strains, addition of DTT causes an increase in fluorescence levels, consistent with blockage of some non-photochemical quenching.

Induction of non-photochemical quenching in our treatments followed more complex kinetics than the simple, near-instantaneous light dependence of qP. We therefore fit the time/light courses of Y(NPQ) (Fig. 5i, j) (Kramer et al. 2004; Klughammer and Schreiber 2008) with a more complex equation (Table 2). We chose the Y(NPQ) formulation because it is bounded between 0 and 1, rather than the unbounded Stern–Volmer NPQ formulation. We parameterized the amplitude of Y(NPQ) at a given light level again using a Michaelis–Menten formulation with a half-saturation light level ke npq. We captured the rate of approach to this Y(NPQ) amplitude using a first-order rate constant (s⁻¹) for induction of non-photochemical quenching. We also observed a slower phase that accumulated as a linear function of cumulative time under irradiance (Fig. 5i, j), particularly in the temperate NCMA 1646 and in arctic NCMA 2099 when grown at 10 °C. We therefore included k npq slow as a zero-order rate constant of cumulative time, accumulating a slow induction phase of Y(NPQ) (Table 2). Given the effect of DTT upon the temperate strain and upon the arctic strain growing at 10 °C, and the limited DTT effect upon the arctic strain growing at 2 °C, we suspect this k npq slow zero-order induction rate represents induction of xanthophyll cycling.

The temperate NCMA 1646 at 20 °C and 20–36 μmol photons m⁻² s⁻¹ growth light showed a limited amplitude for Y(NPQ) which did not saturate under our range of treatment light levels, shown by a poorly constrained ke npq of 2616 μmol photons m⁻² s⁻¹. In contrast, at 185 μmol photons m⁻² s⁻¹ growth light, temperate NCMA 1646 demonstrated stronger induction of Y(NPQ) with a ke npq of 166 μmol photons m⁻² s⁻¹, already showing half saturation of Y(NPQ) induction at the culture growth light. The arctic NCMA 2099 grown at 2 °C and 20–36 μmol photons m⁻² s⁻¹ had a ke npq of 49 μmol photons m⁻² s⁻¹, again showing half-saturation of Y(NPQ) induction near growth light levels, consistent with findings from ice algae (Petrou et al. 2010). Under similar growth light conditions but at a higher temperature (10 °C), the arctic NCMA 2099 showed a more gradual induction of NPQ with a higher ke npq of 159 μmol photons m⁻² s⁻¹. Addition of DTT had little effect upon ke npq in the arctic NCMA 2099 at 2 °C but greatly suppressed light induction of Y(NPQ) at 10 °C driving ke npq up to 1495 μmol photons m⁻² s⁻¹. Y(NPQ) induction was therefore not directly dependent upon sustained xanthophyll cycling in arctic NCMA 2099 at 2 °C, but was during growth at the higher temperature. Addition of lincomycin caused a significant drop in Y(NPQ) in arctic NCMA 2099, consistent with findings (Bachmann et al. 2004; Lavaud et al. 2016) that accumulation of NPQ depends directly or indirectly upon sustained chloroplastic protein synthesis.

We suspected the lack of a DTT effect upon arctic NCMA 2099 growing at 2 °C resulted from full pre-induction of xanthophyll deepoxidation in these cells before the onset of any light treatment. Therefore, in Fig. 7 we present normalized, averaged whole-cell spectra captured from the four combinations of strain and growth. From these whole-cell spectra, we extracted (Fig. 7b, c) second-derivative spectra to detect inflection points of spectra. In the second-derivative spectra from 470 to 500 nm in the carotenoid region, we detected a statistically significant difference at 487 nm between arctic NCMA 2099 grown at 2 °C versus 10 °C, likely reflecting differences in xanthophyll cycle pigment contents of zeaxanthin (485 nm) and lutein (494 nm) (Jesus et al. 2008; Six et al. 2009; Méléder et al. 2013) between these growth conditions.

