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1 **TAXONOMIC VARIABILITY IN THE ELECTRON REQUIREMENT**

2 **FOR CARBON FIXATION ACROSS MARINE PHYTOPLANKTON**

3

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15 Running Title: Phytoplankton electron requirements

16

17 Abstract

18 Fast Repetition Rate fluorometry (FRRf) has been increasingly used to measure marine
19 primary productivity by oceanographers to understand how carbon (C) uptake patterns vary
20 over space and time in the global ocean. As FRRf measures electron transport rates through
21 photosystem II ($ETR_{PSII}$), a critical, but difficult-to-predict conversion factor termed the
22 "electron requirement for carbon fixation" ($\Phi_{e,C}$) is needed to scale $ETR_{PSII}$ to C-fixation
23 rates. Recent studies have generally focused on understanding environmental regulation of
24 $\Phi_{e,C}$, while taxonomic control has been explored by only a handful of laboratory studies
25 encompassing a limited diversity of phytoplankton species. We therefore assessed $\Phi_{e,C}$ for a
26 wide range of marine phytoplankton ($n$=17 strains) spanning multiple taxonomic and size-
27 classes. Data mined from previous studies were further considered to determine whether $\Phi_{e,C}$
28 variability could be explained by taxonomy versus other phenotypic traits influencing growth
29 and physiological performance (e.g. cell size). We found that $\Phi_{e,C}$ exhibited considerable
30 variability (~4-10 mol $e^-$ [mol C]$^{-1}$), and was negatively correlated with growth rate ($R^2 = 0.7$,
31 $p < 0.01$). Diatoms exhibited a lower $\Phi_{e,C}$ compared to chlorophytes during steady-state,
32 nutrient-replete growth. Inclusion of meta-analysis data did not find significant relationships
33 between $\Phi_{e,C}$ and class, or growth rate, although confounding factors inherent to
34 methodological inconsistencies between studies likely contributed to this. Knowledge of
35 empirical relationships between $\Phi_{e,C}$ and growth rate coupled with recent improvements in
36 quantifying phytoplankton growth rates *in-situ*, facilitate up-scaling of FRRf campaigns to
37 routinely derive $\Phi_{e,C}$ needed to assess ocean C-cycling.

38 *Key index words:* Fast Repetition Rate fluorometry, electron requirements, carbon fixation,
39 phytoplankton, FRRf

40 *Abbreviations:* FRRf, Fast Repetition Rate fluorometry; C, carbon; ETR, electron transport
41 rate; PSII, photosystem II; $\Phi_{e,C}$, electron requirement for carbon fixation; MPP, marine
42 primary production; Chl-*a*, Chlorophyll-*a*; NPQ, non-photochemical quenching

43 Introduction

44 Marine primary production (MPP) is a fundamental ecosystem process that supports food
45 webs and regulates the global climate. For decades, various approaches have been applied to
46 quantify phytoplankton photosynthesis - by far the single largest contributor to MPP in the
47 global oceans (Regaudie-de-Gioux et al. 2014). However, disparate approaches commonly
48 evaluate different components of photosynthesis over variable timeframes, and measure
49 photosynthetic rates in different units or "currencies" (*sensu* Suggett et al. 2009a), such as
50 carbon (C)-fixed, oxygen ($O_2$)-evolved and electron transport through Photosystem II
51 ($ETR_{PSII}$). Reconciling estimates of MPP between disparate approaches is therefore not
52 trivial, being further compounded by inherent assumptions and caveats specific to each
53 method (Marra 2012, Regaudie-de-Gioux et al. 2014, Hughes et al. 2018a).

54 Use of $^{14}C$ bottle incubations (Steeman-Nielsen 1952) to trace incorporation of radio-labelled
55 carbon into organic matter remains the "gold-standard" method to measure aquatic
56 photosynthesis despite well-recognised methodological limitations (Longhurst et al. 1995,
57 Melrose et al. 2006, Marra 2009, Milligan et al. 2015). As such, $^{14}C$ measurements underpin
58 the calibration of virtually all satellite algorithms that yield MPP from ocean colour
59 (Campbell et al. 2002, Quay et al. 2010, Saba et al. 2011). Desire to better understand ocean
60 sequestration of atmospheric carbon under a rapidly-changing climate has driven efforts to
61 develop conversion factors allowing for accurate retrieval of C-fixation rates from
62 photosynthetic currencies that can be measured with greater resolution and precision
63 (Regaudie-de-Gioux et al. 2014, Hughes et al. 2018a).

64 Limited sampling resolution afforded by incubation-dependent techniques such as $^{13}C$, $^{14}C$
65 and $O_2$-bottle methods has resulted in bias and uncertainties within satellite-based MPP
66 models as they permit insufficient measures of synchronous *in-situ* photosynthetic rates to

67 robustly "ground-truth" ocean colour algorithms (Saba et al. 2011, Jacox et al. 2015). To
68 overcome this, oceanographers have increasingly gravitated towards bio-optical techniques
69 such as Fast Repetition Rate fluorometry (FRRf, Kolber et al. 1998) that permit significantly
70 greater sampling resolution (reviewed by Hughes et al. 2018a). FRRf and analogous active
71 Chlorophyll-*a* (Chl-*a*) fluorometry techniques, actively probe the photochemical status of the
72 oxygen-evolving complex at photosystem II (PSII) (see Huot and Babin 2010, Hughes et al.
73 2018a), to estimate $ETR_{PSII}$ - the photosynthetic currency of electron transport through PSII.
74 Retrieving rates of C-fixation from $ETR_{PSII}$ measured by FRRf therefore requires a specific
75 conversion factor, termed the "electron requirement for carbon fixation", $\Phi_{e,C}$ (Lawrenz et al.
76 2013), also termed $K_C$ (Hancke et al. 2015), describing the number of moles of electrons used
77 to fix one mole of C biomass.

78 The theoretical lower limit for $\Phi_{e,C}$ is 4-5 mol $e^-$ (mol $C)^{-1}$ (Kolber and Falkowski 1993)
79 based on the minimum number of electrons derived from two $H_2O$ molecules in the
80 production of one $O_2$ molecule. This lower limit however assumes that all electrons generated
81 at PSII are transferred to $NADP^+$ via Photosystem I (PSI), following a pathway referred to as
82 linear electron flow (LEF) which generates the energy (ATP) and reductant (NADPH)
83 required to fix C during the Calvin Cycle (Behrenfeld et al. 2008). In fact, PSII electrons can
84 also flow to diverse alternative pathways that either: i) do not directly result in C-fixation,
85 e.g. nutrient reduction or cyclic electron flow, or ii) fix C less efficiently per electron, e.g.
86 photorespiration (Hughes et al. 2018a). Thus, $ETR_{PSII}$ represents the total number of electrons
87 available to multiple electron sinks, and $\Phi_{e,C}$ summarises the net distribution of electrons
88 between C-fixing and non C-fixing pathways (Hughes et al. 2018a). In highly-dynamic
89 environments phytoplankton tend to exhibit great flexibility in their capacity to adjust
90 photosynthetic electron flow (Cardol et al. 2011). When conditions for photosynthesis are
91 optimal (e.g. when light is not excessive and downstream electron acceptors are not limited

92 by $CO_2$-availability or nutrient stress), LEF dominates (McDonald et al. 2011), and thus $\Phi_{e,C}$
93 should be nearer to the theoretical minimum. Under less-optimal conditions, phytoplankton
94 cells upregulate alternative electron pathways which act as photo-protective sinks (Roberty et
95 al. 2014), or mechanisms to generate more ATP relative to NADPH (Cardol et al. 2011,
96 Hughes et al. 2018a), allowing cells to meet increased energy requirements for cellular
97 maintenance, or power nutrient-acquisition processes including carbon concentrating
98 mechanisms (Langner et al. 2009, Halsey et al. 2010, Halsey and Jones 2015). Alternative
99 electron pathways decouple $ETR_{PSII}$ from C-fixation resulting in increased $\Phi_{e,C}$ (Hughes et al.
100 2018a). Indeed, measured values of $\Phi_{e,C}$ for natural phytoplankton assemblages routinely
101 exceed the theoretical minimum, evidenced by a global mean value of ~11 mol $e^-$ (mol C)$^{-1}$,
102 and often correlate with environmental variables known to regulate photosynthesis (Lawrenz
103 et al. 2013), however the strength of correlation is highly-variable between studies. Schuback
104 et al. (2015) demonstrated a correlation between the extent of non-photochemical quenching
105 (NPQ) – dissipation of excess excitation energy as heat – and $\Phi_{e,C}$, possibly reflecting a
106 positive feedback loop between upregulation of alternative electron pathways and activation
107 of NPQ processes at PSII (Nawrocki et al. 2015). Subsequent studies have established further
108 correlations between NPQ and $\Phi_{e,C}$ (e.g. Schuback et al. 2017, Hughes et al. 2018b,
109 Schuback and Tortell 2019), yet discrepancies in the slopes of the relationship between
110 studies suggest that the relationship between the two parameters is not easily predictable at a
111 given space and time (Hughes et al. 2018b). Developing a predictive understanding of $\Phi_{e,C}$
112 remains a key priority for end-users of active fluorometry, and an essential step towards
113 generating high resolution estimates of C-fixation that can be used to support remote sensing
114 models of MPP (Hughes et al. 2018a).

115 Whilst recent efforts in the field have predominantly focussed on examining $\Phi_{e,C}$ variability
116 in response to light and nutrient availability (Lawrenz et al. 2013, Schuback et al. 2015, 2016,

117 2017, Zhu et al. 2016, 2017, 2019, Hughes et al. 2018b), a few laboratory studies examining
118 a limited number of phytoplankton strains (Suggett et al. 2009a, Napoleon et al. 2013, Hoppe
119 et al. 2015) have provided intriguing evidence for possible taxonomic regulation of $\Phi_{e,C}$ – an
120 idea also raised by several field observations (Suggett et al. 2006, 2009a, Lawrenz et al. 2013,
121 Robinson et al. 2014). Conclusively identifying taxonomic regulation of $\Phi_{e,C}$ from field
122 studies is, however, problematic because i) seawater samples usually contain a wide diversity
123 of phytoplankton in various physiological states, and ii) taxonomic dominance by broad
124 functional groups is selected for via specific environmental conditions (Finkel et al. 2009).
125 Additionally, field measurements are often constrained by methodological limitations
126 (reviewed by Lawrenz et al. 2013, Hughes et al. 2018a) e.g. such as reliance upon lengthy
127 incubations to measure C-fixation in low biomass samples, which can introduce uncertainty
128 in $\Phi_{e,C}$, making it difficult to separate "real" variability in $\Phi_{e,C}$ from that introduced via
129 methodological artefacts. Studies of unialgal cultures under controlled laboratory conditions
130 allow for robust assessment of taxonomic regulation of $\Phi_{e,C}$ and allow for far better control of
131 methodological artefacts, yet curiously have been under-utilised in studies of $\Phi_{e,C}$ to date
132 (Hughes et al. 2018a). As a result, there is simply not enough statistical power within existing
133 laboratory or field data to determine i) if, and to what extent, taxonomic variation regulates
134 $\Phi_{e,C}$, ii) the taxonomic resolution needed to explain such variability, or iii) whether an
135 overarching trait governing photosynthetic performance (e.g. cell size) associated with
136 changes in taxa can prove a useful metric to explain variability in $\Phi_{e,C}$.