After high-light exposure, we measured the relaxation of Y(NPQ) as kr npq, (s⁻¹), a first-order rate constant (Table 3). The arctic NCMA 2099 at 2 or 10 °C showed a kr npq similar within confidence intervals to the kr npq fitted for the temperate NCMA 1646 at 20 °C. Thus, even though the DTT susceptibility, and thus dependence upon ongoing xanthophyll cycling, was distinct for the arctic NCMA 2099 at 2 °C, it retained the flexibility to relax NPQ on timescales comparable to the temperate NCMA 1646. Finer scale comparisons of relaxation over 2 versus 20 s (data not shown) did show that in arctic NCMA 2099 at 2 °C NPQ relaxed somewhat slower than the temperate NCMA 1646 growing at 20 °C (ex. compare Fig. 2a with Fig. 2b), but over scales of 300 s or more, relaxation kinetics were comparable. The arctic NCMA 2099 thus retains the regulatory flexibility to induce and then relax NPQ under fluctuating light.

In Fig. 8, we summarize the function of Photosystem II in the two strains by plotting the light and time response for PSII electron transport (PSII ETR) (Suggett et al. 2003, 2009; Huot and Babin 2010), in the absence (Fig. 8a, c, e, g) and presence (Fig. 8b, d, f, h) of lincomycin to show the influence of PSII repair on short-term
maintenance of PSII ETR across the strains and growth conditions. With cumulative time and photon dose, PSII repair has a detectable influence on PSII ETR in the temperate NCMA 1646, after growth at 20–36 μmol photons m⁻² s⁻¹ (compare Fig. 8a, no lincomycin, with 8B, with lincomycin), and a bigger influence after growth at 185 μmol photons m⁻² s⁻¹ (compare Fig. 8c, no lincomycin, with Fig. 8d, with lincomycin). Ongoing PSII repair was thus a significant factor to maintain PSII ETR during higher illumination treatments for the temperate NCMA 1646. In the arctic NCMA 2099, PSII ETR at a given light level was much lower because PSII closure (Fig. 5k, l) imposed by slow electron transport away from PSII (Table 3, τ₁, τ₂) and strong induction of NPQ (Fig. 5i, j) limit PSII ETR. In arctic NCMA 2099 after growth at 2 or 10 °C, PSII repair was a minor to negligible factor in maintaining this limited PSII ETR over the course of a 2700-s high-light challenge. Instead, PSII closure and non-photochemical quenching were the dominant influences on PSII ETR. To be fair, for the arctic NCMA 2099 the highest light treatment levels were equivalent to levels at which PSII repair was just manifesting as significant in the temperate NCMA 1646 (Compare Fig. 8e–h with Fig. 8a–d). But at yet higher light levels, complete PSII closure and strong non-photochemical quenching meant near complete suppression of variable chlorophyll fluorescence signals in
the arctic NCMA 2099, rendering any effect of instantaneous PSII repair functionally negligible for instantaneous $\text{PSII}_{\text{ETR}}$.

**Discussion**

The arctic strain under low light at 2 or 10 °C showed a classic excitation pressure acclimatory response (Huner et al. 1998) with chl b/a ratios and effective absorbance cross section for PSII photochemistry ($\sigma_{\text{PSII}}$) both comparable to the temperate NCMA 1646 growing under much higher light (Table 1). Interestingly in the arctic NCMA 2099 at 10 °C, near the upper temperature limit for this psychrophile (Lovejoy et al. 2007), fitted $k_{\text{recinact}}$ decreased, albeit with poorly constrained confidence intervals, reflecting scatter among the repeated time courses. Nevertheless, this possible decrease in functional $k_{\text{recinact}}$ in at least some culture replicates grown at supra-optimal temperatures is consistent with the decrease in $F_{\text{v}}/F_{\text{M}}$ reflecting sustained photoinhibition (Table 2) under this growth condition. We observed similar patterns in marine diatoms (Wu et al. 2012) grown across temperature ranges, with $k_{\text{rec}}$ peaking at the optimal growth temperature for the species, rather than showing a Q10-type response with increasing temperature.