137 To bridge this knowledge gap, we cultured a diverse selection of phytoplankton strains
138 spanning multiple taxonomic and size -classes, to examine $\Phi_{e,C}$ under controlled laboratory
139 conditions. We hypothesised that $\Phi_{e,C}$ would follow a predictable taxonomic pattern, whereby
140 greater values would be observed for taxa with greater energetic requirements for cellular
141 maintenance (and thus lower growth rates). Growth rates have been shown to be highly

142 correlated with algal lineage (Litchman et al. 2007). We further combined our new
143 observations with data mined from previous studies to evaluate the extent to which variation
144 of $\Phi_{e,C}$ could be explained by taxonomy, versus additional traits including cell size and non-
145 photochemical quenching (NPQ). Such knowledge is needed to improve the accuracy with
146 which FRRf measurements can be scaled-up for remote sensing purposes in order to estimate
147 MPP, particularly as capacity to resolve phytoplankton groups and size classes from satellite
148 data is fast developing (e.g. Bracher et al. 2017).

149 Materials and Methods

150 *Phytoplankton culturing*

151 We examined a range of phytoplankton taxa, encompassing 7 taxonomic classes and a wide
152 range of cell sizes/volumes (Table 1). Measurements were initially performed on 12 species
153 of non-axenic phytoplankton cultures obtained from the Australian National Algal Culture
154 Collection (ANACC), representing 5 eukaryotic microalgal classes: *Dunaliella tertiolecta*
155 CCMP1320, *Tetraselmis* sp. CS-91 and *Tetraselmis* sp. CS-352 (Chlorophyceae);
156 *Thalassiosira weissflogii* CCMP1336, *T. pseudonana* CS-173, *Nitzschia closterium* CS-5 and
157 *Ditylum brightwellii* CS-131 (Bacillariophyceae); *Nannochloropsis oculata*
158 (Eustigmatophyceae); *Phaeocystis pouchetti* CS-165 and *Emiliania huxleyi* CS-370
159 (Prymnesiophyceae); *Rhodomonas salina* CS-692 (Cryptophyceae) and the one prokaryotic
160 group, *Synechococcus* sp. CS-94 (Cyanophyceae). All cultures were grown in a temperature-
161 controlled incubator (Steridium model: E500, Brisbane, Australia) at 20 °C (See Table 1)
162 within 75 mL flasks (Falcon T75, Sigma-Aldrich Pty Ltd, Castle Hill, Australia) and
163 maintained in semi-continuous batch mode via periodic serial dilutions when required (Wood
164 et al. 2005). Strains were grown in f/2 enriched seawater (Guillard and Ryther 1962),
165 prepared with sterile local seawater, plus additional silicate (Si) for Bacillariophyceae
166 (diatoms). Salinity was maintained at 35 PSU for all cultures, and a growth irradiance of 60 ±

167 10 μmol photons $m^{-2}$ $s^{-1}$ was provided by T5 cool-white fluorescent lighting (24W, Combrite
168 SD224-40) set to a 12:12 hour photoperiod. Cultures were maintained in semi-continuous
169 batch culture with periodic dilution to keep cells in exponential growth (monitored by FRRf
170 physiology and periodic cell counts) for approximately three months prior to sampling.
171 During experimentation, samples were collected in mid-exponential growth for: FRRf
172 photophysiology and photosynthetic-irradiance (PE) response, Chl-*a* determination,
173 particulate organic carbon and nitrogen content (POC, PON), cell density and biovolume
174 analysis, spectral light absorption ($a^{Chl}$) and measurements of $\Phi_{e,C}$, detailed below.

175 *Sampling of additional strains*

176 Additionally, we performed measurements of $\Phi_{e,C}$ (plus ancillary measurements where
177 available) on five dinoflagellate (Dinophyceae) strains that were not part of the original study
178 design, yet became available for sampling as a result of other experimental work that
179 coincided with our study. Specifically, this included two strains of the palytoxin-producing
180 benthic dinoflagellate species, *Ostreopsis* cf. *siamensis* (MW3 and F3) that were isolated and,
181 cultured as described in Verma et al. (2016) and Verma et al. (2020), along with three strains
182 of symbiotic dinoflagellates within the family Symbiodiniaceae; *Durusdinium trenchii* (strain
183 SCF082, formerly called *Symbiodinium trenchii* or ITS2 type D1a), and *Cladocopium*
184 *goreaui* (strains SCF058-04 and SCF055-06 – formerly called *Symbiodinium goreaui* or ITS2
185 type C1) described in Ros et al. (2020). *O.* cf. *siamensis* was maintained in the same
186 incubator that housed strains from our main study, with the only difference being the media
187 was substituted for F/10 due to visible cell deformation being observed in cells when grown
188 in F/2. Culturing conditions for Symbiodiniaceae were slightly modified to better represent
189 their sites of collection (and long term growth conditions), with strains grown in a separate
190 incubator under a higher irradiance (180 μmol photons $m^{-2}$ $s^{-1}$ – also provided by a 24W,
191 Combrite SD224-40 fluorescent tube) and temperature (26°C), using Daigo's IMK culture

192 medium (Wako, Osaka, Japan) (Table 1). All dinoflagellate strains were maintained in semi-
193 continuous batch culture with periodic dilution to keep cells in exponential growth
194 (monitored by FRRf physiology).

195 *Growth rates and cell size*

196 Growth rates were calculated from daily measurements of *in-vivo* fluorescence over a total
197 period of three months, using FRRf to monitor minimal fluorescence ($F_o$) as a proxy for Chl-
198 *a* concentration as per Suggett et al. (2009a). Growth rates (μ) were calculated through linear
199 regression of the natural log of $F_o$ against time, where the slope of the regression line
200 describes daily division rate ($d^{-1}$). Growth rates calculated from $F_o$ were periodically validated
201 against cell counts from samples preserved daily throughout the experimental period in
202 Lugol's alkaline solution (to a final concentration of 1%), with the exception of
203 *Synechococcus* sp. Overall, growth rates determined from $F_o$ showed strong correlations for
204 the 12 strains grown (range of $R^2$: 0.71–0.92, mean $R^2$ = 0.87; data not shown). For most
205 strains, a minimum of 50 cells were imaged via microscopy and measured using ImageJ
206 software (US National Institutes of Health). Cell volume ($\mu m^3$) was determined from
207 calculations based on geometric shape codes of taxa from (Sun and Liu, 2003), selecting
208 codes from taxa with similar shapes in their study.

209 *Chl-a analysis*

210 Chl-*a* for all samples was determined by filtering 15 mL aliquots onto a Whatman GF/F filter
211 (0.7 μm nominal pore size) before immediate extraction of pigments in 90% acetone and
212 storage at 4°C in darkness. For the >10 μm fraction, a similar procedure was conducted, but
213 instead used 10 μm polycarbonate filters (Merck Millipore, Bayswater, VIC, Australia). After
214 48 h of extraction in acetone, Chl-*a* was determined fluorometrically using a Trilogy
215 fluorometer fitted with Chl-*a* non-acidification module (Turner Designs, California, USA)
216 and calibrated against pure Chl-*a* standards (Sigma-Aldrich Pty Ltd).

217 *POC and PON analysis*

218 Twenty mL aliquots were filtered onto pre-combusted GF/F filters under low vacuum (~50
219 mm Hg), dried for 48 h at 60 °C, packaged in pre-combusted aluminium foil and stored in
220 darkness inside air-tight plastic bags containing silica gel packets. Subsequent analysis of
221 POC and PON content was conducted at the Research Corporation of the University of
222 Hawaii, using an elemental analyser (MAT Conflo IV, Thermo Finnigan, California USA)
223 coupled to a mass spectrometer (Delta+ XP, Thermo Finnigan). Unfortunately, no value is
224 reported for *Synechococcus* sp. (CS-94) because this sample was lost prior to analysis.

225 *Fast Repetition Rate fluorometry (FRRf) photophysiology*
226
227 A FastOcean FRRf fluorometer attached to a FastAct laboratory system (Chelsea
228 Technologies Group, London, UK) was programmed to deliver single turnover saturation of
229 PSII from 100 flashlets (1 μs pulse with a 2 μs interval between flashes), followed by a
230 relaxation phase of 40 flashlets (1 μs pulse with a 50 μs interval between flashes) (as per
231 Suggett et al. 2015, Hughes et al. 2018b). A total of 20 sequences were performed per
232 acquisition, with an interval of 150 ms between sequences. For all eukaryotic phytoplankton
233 in this study, the blue LED (450 nm) was the sole excitation source used, while a
234 combination of blue and red LEDs (450 nm + 624 nm) was applied to *Synechococcus* to
235 ensure full closure of PSII reaction centres. The biophysical model of Kolber et al. (1998)
236 was fitted to all FRRf acquisitions using FastPro8 software (V.1.0.55) to determine minimum
237 ($F_o$, $F'$) and maximum fluorescence ($F_m$, $F_m'$) and the functional absorption cross-section of
238 PSII ($\sigma_{PSII}$, $\sigma_{PSII}'$) (where the prime notation denotes that samples were measured during
239 exposure to actinic light). FastPro8 software was also used to subtract background
240 fluorescence (obtained from 0.2 μm filtered samples) from the total variable fluorescence
241 signal.

242 *Electron transport rate ($ETR_{PSII}$)*

243 FRRf-derived photosynthetic electron transport rates ($ETR_{PSII}$, electrons $m^{-3}$ $s^{-1}$) were
244 determined using the biophysical "sigma-based" algorithm originally developed by Kolber
245 and Falkowski (1993).

246 $$ETR_{PSII} = E \cdot \overline{\sigma_{PSII}}' \cdot [RCII]_{(FRRf)} \cdot (1 - C) \quad (1)$$

247 where $E$ is irradiance (photons $m^{-2}$ $s^{-1}$), $\sigma_{PSII}'$ is the functional absorption cross-section of PSII
248 under actinic light ($nm^2$ $PSII^{-1}$), $[RCII]_{(FRRf)}$ is the concentration of PSII reaction centres (mol
249 RCII $m^{-3}$), estimated fluorometrically according to Oxborough et al. (2012) as:

250 $$[RCII]_{(FRRf)} = K_a \cdot \frac{F_o}{\sigma_{PSII}} \quad (2)$$

251 where $K_a$ is an instrument-specific constant ($m^{-1}$). The parameter (1-$C$) in Eq. (1) describes
252 the fraction of open [RCII], calculated here as qP (where qP = $(F_m'-F')/(F_m'-F_o')$). Both
253 [RCII] and (1-$C$) are necessary in order to measure gross photosynthesis, yielding $ETR_{PSII}$
254 with units of electrons $m^{-3}$ $s^{-1}$, assuming an efficiency of one charge-separation event per
255 photon absorbed and delivered to [RCII] (Kolber and Falkowski 1993). As values of $\sigma_{PSII}'$
256 are spectrally-weighted towards the fluorometer's measurement LED (see Suggett et al.
257 2004), all measurements of $\sigma_{PSII}'$ were spectrally-adjusted to account for the bias of light
258 absorption towards the FastOcean's 450 nm excitation LED (and for combined 450 + 624 nm
259 LEDs used to measure *Synechococcus*). For this, absorption spectra for the initial 12
260 phytoplankton strains sampled were measured using the quantitative filter pad technique
261 (Roesler, 1998), with absorbance measured from 400-750 nm using a fibre-optic
262 spectrometer (UV/VIS, Ocean Optics, Florida, USA) against a separate sample blank.
263 Measured absorbance spectra were converted into Chl-*a* specific absorption coefficients
264 ($a^{Chl}$) as:

265 $$a^{Chl}(\lambda) = \frac{2.303 \cdot A(\lambda)}{L \cdot \beta \cdot Chl - a} \quad (3)$$

266 where $A(\lambda)$ represents the wavelength-dependent absorbance, $L$ is the optical pathlength of
267 filter particulates (sample volume filtered [$m^3$] / filter clearance area [$m^2$]) and β the
268 pathlength amplification factor (see Roesler, 1998). From this we obtained spectrally-
269 resolved values of PSII effective absorption, $\sigma_{PSII}'(\lambda)$ for all strains except *Synechococcus* as:

270 $$\sigma_{PSII}'(\lambda) = \left( \sigma_{PSII}'(450\ \text{nm}) \middle/ \alpha^{Chl(\lambda)}(450\ \text{nm}) \right) . \alpha^{Chl(\lambda)} \quad (4)$$

271 For *Synechococcus* – where $\sigma_{PSII}$ was obtained from a combination of 450 and 624 nm
272 excitation LEDs - spectrally resolved values of PSII effective absorption were obtained as:

273 $$\sigma_{PSII}'(\lambda) = \left( \sigma_{PSII}'(450 + 624\ \text{nm}) \middle/ \alpha^{Chl(\lambda)}(450 + 624\ \text{nm}) \right) . \alpha^{Chl(\lambda)} \quad (5)$$

274 Values of $\sigma_{PSII}'(\lambda)$ for all strains were then spectrally-adjusted to the spectral output of the
275 white LED within the FRRf optical head (the light source used to drive photosynthesis during
276 the incubations) as,

277 $$\overline{\sigma_{PSII}'} = \left( \sum_{400}^{700} \sigma_{PSII}'^{(\lambda)} . E(\lambda) \right) \Delta\lambda \Big/ \sum_{400}^{700} E(\lambda) \Delta\lambda \quad (6)$$

278 For the five dinoflagellate strains that were sampled in addition to strains from the main
279 study, it was not possible to use the same quantitative filter pad technique due to limited
280 available sample volume. Therefore, we followed the procedure outlined in Hughes et al.
281 (2018b) to perform a spectral correction based on previously-collected fluorescence
282 excitation spectra (400–700 nm) collected from dinoflagellate cultures pre-treated with 3-
283 (3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) where fluorescence was measured at 730 nm
284 (Suggett et al. 2009b, Wu et al. 2014). In doing so we assumed that the shapes of absorption

285 and fluorescence excitation scale equivalently to the functional absorption cross-section over
286 the spectrum, although acknowledge that absorption by photoprotective pigments would not
287 contribute to the fluorescence spectrum (see Lutz et al. 2001). However, as strains in our
288 study were grown under a relatively low irradiance (60 μmol photons $m^{-2}$ $s^{-1}$ – with the
289 exception of Symbiodiniacea that were grown under 180 μmol photons $m^{-2}$ $s^{-1}$), it is
290 reasonable to suggest that differences between absorption and fluorescence excitation arising
291 from photoprotective pigments would be minimal, and thus, would introduce only minor
292 uncertainty in $\Phi_{e,C}$ during the spectral correction procedure. As the spectral library used
293 contained no representatives of the genus *Ostreopsis*, we used the mean spectral correction
294 factor (SCF) calculated from all dinoflagellate representatives in the library and applied that
295 to the strains MW3 and F3 from this genus (Table 2). We highlight that this may also
296 introduce minor uncertainty into their reported $\Phi_{e,C}$ values, yet arguably represents an
297 improvement over not applying an SCF altogether - which would likely introduce far larger
298 uncertainty (see Silsbe et al. 2015).

299 *FRRf photosynthesis-irradiance (PE) curves*

300 Steady-state fluorescence light curves were performed using an identical protocol as that
301 described in Suggett et al. (2015). The model of Platt et al. (1980) was then fit to the data
302 using Sigmaplot curve-fitting software (Version 12.5, Systat Software Inc, California, USA).
303 Least squares non-linear regression analysis of the model fit was performed to derive the
304 maximum rate of photosynthesis, $ETR_{PSII}^{max}$ and the light utilisation efficiency, α (electrons
305 $m^{-3}$ $s^{-1}$), allowing for subsequent calculation of the light saturation parameter, $E_k$ ($ETR_{PSII}^{max}$/
306 α) with units of μmol photons $m^{-2}$ $s^{-1}$.

307 *Simultaneous FRRf-$^{14}$C incubations* ($\Phi_{e,C}$)

308 We followed the recommendations of Suggett et al. (2009a) and measured both $ETR_{PSII}$ and
309 $^{14}$C-uptake simultaneously (i.e. a "dual incubation") upon the same sample, thus avoiding

310 discrepancies between spectral quality and intensity that can arise when performing FRRf and
311 $^{14}C$ measurements in separate incubations using multiple light sources. This was achieved by
312 incubating radio-labelled samples within the optical head of the FastOcean FRRf and using
313 the FRRf's cool-white LED array to drive photosynthesis in the sample (Supplementary Fig.
314 S1). Not only does the dual incubation method avoid the need to apply spectral correction
315 factors which can be error-prone, but also avoids potential issues with sample heterogeneity
316 (Lawrenz et al. 2013, Hughes et al. 2020).

317 To quantify $^{14}C$-uptake, we adopted the small-volume method of Lewis and Smith (1983)
318 with several modifications. For each strain, triplicate 3 mL samples were placed in a
319 borosilicate test-tube and acclimated under low light (~2-3 μmol photons $m^{-2}$ $s^{-1}$) for 15 min
320 to relax non-photochemical quenching processes before FRRf assessment of dark-acclimated
321 physiology as per Hughes et al. (2018b). Samples were then spiked to a final concentration of
322 0.4 μCi $mL^{-1}$ $NaH^{14}CO_3$ (Perkin-Elmer, Melbourne, Australia). The radio-labelled sample
323 was then incubated for 20 min inside the FRRf at a single irradiance corresponding to the
324 growth conditions of that specific strain (60 μmol photons $m^{-2}$ $s^{-1}$ for most strains, 180 μmol
325 photons $m^{-2}$ $s^{-1}$ for the family Symbiodiniaceae) and $ETR_{PSII}$ was determined every 5 s during
326 this period. At the end of the incubation period, the sample was removed and immediately
327 acidified with 150 μL of 6 M HCl to drive remaining unfixed inorganic $^{14}C$ to $^{14}CO_2$.
328 Samples were then agitated gently on an orbital shaker (100 rpm) and left to de-gas for 24 h
329 prior to fixation with 10 mL scintillation fluid (Ultima Gold LLT, Perkin Elmer). Fixed
330 samples were shaken vigorously for several minutes and left to stand for 3 h before
331 measuring disintegrations per minute (DPM) via liquid scintillation counting (Tri-Carb 2810
332 TR, Perkin-Elmer), using automatic quench correction and a count time of 5 min. $^{14}C$-uptake
333 was calculated on a volumetric basis from the concentration of dissolved inorganic carbon
334 (DIC) and the amount of $^{14}C$ isotope incorporated during the incubation as per Knap et al.

335 (1996). Twenty mL aliquots for DIC analysis were taken from parallel samples grown under
336 identical conditions. Each sample was transferred to a glass scintillation vial, preserved with
337 $HgCl_2$ at a final concentration of 0.5%, double-wrapped in parafilm to prevent gas exchange
338 and stored in darkness until subsequent analysis of DIC using a dissolved gas analyser
339 (Picarro 1301, Picarro Instruments, California, USA). Both $ETR_{PSII}$ and $^{14}C$-uptake were
340 scaled to hourly-integrated rates as per Suggett et al. (2009a) so that $\Phi_{e,C}$ (mol $e^-$ [mol C]$^{-1}$)
341 could be determined as:

342 $$\Phi_{e,C} = \frac{ETR_{PSII}\ (\text{mol } e^- \ m^{-3}\ h^{-1})}{14C\ \text{uptake}\ (\text{mol C } m^{-3}\ h^{-1})} \qquad (7)$$

343 *Additional FRRf-based parameterisation*

344 For all cultures and conditions, non-photochemical quenching of fluorescence was calculated
345 as the normalised Stern-Volmer coefficient ($NPQ_{NSV}$) as per McKew et al. (2013) during the
346 simultaneous $^{14}C$-FRRf incubations and represents an integrated value over the entire
347 incubation period. In addition, PSU size (mol Chl-*a* [mol RCII]) was calculated from
348 $[RCII]^{(FRRf)}$ and the concentration of Chl-*a* according to Oxborough et al. (2012).

349 *Meta-analysis of* $\Phi_{e,C}$

350 We searched the literature for parallel measurements of $ETR_{PSII}$ and $^{14}C$-uptake upon
351 laboratory-grown phytoplankton cultures. Principal component analysis (PCA) was used to
352 visually assess patterns in $\Phi_{e,C}$ (Supplementary Fig. S2). Upon initial inspection, it was
353 evident that methodological inconsistencies between studies were potentially driving
354 variability in $\Phi_{e,C}$. To minimise methodological bias from our pooled assessment of $\Phi_{e,C}$, we
355 applied screening criteria to ensure consistency with our existing data set. Specifically, data
356 was only included if the following conditions were met: i) phytoplankton were grown under
357 nutrient-replete conditions, ii) $ETR_{PSII}$ was measured by single-turnover instrumentation
358 protocols (Kromkamp and Forster 2003), iii) $^{14}C$ incubations were derived from short

359 incubations <30 min to minimise respiration of C-fixed, iv) the concentration of functional
360 PSII reaction centres [RCII] was determined, rather than assuming a constant relative to
361 measured Chl-*a* concentration, and v) appropriate spectral correction factors were applied
362 (see above). Although our measurements were performed at irradiances representing light-
363 limited photosynthesis (i.e. where $E/E_k < 1$ – we revisit this point later in the results section),
364 much reported data is pooled from incubations performed under a range of irradiances
365 spanning both light-limited and light-saturated photosynthesis ($E/E_k > 1$) (e.g. Suggett et al.
366 2009a), therefore we did not include $E/E_k$ in our screening criteria.