Literature values for ribosomal translation rates (Guet et al. 2008) suggest that the rate constant for translation of PsbA protein is on the order of $2 \times 10^{-2}$ s$^{-1}$, orders of magnitude larger than the measured rate constants for PSII repair or PsbA clearance in our study, indicating that PsbA protein clearance is likely the rate limiting step upon PSII repair in these organisms. The arctic strain in particular suffered severe limitation on their clearance of PsbA protein and functional repair of PSII during light treatments (Fig. 6b; triangles). In Fig. 6c, we show that the arctic strain compensates by increasing the ratio of PsbA/[$\text{PSII}_{\text{active}}$], investing in reserve pools of excess PSII subunits (Behrenfeld et al. 1998) to support some PSII repair even when slow clearance of inactivated protein lags behind photoinactivation. Thus, the two strains balance their PSII repair cycles differently. The arctic strain maintains a large reserve of PSII subunits to compensate for restricted protein clearance, even under moderate growth light conditions. In parallel, the arctic strain accumulates high levels of FtsH protease subunits, possibly to partially counter kinetic limitations on protein turnover at low temperature. In studies of marine diatoms (Wu et al. 2011, 2012; Campbell et al. 2013) and currently in some marine picocyanobacteria (Cocksheutt et al., unpub.), we are finding evidence for sustained presence of significant pools of [PSII]$_{\text{inactive}}$ under various growth conditions, prior to the onset of any higher light treatment. We suggest that our modified integral equation for fitting $k_{\text{recinact}}$ will prove generally useful for analyzing PSII repair in marine phytoplankters.

In the arctic strain Micromonas NCMA 2099, instantaneous repair of PSII had only a marginal influence on the maintenance of $\text{PSII}_{\text{ETR}}$ under an upward light shift since PSII closure and strong induction of non-photochemical quenching suppressed $\text{PSII}_{\text{ETR}}$. Under growth at 2 °C, arctic Micromonas NCMA 2099 maintains a constitutive induction of xanthophyll cycling, shown by second-derivative whole-cell spectra, which supports a strong induction of non-photochemical quenching under low to moderate light, that does not depend upon sustained xanthophyll cycling and thus can function even if enzymatic activities are restricted at low temperature. This NPQ, however, can relax during subsequent darkness with kinetics almost comparable to the temperate Micromonas NCMA 1646, thereby limiting the opportunity cost of sustained down regulation of PSII function after a decrease in light (Raven 2011).

The temperate strain, in contrast, uses rapid protein clearance to maintain almost all of its PSII protein in the form of [PSII]$_{\text{active}}$ under growth conditions, thereby using faster kinetics to achieve a better functional return on standing protein investment, with much less dependence upon induction of NPQ under physiologically relevant light intensities. It remains to be seen whether the distinct Photosystem II maintenance strategies of arctic and temperate Micromonas will be mirrored in other psychrophile/
temperate taxon pairings (Jungblut et al. 2009; Lovejoy et al. 2011; Dolhi et al. 2013).

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Authors’ contributions All authors contributed to drafts of the manuscript. Guangyan Ni grew the cultures, collected the growth, biooptical and gas exchange data, and generated drafts of the introduction, materials and methods, figures and figure legends. Gabrielle Zimbalditi performed the protein extractions and immunoquantitation work, assisted in data analysis and first-derivative spectra figures. Gang Li assisted in the experimental work and with the R data processing pipeline for converting PSIWORX-R data output to formatted time courses for analyses. Audrey Barnett wrote the PSIWORX-R scripts for extracting chlorophyll fluorescence rise and relaxation parameters from data traces from PSI fluorometers, and contributed to the introduction and discussion. Christopher M. Arsenault assisted with R-scripting and performed the data analyses on whole-cell spectra to determine a\(^\text{a}\) and the second-derivative spectra figures. Gang Li assisted in the experimental work, data collection and analyses and figure generation. Amanda M. Cockshutt designed and oversaw the protein extraction and immunoquantitation work, assisted in data analysis and figure generation and edited the manuscript. Douglas A. Campbell planned the experiments, wrote the R data processing pipeline to extract kinetic data from the fluorescence time courses, prepared some figures and wrote the main draft of the results and discussion.

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