367 *Statistical analysis*

368 A one-way analysis of variance (ANOVA) was used to test for strain-specific differences in
369 $\Phi_{e,C}$, using a Tukey test when significant differences were detected. All $\Phi_{e,C}$ data were $\log_{10}$
370 transformed prior to statistical analysis to improve assumptions for ANOVA (IBM SPSS
371 v20.0). Differences in $\Phi_{e,C}$ between phytoplankton classes and size classes were assessed by
372 using the non-parametric Kruskal-Wallis test due to violation of the Levene's test for
373 homogeneity of variance. Comparison of $\Phi_{e,C}$ between diatoms and chlorophytes (main study
374 data only) was performed using student's t-test where assumption of normality and equal
375 variance were verified (tested using Kolmogorov-Smirnov and Levene's tests respectively).
376 Relationships between $\Phi_{e,C}$ and both growth rate, and $NPQ_{NSV}$ were assessed by linear
377 regression (R-software v. 3.2.1). Factors explaining variation in growth rates between strains
378 were assessed using distance-based redundancy analysis (dbRDA) – multivariate multiple
379 regression of principal coordinate axes on predictor variables (PRIMER v6.0, PRIMER-E,
380 Plymouth, UK) using $\Phi_{e,C}$, POC:PON and cell volume as predictive variables. Principal
381 component analysis (PCA, PRIMER v6.0) was used to identify patterns in data collated
382 during the meta-analysis of the wider literature. Upon incorporating the additional data from

383 the meta-analysis, differences in $\Phi_{e,C}$ between taxonomic and size classes were evaluated
384 using the non-parametric, Kruskal-Wallis test.

385 Results

386 *Phytoplankton growth, elemental stoichiometry and photophysiology*
387 Growth rates varied considerably between strains, ranging from 0.24 (*Tetraselmis* sp.) to 0.84
388 $d^{-1}$ (*N. closterium*). Diatoms, together with *Synechococcus* sp., consistently exhibited the
389 highest growth rates (0.75 - 0.84 $d^{-1}$), compared to chlorophytes which were generally low
390 (0.24 - 0.57 $d^{-1}$), with prymnesiophytes intermediate (0.51 – 0.71 $d^{-1}$) (Table 2). Growth rates
391 for strains measured outside of the main study are not reported here since we did not have
392 sufficient fluorometry data prior to sampling from which to derive a comparable growth rate.
393 Measured POC:PON ratios ranged from 4.97 to 9.59 across the 12 strains grown in the main
394 study (i.e. excluding additional strains sourced from other studies) (Table 2). Diatoms had
395 notably lower POC:PON ratios than most other classes, with all but *D. brightwellii* exhibiting
396 values <6, together with the cryptophyte, *R. salina*.

397 Generally, and as expected (see Suggett et al. 2009b, 2015), we observed inverse covariation
398 between $F_v/F_m$ and $\sigma_{PSII}$ associated with cell volume (Table 2, Supplementary Fig S3), with
399 the exception of the dinoflagellates that had relatively low $F_v/F_m$ values together with larger
400 $\sigma_{PSII}$ despite their large cell volumes (Table 2). *Synechococcus* sp. also appeared to contradict
401 this trend, most likely since the low value for $\sigma_{PSII}$ reflects the unique combination of
402 excitation LEDs employed in the FRRf protocol for this strain (450 nm + 624 nm).
403 Photosynthetic unit (PSU) size ranged from 254.8 - 842.5 mol Chl-*a* (mol RCII)$^{-1}$, however
404 most (14 out of 17) strains had a PSU size between 300 - 650 mol Chl-*a* (mol RCII)$^{-1}$ (Table
405 2). The cyanobacterium *Synechococcus* exhibited the smallest PSU size, likely since more

406 Chl-*a* is typically associated with PSI rather than PSII as opposed to the eukaryotic strains in
407 this study (Pakrasi et al. 1985).

408 Interestingly, the light-saturation parameter, $E_k$ (i.e. the irradiance at which photosynthesis –
409 measured here as $ETR_{PSII}$ - becomes light-saturated), when spectrally-corrected to match the
410 growth conditions, was between 130 - 200 µmol photons $m^{-2}$ $s^{-1}$ for the majority of strains,
411 thus up to three-fold higher than growth irradiance (60 - 180 µmol photons $m^{-2}$ $s^{-1}$, Table 2).
412 Only three strains (*R. salina* and both prymnesiophytes) exhibited $E_k$ values close to growth
413 irradiance, ranging from 52.2 - 58.8 µmol photons $m^{-2}$ $s^{-1}$, whilst the single largest $E_k$ value in
414 this study (~350 µmol photons $m^{-2}$ $s^{-1}$) was measured in *Tetraselmis* sp. (CS-352). Since
415 $ETR_{PSII}$ and $^{14}C$-uptake incubations were performed at irradiance levels close to growth
416 conditions, the irradiance ($E$) relative to $E_k$, (i.e. $E/E_k$) was consequently <1 for most strains
417 except *R. salina* (1.26), *E. huxleyi* (1.12) and *P. pouchetti* (1.01), thus practically all $\Phi_{e,C}$
418 values reported in this study correspond to measurements during light-limited electron
419 transport rates (Table 2).

420 *Simultaneous $^{14}C$-uptake and $ETR_{PSII}$ incubations* ($\Phi_{e,C}$)

421 The mean measured value of $\Phi_{e,C}$ for this study was 5.7 ± 0.3 mol $e^-$ (mol C)$^{-1}$ ($n$ = 17), but
422 ranged from 3.8 to 10.7 mol $e^-$ (mol C)$^{-1}$; with *T. weissflogii* and *R. salina* exhibiting $\Phi_{e,C}$
423 values fractionally below the theoretical minimum of 4 mol $e^-$ (mol C)$^{-1}$ (Fig. 1). Measured
424 $\Phi_{e,C}$ for the dinoflagellate *Durusdinium trenchii* was significantly higher (10.7 mol $e^-$ [mol
425 C]$^{-1}$) than all other species in this study (ANOVA, $F_{16,34}$ = 8.42, $P$ < 0.05), whilst the values
426 for *P. pouchetti* and *N. oculata* were also larger (7.8 and 7.2 mol $e^-$ [mol C]$^{-1}$, respectively)
427 than a number of other strains (ANOVA, $F_{16,34}$ = 8.42, $P$ < 0.05) (Fig. 1). For the remaining
428 strains, $\Phi_{e,C}$ values were ~4-5 mol $e^-$ (mol C)$^{-1}$ and not statistically distinguishable from one
429 another (Fig. 1). Overall, the representative dinoflagellate and prymnesiophyte strains

430 exhibited a wider range of $\Phi_{e,C}$ values compared to strains within other classes (Fig. 2a),
431 suggesting that taxonomic class alone was not a reliable predictor for $\Phi_{e,C}$ for these groups.
432 Indeed, no statistically-significant differences in $\Phi_{e,C}$ values were found between taxonomic
433 classes when considering data from all strains (Kruskal-Wallis, $H_3 = 7.64$, $P = 0.241$). Given
434 the reduced statistical power due to the limited number of replicates within certain classes,
435 the analysis was repeated including only those classes with $n=3$ strains or higher: i.e. diatoms,
436 dinoflagellates and chlorophytes. We still found no difference between classes (Kruskal-
437 Wallis, $H_2 = 4.15$, $P = 0.08$). Because we could not entirely discount the possibility that
438 inclusion of the dinoflagellate strains introduced a confounding factor of growth
439 environment, we also examined $\Phi_{e,C}$ between diatoms and chlorophytes, finding that diatoms
440 exhibited a lower mean $\Phi_{e,C}$ (t-test, $t_5 = -2.77$, $P < 0.05$) (Fig 2a). When binned into arbitrary
441 size classes, $\Phi_{e,C}$ exhibited no statistical differences (Kruskal-Wallis, $H_3 = 4.11$, $P = 0.25$),
442 although the 1,000-9,999 $\mu m^3$ size class exhibited the greatest range of $\Phi_{e,C}$ values (3.8-10.8)
443 (Fig 2b)

444 *Relationship between $\Phi_{e,C}$ and other traits*

445 Consistent with our original expectation, we observed a significant inverse relationship
446 between $\Phi_{e,C}$ and growth rate ($R^2 = 0.49$, $P < 0.05$, Fig. 3a); however, *R. salina* clearly
447 contributed to lack of convergence of a linear fit, and the relationship improved considerably
448 if this strain outlier was removed ($R^2 = 0.70$, $P < 0.05$, Fig. 3a). Linear regression showed no
449 significant relationship between $\Phi_{e,C}$ and either cell volume ($P = 0.28$, Fig. 3b), or $NPQ_{NSV}$
450 ($P = 0.26$, Fig. 3c). We further examined whether a greater extent of variability in growth
451 rates could be explained from $\Phi_{e,C}$, when combined with additional traits associated with
452 biophysical constraints (cell volume) and elemental composition (POC:PON ratio) that
453 govern the efficiency with which light energy is converted to biomass (see Litchman et al.
454 2007). Approximately 55% of variability in growth rates for all strains combined mapped

455 onto 3 variables, thus offering only a slight improvement over $\Phi_{e,C}$ alone; again, removal of
456 *R. salina* improved this to 87% (Fig. 4). Thus overall, the inclusion of additional variables
457 resulted in only a marginally improved ability to reconcile variability between $\Phi_{e,C}$ and
458 growth rates.

459 *Resolving further trends in $\Phi_{e,C}$ by including meta-data*
460 Incorporation of data mined from the extended literature (Supplementary Table S1),
461 increased the total number of observations to $n = 25$ (from our study alone, $n = 17$),
462 representing $\Phi_{e,C}$ measurements from taxonomic classes we previously evaluated
463 (Chlorophyceae, Bacillariophyceae, Dinophyceae and Cryptophyceae), plus one new class
464 (Pelagophyceae). All of these additional data fell into three of our four arbitrarily-defined size
465 classes, specifically 0-99 $\mu m^3$, 100-999 $\mu m^3$ and 1,000-9,999 $\mu m^3$. These additional data
466 introduced variability of $\Phi_{e,C}$ within most taxonomic classes (Fig. 5a) compared to our
467 original data set (Fig. 2a). Chlorophytes exhibited the widest range of $\Phi_{e,C}$ values (~5-16 mol
468 $e^-$ [mol C]$^{-1}$), closely followed by dinoflagellates (~4-15 mol $e^-$ [mol C]$^{-1}$) while diatoms were
469 still characterised by a relatively narrow range of $\Phi_{e,C}$ values (~4-7 mol $e^-$ [mol C]$^{-1}$);
470 however diatoms and chlorophytes (which together comprised nearly half the total
471 observations in this study) were no longer statistically distinguishable (t-test, $t_8 = 0.86$, $P =$
472 $0.52$).

473 Consideration of the full meta-data did not allow for statistical differences to be determined
474 between size classes (Kruskal-Wallis $H_3 = 0.76, P = 0.86$) (Fig. 5b). Furthermore, linear
475 regression revealed no significant relationship between $\Phi_{e,C}$ and either growth rate, cell
476 volume or $NPQ_{NSV}$ (Fig. 6a-c), suggesting that under light-limited conditions, $\Phi_{e,C}$ cannot be
477 estimated using these other parameters that are easier to measure.

478 Discussion

479 Studies are increasingly turning towards examining and modelling $\Phi_{e,C}$ to retrieve estimates
480 of C-fixation from FRRf-assessments of phytoplankton photosynthetic rates (as $ETR_{PSII}$)
481 (Hughes et al. 2018b). However, these studies have been predominately empirically-based
482 from measurements on natural phytoplankton communities, where it is difficult to reconcile
483 conflated regulatory effects of taxa and environment upon $\Phi_{e,C}$ (Lawrenz et al. 2013, Zhu et
484 al. 2017, 2019, Hughes et al. 2019). By growing 17 strains of phytoplankton belonging to
485 seven classes under controlled laboratory conditions, and mining data from existing studies,
486 we demonstrate taxonomic regulation of $\Phi_{e,C}$ at the species, and possibly class level, and
487 further show that growth rate is an important factor related to $\Phi_{e,C}$ variability. Whilst this is
488 still a statistical approach based on empirical data, by measuring $\Phi_{e,C}$ under controlled
489 environmental conditions, we demonstrate that diatoms appear to exhibit a lower $\Phi_{e,C}$
490 compared to chlorophytes during steady-state, nutrient-replete growth. We next discuss these
491 observations and how they can potentially improve our ability to apply FRRf for the
492 widespread assessment of MPP.

493 *Species-specific variability in* $\Phi_{e,C}$

494 We observed $\Phi_{e,C}$ values ranging from ~3.8-10.7 mol $e^-$ (mol $C)^{-1}$ from the phytoplankton
495 cultures, which fall within the range of values reported by the (few) laboratory studies to date
496 (Fujiki et al. 2007, Suggett et al. 2009a, Brading et al. 2011, Hoppe et al. 2015; see
497 Supplementary Table S1), and well within the range of observations from the field (e.g.
498 Lawrenz et al. 2013, Robinson et al. 2014, Zhu et al. 2016, Hughes et al. 2018b). In
499 agreement with previous findings (Suggett et al. 2009a, Napoleon et al. 2013), our study
500 identified species-specific differences in $\Phi_{e,C}$, with *D. trenchii*, *N. oculata*, and *P. pouchetti*
501 exhibiting higher $\Phi_{e,C}$ values than other strains. Our measured $\Phi_{e,C}$ for *D. trenchii* (10.7 mol
502 $e^-$ [mol $C]^{-1}$) was higher than those reported by Brading et al. (2013) for other members of
503 Symbiodiniaceae – i.e., *Symbiodinium* strains (ITS types A13 and A20) under light-limited

504 photosynthesis (~5 mol $e^-$ [mol C]$^{-1}$). Our findings that low intraspecific variability of *C.*
505 *goreaui* (i.e. exhibiting a similar $\Phi_{e,C}$), supports observations by Brading et al. (2013) for
506 *Symbiodinium*. To our knowledge no previous study has reported $\Phi_{e,C}$ for *N. oculata*, or *P.*
507 *pouchetti*, thus it is not possible to evaluate our $\Phi_{e,C}$ values against previous measurements.
508 However, for all remaining strains, $\Phi_{e,C}$ values (~4-6 mol $e^-$ [mol C]$^{-1}$) generally agreed well
509 with the theoretical minimum value of 4-5 mol $e^-$ (mol C)$^{-1}$ (Hughes et al. 2018a), indicating
510 a close-coupling of $ETR_{PSII}$ to C-fixation, although values of $\Phi_{e,C}$ for *T. weissflogii* and *R.*
511 *salina* fell just under this minimum threshold (3.9 and 3.8 mol $e^-$ [mol C]$^{-1}$ respectively).
512 Values of $\Phi_{e,C}$ less than 4 occur relatively frequently in FRRf studies yet are difficult to
513 reconcile with existing knowledge of the oxygenic photosynthesis pathway, and are thus
514 commonly attributed to artifacts in methodology (see Hughes et al 2018a). By performing
515 measurements under carefully-controlled laboratory conditions, we have eliminated most
516 common sources of error (reviewed by Hughes et al. 2018a), but return to this point later
517 when discussing possible effects of cellular pigment concentration upon determination of
518 $ETR_{PSII}$ (and thus $\Phi_{e,C}$).

519 The observed inverse relationship between growth rate and $\Phi_{e,C}$ in this study supported our
520 original expectation that taxa investing electrons more efficiently into fixed-C (i.e. those with
521 a low $\Phi_{e,C}$), can sustain higher rates of growth. As the ratio of gross primary production (GPP
522 - the total amount of C fixed during photosynthesis) to net primary production (NPP – the
523 amount of fixed C retained over the duration of the cell cycle) remains fairly constant
524 irrespective of growth rate (Halsey et al. 2010) this is not unexpected, and was demonstrated
525 particularly well by diatoms, which consistently exhibited high growth rates together with a
526 low and relatively narrow range of $\Phi_{e,C}$ values. Considering the design of this study with $\Phi_{e,C}$
527 measurements made under nutrient-replete and a constant, relatively low irradiance, such an
528 outcome appears logical. Under such favourable conditions, linear electron flow, which

529 generates the ATP and NADPH needed for carbon-assimilation (and thus, ultimately for
530 cellular growth) is expected to be the dominant component of $ETR_{PSII}$, with little competition
531 from alternative (i.e. non-C-fixing) electron sinks (McDonald et al. 2011) that would be
532 expected to increase $\Phi_{e,C}$ (Hughes et al. 2018a).

533 The nutrient replete and light limited growth conditions used in our study likely also explains
534 why the overall range of $\Phi_{e,C}$ values measured (~4-10 mol $e^-$ [mol C]$^{-1}$), was relatively low
535 compared to field observations (~2-50 mol $e^-$ [mol C]$^{-1}$). In their natural environment,
536 phytoplankton cells regularly experience non-steady-state environmental conditions, with
537 transient fluctuations in both light and nutrient availability, and thus operate under more
538 "extreme" conditions than those examined here. Numerous field (Moore et al. 2006,
539 Schuback et al 2015, 2017, Zhu et al 2016, 2017, 2019) and laboratory (Suggett et al. 2009a,
540 Brading et al. 2013) studies have shown excess light to be a strong factor decoupling $ETR_{PSII}$
541 and rates of C-fixation. In this study, $\Phi_{e,C}$ was measured at an irradiance approximating
542 growth conditions, representing light-limited photosynthesis (i.e. $E/E_k < 1$) for the majority of
543 strains. However, in nature, irradiance can fluctuate over a range of time-scales (Falkowski,
544 1984), and thus phytoplankton cells are often subjected to extended periods of light-saturated
545 photosynthesis where $E/E_k > 1$) (Moore et al. 2006). Thus, it is unlikely that our observations
546 would hold true under a dynamic light field, where energy dissipation mechanisms (including
547 non-C-fixing pathways which increase $\Phi_{e,C}$) play an important role in regulating
548 photosynthetic performance (e.g. Cardol et al. 2011). This has obvious implications for using
549 a single conversion factor when converting underway FRRf measurements of upper ocean
550 $ETR_{PSII}$ into estimates of fixed C. Currently, little is known as to how $\Phi_{e,C}$ may ultimately
551 scale to a daily mean value under such a fluctuating light regime (but see Hoppe et al. 2015,
552 Zhu et al. 2016, Schuback et al. 2016). Certainly, it is highly likely that taxa-specific
553 differences would be a key factor here, as demonstrated by Wagner et al (2006) who showed

554 that the diatom, *Phaeodactylum tricornutum*, was nearly twice as efficient at converting
555 photosynthetic energy into biomass than the chlorophyte, *Chlorella vulgaris*, when grown
556 under fluctuating light, attributing this difference to the highly-efficient NPQ of the diatom.
557 Clearly an important step towards using FRRf to validate remote sensing estimates of MPP is
558 to better understand the uncertainty related to how $\Phi_{e,C}$ responds to fluctuating light, and
559 comparing this with uncertainties of other methods which also scale up incubation time(s)
560 and conditions to daily MPP measures.

561 *Evidence for class-dependent variability in* $\Phi_{e,C}$

562 Our study provided some evidence to suggest that diatoms may exhibit a lower $\Phi_{e,C}$ than
563 chlorophytes – although it is important to note that this trend became apparent only after
564 exclusion of dinoflagellate strains from the analysis on the basis of a possible confounding
565 factor of growth condition (discussed below). Our evidence for $\Phi_{e,C}$ regulation at the class
566 level contradicts findings of Napoleon et al. (2013), who reported inter-specific differences in
567 $\Phi_{e,C}$ for both diatoms (*Pseudo-nitzschia pungens* and *Asterionellopsis glacialis*), and
568 dinoflagellates (*Heterocapsa* sp. and *Karenia mikimitoi*), but no difference between classes.
569 Previous laboratory studies of $\Phi_{e,C}$ (e.g. Suggett et al. 2009a, Brading et al. 2013) either
570 contain too few representatives per phytoplankton class, or examine strains from only a single
571 class, to make meaningful inferences regarding taxonomic regulation of $\Phi_{e,C}$ beyond that of
572 strain/species level.

573 Inclusion of such meta-data (Fig. 5) in our analysis only increased the observed variance, and
574 indeed we could not detect statistical differences in $\Phi_{e,C}$ between classes. Interestingly, $\Phi_{e,C}$
575 for diatoms was still confined to a relatively narrow range of values despite the increased
576 number of observations ($n$ = 5). Suggett et al. (2006) showed that $\Phi_{e,C}$ values increased
577 during a spring bloom transition from diatoms to (dino)flagellates, whilst Hughes et al.
578 (2018b) demonstrated via nutrient-enrichment bioassays, that decreasing $\Phi_{e,C}$ was largely

579 driven by a taxonomic shift from a co-dominated phytoplankton assemblage of diatoms and
580 dinoflagellates, towards a diatom-only assemblage. Studies therefore appear to consistently
581 report low $\Phi_{e,C}$ values for diatoms (Suggett et al. 2009a, Hoppe et al. 2015, Hughes et al.
582 2018b) perhaps suggesting that lower conversion factors (i.e. $\Phi_{e,C}$) could be routinely applied
583 to diatom-dominated assemblages to retrieve C-fixation rates from $ETR_{PSII}$ (at least under
584 specific conditions, for example where nutrients are replete and photosynthesis is light-
585 limited).

586 Overall, our range of $\Phi_{e,C}$ values both within, and between taxonomic classes, appears
587 consistent with previous studies of diatoms, chlorophytes and dinoflagellates under
588 conditions of balanced, nutrient-replete growth (Suggett et al. 2009a, Brading et al. 2013,
589 Hoppe et al. 2015). Variability of $\Phi_{e,C}$ between classes could be expected to be driven by
590 variable demands for energy (ATP) and reductant (NADPH) to maintain optimal growth
591 (Halsey et al. 2013), thus reflecting adaptive strategies to maintain photosynthetic fitness,
592 which have their origins in evolutionary history. Indeed, it has recently been shown that
593 adaptations in light-harvesting apparatus partially explain phylogenetic differences in the
594 proportions of macromolecular pools (i.e. carbohydrates, lipids and proteins) between classes,
595 consistent with observed C:N ratios in the field (see Finkel et al. 2016). Incorporation of N
596 into biomass typically consumes electrons for reductive assimilation of an external N source
597 (see Anderson 1995), thus $\Phi_{e,C}$ is expected to increase in parallel with cellular nitrogen
598 content (Jakob et al. 2007, Hughes et al. 2018a). However, in our study C:N ratio did not
599 appear to be a factor driving $\Phi_{e,C}$ variability, as the lowest measured POC:PON ratios (i.e.
600 highest proportion of N per C biomass) often corresponded to strains with lowest $\Phi_{e,C}$ values
601 (e.g. *R. salina*). It is however possible that taxa with larger pools of protein, would have a
602 larger $\Phi_{e,C}$ due to the increased cellular maintenance costs for protein turnover (e.g. Quigg
603 and Beardall 2003), which can be directly coupled to the use of photo-produced ATP, instead

604 of via respiration. Whilst this notion would warrant testing, the relative production of ATP
605 from photosynthetic versus respiratory metabolism would be difficult to quantify (Quigg and
606 Beardall 2003), yet knowledge of protein content could provide additional insight here.
607 Variability in $\Phi_{e,C}$ could also be expected to be driven by taxonomic differences in the
608 downstream efficiency of $CO_2$-assimilation itself. Dinoflagellates are the only phytoplankton
609 class in this study to possess form II of RuBisCO, characterised by poor $CO_2$:$O_2$
610 discrimination, and are thus more likely to exhibit photorespiration (Brading et al. 2013). It
611 remains unclear, however, whether photorespiration is significant for microalgae or is instead
612 overcome by the expenditure of photo-produced ATP to fuel carbon-concentrating
613 mechanisms (e.g. Badger et al. 1980).

614 *No overarching explanation for* $\Phi_{e,C}$ *variability through cell size*

615 Cell size is considered a "master trait" that constrains many physiological and ecological
616 characteristics of phytoplankton, including photosynthetic performance (Finkel et al. 2009).
617 Cell size, captured in this study as biovolume, influences PSII light absorption efficiency
618 (Ciotti et al. 2002), the efficiency with which absorbed light is used to drive photochemistry
619 (Suggett et al. 2009b) and, thus ultimately photosynthetic electron transport (Suggett et al.
620 2009a, Rattan et al. 2012). Generally, when normalised to cell size, photosynthetic rates tend
621 to be lower for phytoplankton of larger size classes (Bouman et al. 2005, Barnes et al. 2015),
622 as surface-area-to-volume ratio imposes biophysical constraints upon light absorption and
623 nutrient-uptake (Marra et al. 2007). Interestingly, we found that $\Phi_{e,C}$ scaled to growth rate,
624 suggesting biophysical constraints over $\Phi_{e,C}$, yet we did not observe the expected relationship
625 between cell volume and growth rate that would be expected from cell size constraints.
626 Commonly, a reduction in growth rate corresponding to increasing cell size is documented
627 for phytoplankton (Geider et al. 1986), thus our observations would appear to support that
628 $\Phi_{e,C}$ was not subjected to biophysical constraints of cell size. However, we also point out that

629 it has been previously demonstrated that growth rate may be taxa-dependent, with diatoms
630 and dinoflagellates of equivalent size exhibiting up to a three-fold difference in cell division
631 rate under identical conditions (Banse, 1982).

632 A notable exception to the generally close-coupling of $\Phi_{e,C}$ and cellular growth rate was
633 observed for the cryptophyte, *R. salina*, which despite having one of the lowest $\Phi_{e,C}$ values
634 (~4 mol $e^-$ [mol C]$^{-1}$), also exhibited a low growth rate during the study (0.39 $d^{-1}$). A possible
635 explanation is that the small volume $^{14}C$-method used in this study captures total organic
636 carbon fixed over the incubation period, and does not discriminate between particulate
637 organic carbon (POC) retained for cellular growth, and any extracellular release of newly-
638 fixed carbon as dissolved organic carbon (DOC). A study by Fukuzaki et al. (2014) found a
639 closely-related cryptophyte species (*Rhodomonas ovalis)* to be a prolific producer of
640 fluorescent dissolved organic matter (of which DOC was likely the largest component) when
641 compared to a range of other phytoplankton taxa. Unaccounted-for DOC release by *R. salina*
642 would decouple GPP from NPP and could thus explain why *R. salina* grew relatively slowly
643 despite exhibiting a low $\Phi_{e,C}$ value, however this was not explicitly measured and warrants
644 further targeted examination.

645 *Optimality of growth conditions*

646 Given the negative linear relationship between growth rate and $\Phi_{e,C}$ (Fig. 3a), but not cell
647 volume, it raises the interesting question as to whether growth rates in our study were a proxy
648 for perceived quality of growth condition for each strain, and whether this may be responsible
649 for driving observed variability in $\Phi_{e,C}$. Variation in phytoplankton growth rates has been
650 shown to influence lifetimes of newly-fixed carbon (Halsey et al. 2010, 2011, 2013) leading
651 to a variable C-uptake rate somewhere between GPP and NPP as measured by short
652 incubations of 20 – 60 min, reflecting varying extents to which energy and reductant are

653 utilised for cellular maintenance. In the studies of Halsey et al. (2010, 2011, 2013) different
654 growth rates were achieved by nutrient limitation - a factor that is unlikely to be an issue for
655 our study as strains were maintained under nutrient replete conditions. A key environmental
656 variable that should be considered in the context of this study however is temperature.
657 Temperature exerts considerable influence over key phytoplankton traits including growth,
658 electron transport and carbon fixation (Baker et al. 2016), with considerable variability in
659 physiological responses to sub- and supra-optimal temperatures, observed both within and
660 between phytoplankton species (e.g. Pittera et al. 2014, Baker et al. 2016, Varkey et al.
661 2016). All strains in this study (with the exception of Symbiodiniaceae) were grown at a
662 single temperature (20 °C), thus variability introduced in $\Phi_{e,C}$ (and indeed growth rates) due
663 to differing thermal preferenda of strains cannot be adequately accounted-for without thermal
664 performance curves (e.g. Baker et al. 2016), and should be considered when interpreting
665 trends reported here. A final area of consideration is how pigment chemodiversity among
666 phytoplankton strains and spectral quality of the growth conditions (cool white fluorescent
667 tube) may have contributed to variability in growth rate(s), and therefore potentially
668 influenced the observed relationship between growth rate and $\Phi_{e,C}$ . Unfortunately the
669 spectral quality of the incubator was not measured at the time of the study therefore not
670 permitting a spectral-adjustment of growth rates – although, we estimate based on previous
671 measurements of similar light sources that differences in absorption could account for 25-
672 50% variability in growth rates across taxa in this study (data not shown).

673 *Consideration of "package effects" upon* $K_a$

674 A recent study by Boatman et al. (2019) demonstrated that packaging of chlorophyll and
675 light-harvesting pigments into phytoplankton cells, referred to as the "package effect" (Kirk
676 1975, Bricaud et al. 1995) represents a potential source of error when calculating $ETR_{PSII}$
677 (and hence $\Phi_{e,C}$) when using fluorometric estimation of $[RCII]_{(FRRf)}$ (e.g. Oxborough et al.

678 2010). Specifically, this error arises when applying an instrument-specific constant, $K_a$, ($m^{-1}$)
679 not corrected to account for re-absorption of photons generated by PSII fluorescence within
680 the cell before exiting – a phenomenon that increases in magnitude with increasing package
681 effect (Boatman et al. 2019). While a corrective procedure is described by Boatman et al.
682 (2019), this requires the measuring fluorometer to be equipped with dual narrow-band pass
683 filters (at 680 and 730 nm), an atypical configuration for many FRRf instruments currently in
684 use, including the model used here.

685 Boatman et al. (2019) reported post-corrected values for $K_a$ ranging from ~8,000–25,000 $m^{-1}$
686 across 11 phytoplankton strains grown under nutrient-replete conditions. This represents a far
687 wider range of $K_a$ values than previously established by field studies (Oxborough et al. 2010)
688 used to derive the default value (11,800 $m^{-1}$) for the instrument used in our study.
689 Theoretically, in a worst case scenario, left uncorrected, package effects may translate to a
690 roughly two-fold underestimate of $\Phi_{e,C}$ reported here for species with the highest pigment
691 packaging. For example, Boatman et al. (2019) reported a corrected $K_a$ value for *T.*
692 *pseudonana* of 25,743 $m^{-1}$, which if applied for the same species in our study would adjust
693 $\Phi_{e,C}$ from 5.6 to 13.6 mol $e^-$ (mol C)$^{-1}$. However, it is important to note that pigment
694 packaging levels for a given isolate are dependent upon growth condition (Topel et al. 2005)
695 and physiological condition (Berner et al. 1989), thus interchanging $K_a$ values between
696 studies is unlikely to prove robust. Moreover, only four species are common to our current
697 data set and that of Boatman et al. (2019): *T. pseudonana, T. weissflogii, D. tertiolecta* and *E.*
698 *huxleyi*, thereby precluding thorough evaluation of the error in our dataset arising from
699 uncorrected package effects. Certainly, the packaging-adjusted $K_a$ values reported by
700 Boatman et al. (2019) for nutrient replete cultures were larger than our default $K_a$ value for all
701 but one species in their study (*Chlorella vulgaris*), suggesting that absolute $\Phi_{e,C}$ reported here
702 may be consistently underestimated. However, we also point out that our study includes taxa

703 not assessed by Boatman et al. (2019), notably dinoflagellates, a cryptophyte (*R. salina*) and a
704 cyanobacterium (*Synechococcus* sp.) for which package effects on $K_a$ remains undetermined,
705 and in the case of *R. salina* for example, this could be a contributing factor towards why
706 measured a $\Phi_{e,C}$ value falling just under the theoretical minimum value of 4 mol $e^-$ (mol C)$^{-1}$.
707 Interestingly, it appears that the extent to which package effects influences $K_a$ is not
708 predictable by cell size, but rather by optical characteristics of a given taxon (Boatman et al.
709 2019). Despite limited ability to resolve how package effects may, or may not, be driving
710 trends observed here, it remains an important consideration when interpreting reported values
711 for $\Phi_{e,C}$ derived from fluorometric estimates of [RCII].

712 *$NPQ_{NSV}$ provides limited predictive capability for $\Phi_{e,C}$ under light-limited conditions*
713 In efforts to identify empirical relationships between $\Phi_{e,C}$ and (more easily measured)
714 biophysical properties, we observed poor correspondence between $NPQ_{NSV}$ and $\Phi_{e,C}$: an
715 outcome that appears to contradict observations from natural phytoplankton communities
716 (Schuback et al. 2015, 2016, 2017, Zhu et al 2016, Hughes et al. 2018b, Wei et al. 2019).
717 Such an empirical relationship presumably depends upon a mechanistic link between the
718 upregulation of alternative electron pathways in response to high excitation pressure which
719 increases $\Phi_{e,C}$, and the simultaneous generation of ΔpH that activates thermal-dissipation
720 mechanisms in the PSII antenna (detected as an increased $NPQ_{NSV}$ signature) (Nawrocki et al.
721 2015, Schuback et al. 2015, Hughes et al. 2018a). It is therefore perhaps unsurprising that we
722 found no correlation between $NPQ_{NSV}$ and $\Phi_{e,C}$ in our study since incubations were
723 predominately performed during conditions of low excitation pressure (i.e. light-limited
724 photosynthesis).

725 Field observations of a correlation between $\Phi_{e,C}$ and $NPQ_{NSV}$ often appear to be driven by
726 cells experiencing dynamic stress to nutrient availability (Schuback et al. 2016, Hughes et al.
727 2018b) or light exposure (Zhu et al. 2016). While phytoplankton strains were maintained

728 under steady-state conditions in the present study, we still observed a fairly large range of
729 $NPQ_{NSV}$ values (0.35-1.81) across all strains, indicating some degree of taxonomic control
730 over this fluorescence parameter. $NPQ_{NSV}$ quantifies the thermal dissipation of absorbed
731 energy, but the regulation and mechanisms by which this is accomplished, vary considerably
732 between taxa (Kaňa et al. 2012). For example, NPQ in diatoms is characterised by a
733 diadinoxanthin-based xanthophyll cycle which is rapidly triggered by ΔpH (Lavaud and
734 Groth 2006), compared to the violaxanthin-based xanthophyll cycle found in chlorophytes
735 over which ΔpH has less control (Finazzi et al. 2006), or the entirely different NPQ found in
736 cyanobacteria associated with the orange carotenoid protein (OCP, Kiriliovsky et al. 2007).

737 In comparison to the $NPQ_{NSV}$ values measured in this study, Hughes et al. 2018b measured a
738 similar range of $NPQ_{NSV}$ for a predominately diatom-dominated natural assemblage, although
739 this corresponded to a much larger range of $\Phi_{e,C}$ values (~4-16 mol $e^-$ [mol C]$^{-1}$), and
740 presumably included cells in various nutritional (and hence photophysiological) states.
741 Indeed, it is increasingly clear that the slopes of the relationship reported between $\Phi_{e,C}$ and
742 $NPQ_{NSV}$ are highly variable between studies, complicating our ability to utilise $NPQ_{NSV}$ as a
743 reliable predictor of $\Phi_{e,C}$ unless the cause of this variance is better understood. Measures of
744 ambient light and dissolved nutrients are clearly important contextual observations during
745 field studies. The taxonomic differences in $NPQ_{NSV}$ highlighted here may explain at least
746 some of that variability across prior recent field studies, and merit further investigation.
747 Certainly, it could be valuable to determine if a minimum, taxa-specific "threshold" for
748 $NPQ_{NSV}$ exists that could be used to identify $\Phi_{e,C}$ values close to the theoretical minimum
749 regardless of variability in slopes.

750 *Conclusions*

751 By conducting a novel assessment of $\Phi_{e,C}$ variability across phytoplankton taxa from both the
752 current and prior culture-based studies, we have demonstrated that inter-specific differences,

753 together with growth rate may be important factors contributing to observed variability of

754 $\Phi_{e,C}$ amongst marine phytoplankton. We also highlight a possible role for inter-class

755 differences that require further validation. Our empirical observations of co-variance between

756 growth rate and $\Phi_{e,C}$ potentially provides a new means with which $\Phi_{e,C}$ could be predicted, an

757 important step for scaling-up the application of FFRf to improve knowledge of C-cycling in

758 the global oceans. Improved measures in determining phytoplankton growth rates

759 (Behrenfeld et al. 2008) and cell size (Bracher et al. 2017) at global scales may prove key

760 elements in realising this goal. Previous studies have considered $\Phi_{e,C}$ variance predominantly

761 due to environmental regulation, but we have shown that a broader phenotypic trait indicative

762 of fitness, e.g. growth rate co-varies with $\Phi_{e,C}$. While we are unable to fully resolve the

763 mechanisms responsible for explaining this co-variance, our data provides new insight to

764 explore potential cellular properties regulating $\Phi_{e,C}$ across phytoplankton taxa.

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774 References

775 Anderson, L. A. 1995. On the hydrogen and oxygen content of marine phytoplankton. *Deep*
776 *Sea Res., Part I* 42: 1675-1680.

777 Badger, M. R., Kaplan, A. & Berry, J. A. 1980. Internal inorganic carbon pool of
778 *Chlamydomonas reinhardtii*: Evidence for a carbon dioxide-concentrating mechanism. *Plant*
779 *Physiol*. 66: 407-413.

780 Baker, K. G., Robinson, C. M., Radford, D. T., McInnes, A. S., Evenhuis, C. & Doblin, M.
781 A. 2016. Thermal performance curves of functional traits aid understanding of thermally
782 induced changes in diatom-mediated biogeochemical fluxes. *Front. Mar. Sci.* 3: 44.

783 Banse, K. 1982. Cell volumes, maximal growth rates of unicellular algae and ciliates, and the
784 role of ciliates in the marine pelagial. *Limnol. Oceanogr*. 27: 1059-1071.

785 Barnes, M. K., Tilstone, G. H., Smyth, T. J., Widdicombe, C. E., Gloël, J., Robinson, C.,
786 Kaiser, J. & Suggett, D. J. 2015. Drivers and effects of *Karenia mikimotoi* blooms in the
787 western English Channel. *Prog. Oceanogr*. 137: 456-469.

788 Behrenfeld, M. J., Halsey, K. H. & Milligan, A. J. 2008. Evolved physiological responses of
789 phytoplankton to their integrated growth environment. *Philos. Trans. R. Soc., B* 363: 2687-
790 2703.

791 Bouman, H., Platt, T., Sathyendranath, S. & Stuart, V. 2005. Dependence of light-saturated
792 photosynthesis on temperature and community structure. *Deep Sea Res., Part I* 52: 1284-
793 1299.

794 Bracher, A., Bouman, H. A., Brewin, R. J. W., Bricaud, A., Brotas, V., Ciotti, A. M.,
795 Clementson, L., Devred, E., Di Cicco, A., Dutkiewicz, S., Hardman-Mountford, N. J.,

796 Hickman, A. E., Hieronymi, M., Hirata, T., Losa, S. N., Mouw, C. B., Organelli, E., Raitsos,
797 D. E., Uitz, J., Vogt, M. & Wolanin, A. 2017. Obtaining phytoplankton diversity from ocean
798 color: a scientific roadmap for future development. *Front. Mar. Sci.* 4: 55.

799 Brading, P., Warner, M. E., Davey, P., Smith, D. J., Achterberg, E. P. & Suggett, D. J. 2011.
800 Differential effects of ocean acidification on growth and photosynthesis among phylotypes of
801 *Symbiodinium* (Dinophyceae). *Limnol. Oceanogr*. 56: 927-938.

802 Brading, P., Warner, M. E., Smith, D. J. & Suggett, D. J. 2013. Contrasting modes of
803 inorganic carbon acquisition amongst *Symbiodinium* (Dinophyceae) phylotypes. *New Phytol*.
804 200: 432-442.

805 Bricaud, A., Babin, M., Morel, A. & Claustre, H. 1995. Variability in the chlorophyll-specific
806 absorption coefficients of natural phytoplankton: Analysis and parameterization. *J. Geophys.*
807 *Res.: Oceans* 100: 13321-13332.

808 Campbell, J., Antoine, D., Armstrong, R., Arrigo, K., Balch, W., Barber, R., Behrenfeld, M.,
809 Bidigare, R., Bishop, J., Carr, M.E. & Esaias, W. 2002. Comparison of algorithms for
810 estimating ocean primary production from surface chlorophyll, temperature, and irradiance.
811 *Global Biogeochem. Cycles* 16: 9-1-9-15.

812 Cardol, P., Forti, G. & Finazzi, G. 2011. Regulation of electron transport in microalgae.
813 *Biochim. Biophys. Acta, Bioenerg*. 1807: 912-918.

814 Ciotti, A. M., Lewis, M. R. & Cullen, J. J. 2002. Assessment of the relationships between
815 dominant cell size in natural phytoplankton communities and the spectral shape of the
816 absorption coefficient. *Limnol. Oceanogr*. 47: 404-417.

817 Falkowski, P. G. 1984. Physiological responses of phytoplankton to natural light regimes. *J.*
818 *Plankton Res*. 6: 295-307.

819 Finazzi, G., Johnson, G. N., Dall'osto, L., Zito, F., Bonente, G., Bassi, R. & Wollman, F-A.
820 2006. Nonphotochemical quenching of chlorophyll fluorescence in *Chlamydomonas*
821 *reinhardtii*. *Biochemistry* 45: 1490-1498.

822 Finkel, Z. V., Beardall, J., Flynn, K. J., Quigg, A., Rees, T. A. V. & Raven, J. A. 2009.
823 Phytoplankton in a changing world: cell size and elemental stoichiometry. *J. Plankton Res*.
824 32: 119-137.

825 Finkel, Z. V., Follows, M. J., Liefer, J. D., Brown, C. M., Benner, I. & Irwin, A. J. 2016.
826 Phylogenetic diversity in the macromolecular composition of microalgae. *PloS ONE* 11:
827 e0155977.

828 Fujiki, T., Suzue, T., Kimoto, H. & Saino, T. 2007. Photosynthetic electron transport in
829 *Dunaliella tertiolecta* (Chlorophyceae) measured by fast repetition rate fluorometry: relation
830 to carbon assimilation. *J. Plankton Res*. 29**:** 199-208.

831 Fukuzaki, K., Imai, I., Fukushima, K., Ishii, K-I., Sawayama, S. & Yoshioka, T. 2014.
832 Fluorescent characteristics of dissolved organic matter produced by bloom-forming coastal
833 phytoplankton. *J. Plankton Res*. 36: 685-694.

834 Geider, R. J., Platt, T. & Raven, J. A. 1986. Size dependence of growth and photosynthesis in
835 diatoms: a synthesis. *Mar. Ecol.: Prog. Ser*. 30: 93-104.

836 Guillard, R. R. & Ryther, J. H. 1962. Studies of marine planktonic diatoms: I. *Cyclotella*
837 *nana* Hustedt, and *Detonula confervacea* (cleve) Gran. *Can. J. Microbiol*. 8: 229-239.

838 Halsey, K. H. & Jones, B. M. 2015. Phytoplankton strategies for photosynthetic energy
839 allocation. *Annu. Rev. Mar. Sci*. 7: 265-297.

840 Halsey, K. H., Milligan, A. J. & Behrenfeld, M. J. 2010. Physiological optimization underlies
841 growth rate-independent chlorophyll-specific gross and net primary production. *Photosynth.*
842 *Res*. 103: 125-137.

843 Halsey, K. H., Milligan, A. J. & Behrenfeld, M. J. 2011. Linking time-dependent
844 carbon-fixation efficiencies in *Dunaliella tertiolecta* (chlorophyceae) to underlying metabolic
845 pathways. *J. Phycol*. 47: 66-76.

846 Halsey, K. H., O'malley, R. T., Graff, J. R., Milligan, A. J. & Behrenfeld, M. J. 2013. A
847 common partitioning strategy for photosynthetic products in evolutionarily distinct
848 phytoplankton species. *New Phytol*. 198: 1030-1038.

849 Hancke, K., Dalsgaard, T., Sejr, M. K., Markager, S. & Glud, R. N. 2015. Phytoplankton
850 productivity in an Arctic Fjord (West Greenland): estimating electron requirements for
851 carbon fixation and oxygen production. *PloS ONE* 10: e0133275.

852 Hoppe, C. J. M., Holtz, L-M., Trimborn, S. & Rost, B. 2015. Ocean acidification decreases
853 the light-use efficiency in an Antarctic diatom under dynamic but not constant light. *New*
854 *Phytol*. 207: 159-171.

855 Hughes, D. J., Campbell, D. A., Doblin, M. A., Kromkamp, J. C., Lawrenz, E., Moore, C. M.
856 & Suggett, D. J. 2018a. Roadmaps and detours: Active chlorophyll-a assessments of primary
857 productivity across marine and freshwater systems. *Environ. Sci. Technol*. 52: 12039-12054.

858 Hughes, D. J., Crosswell, J. R., Doblin, M. A., Oxborough, K., Ralph, P. J., Varkey, D. &
859 Suggett, D. J. 2020. Dynamic variability of the electron requirement for carbon fixation in
860 eastern Australian waters. *J. Mar. Syst*. 202: 103252.

861 Hughes, D. J., Varkey, D., Doblin, M. A., Ingleton, T., McInnes, A., Ralph, P. J., van
862 Dongen-Vogels, V. & Suggett, D. J. 2018b. Impact of nitrogen availability upon the electron
863 requirement for carbon fixation in Australian coastal phytoplankton communities. *Limnol.*
864 *Oceanogr*. 63: 1891-1910.

865 Huot, Y. & Babin, M. 2010. Overview of fluorescence protocols: theory, basic concepts, and
866 practice. In D. Suggett, O. Prášil & M. Borowitzka [Eds.], *Chlorophyll a fluorescence in*
867 *aquatic sciences: Methods and applications*. Springer, Netherlands, pp. 31-74.

868 Jacox, M. G., Edwards, C. A., Kahru, M., Rudnick, D. L. & Kudela, R. M. 2015. The
869 potential for improving remote primary productivity estimates through subsurface
870 chlorophyll and irradiance measurement. *Deep Sea Res., Part II* 112: 107-116.

871 Jakob, T., Wagner, H., Stehfest, K. & Wilhelm, C. 2007. A complete energy balance from
872 photons to new biomass reveals a light-and nutrient-dependent variability in the metabolic
873 costs of carbon assimilation. *J. Exp. Bot*. 58: 2101-2112.

874 Kaňa, R., Kotabová, E., Sobotka, R. & Prášil, O. 2012. Non-photochemical quenching in
875 cryptophyte alga *Rhodomonas salina* is located in Chlorophyll a/c antennae. *PLoS ONE* 7:
876 e29700.

877 Key, T., McCarthy, A., Campbell, D. A., Six, C., Roy, S. & Finkel, Z. V. 2010. Cell size
878 trade-offs govern light exploitation strategies in marine phytoplankton. *Environ. Microbiol*.
879 12: 95-104.

880 Kirilovsky, D. & Wilson, A. 2007. A new photoactive protein acting as a sensor of light
881 intensity: the Orange Carotenoid Protein (OCP). *Photosynth. Res*. 91: 291-292.

882 Kirk, J. T. O. 1975. A theoretical analysis of the contribution of algal cells to the attenuation
883 of light within natural waters I. General treatment of suspensions of pigmented cells. *New*
884 *Phytol*. 75: 11-20.

885 Knap, A., Michaels, A., Close, A., Ducklow, H. & Dickson, A. 1996. Protocols for the Joint
886 Global Ocean Flux Study (JGOFS) core measurements. *Intergovernmental Oceanographic*
887 *Commission Manuals and Guides: 29, JGOFS Report; 19*. UNESCO-IOC, Paris, France, 170
888 pp.

889 Kolber, Z. & Falkowski, P. G. 1993. Use of active fluorescence to estimate phytoplankton
890 photosynthesis in situ. *Limnol. Oceanogr.* 38: 1646-1665.

891 Kolber, Z. S., Prášil, O. & Falkowski, P. G. 1998. Measurements of variable chlorophyll
892 fluorescence using fast repetition rate techniques: defining methodology and experimental
893 protocols. *Biochim. Biophys. Acta, Bioenerg.* 1367: 88-106.

894 Kromkamp, J. C. & Forster, R. M. 2003. The use of variable fluorescence measurements in
895 aquatic ecosystems: differences between multiple and single turnover measuring protocols
896 and suggested terminology. *Eur. J. Phycol.* 38: 103-112.

897 Langner, U., Jakob, T., Stehfest, K. & Wilhelm, C. 2009. An energy balance from absorbed
898 photons to new biomass for *Chlamydomonas reinhardtii* and *Chlamydomonas acidophila*
899 under neutral and extremely acidic growth conditions. *Plant, Cell Environ.* 32: 250-258.

900 Lavaud, J. & Kroth, P. G. 2006. In diatoms, the transthylakoid proton gradient regulates the
901 photoprotective non-photochemical fluorescence quenching beyond its control on the
902 xanthophyll cycle. *Plant Cell Physiol.* 47: 1010-1016.

903 Lawrenz, E., Silsbe, G., Capuzzo, E., Ylöstalo, P., Forster, R. M., Simis, S. G. H., Prášil, O.,
904 Kromkamp, J. C., Hickman, A. E., Moore, C. M., Forget, M-H., Geider, R. J. & Suggett, D.
905 J. 2013. Predicting the electron requirement for carbon fixation in seas and oceans. *PLoS*
906 *ONE* 8: e58137.

907 Lewis, M. R. & Smith, J. C. 1983. A small volume, short-incubation-time method for
908 measurement of photosynthesis as a function of incident irradiance. *Mar. Ecol.: Prog. Ser.*
909 13: 99-102.

910 Longhurst, A., Sathyendranath, S., Platt, T. & Caverhill, C. 1995. An estimate of global
911 primary production in the ocean from satellite radiometer data. *J. Plankton Res.* 17: 1245-
912 1271.

913 Litchman, E., Klausmeier, C. A., Schofield, O. M. & Falkowski, P. G. 2007. The role of
914 functional traits and trade-offs in structuring phytoplankton communities: scaling from
915 cellular to ecosystem level. *Ecol. Lett.* 10: 1170-1181.

916 Lutz, V. A., Sathyendaranath, S., Head, E. J., & Li, W. K. 2001. Changes in the in vivo
917 absorption and fluorescence excitation spectra with growth irradiance in three species of
918 phytoplankton. *J. Plankton Res.* 23: 555-569.

919 Malerba, M. E., Palacios, M. M., Palacios Delgado, Y. M., Beardall, J. & Marshall, D. J.
920 2018. Cell size, photosynthesis and the package effect: an artificial selection approach. *New*
921 *Phytol.* 219: 449-461.

922 Marra, J. 2009. Net and gross productivity: weighing in with $^{14}C$. *Aquat. Microb. Ecol.* 56:
923 123-131.

924 Marra, J. 2012. Comment on "Measuring primary production rates in the ocean: Enigmatic
925 results between incubation and non-incubation methods at Station ALOHA" by PD Quay et
926 al. *Global Biogeochem. Cycles* 26: GB2031.

927 Marra, J., Trees, C. C. & O'reilly, J. E. 2007. Phytoplankton pigment absorption: a strong
928 predictor of primary productivity in the surface ocean. *Deep Sea Res., Part I* 54: 155-163.

929 Mcdonald, A. E., Ivanov, A. G., Bode, R., Maxwell, D. P., Rodermel, S. R. & Hüner, N. P.
930 2011. Flexibility in photosynthetic electron transport: the physiological role of plastoquinol
931 terminal oxidase (PTOX). *Biochim. Biophys. Acta, Bioenerg*. 1807: 954-967.

932 Mckew, B. A., Davey, P., Finch, S. J., Hopkins, J., Lefebvre, S.C., Metodiev, M. V.,
933 Oxborough, K., Raines, C. A., Lawson, T. & Geider, G. J. 2013. The trade-off between the
934 light-harvesting and photoprotective functions of fucoxanthin-chlorophyll proteins dominates
935 light acclimation in *Emiliania huxleyi* (clone CCMP 1516). *New Phytol*. 200: 74-85.

936 Melrose, D. C., Oviatt, C. A., O'reilly, J. E. & Berman, M. S. 2006. Comparisons of fast
937 repetition rate fluorescence estimated primary production and $^{14}C$ uptake by phytoplankton.
938 *Mar. Ecol.: Prog. Ser*. 311: 37-46.

939 Milligan, A. J., Halsey, K. H. & Behrenfeld, M. J. 2015. Advancing interpretations of 14C-
940 uptake measurements in the context of phytoplankton physiology and ecology. *J. Plankton*
941 *Res*. 37: 692-698.

942 Moore, C. M., Suggett, D. J., Hickman, A. E., Kim, Y-N , Tweddle, J. F., Sharples, J.,
943 Geider, R. J. & Holligan, P. M. 2006. Phytoplankton photoacclimation and photoadaptation
944 in response to environmental gradients in a shelf sea. *Limnol. Oceanogr*. 51: 936-949.

945 Napoleon, C., Raimbault, V. & Claquin, P. 2013. Influence of nutrient stress on the
946 relationships between PAM measurements and carbon incorporation in four phytoplankton
947 species. *PLoS ONE* 8: e66423.

948 Nawrocki, W. J., Tourasse, N. J., Taly, A., Rappaport, F. & Wollman, F. A. 2015. The plastid
949 terminal oxidase: its elusive function points to multiple contributions to plastid physiology.
950 *Annu. Rev. Plant Biol*. 66: 49-74.

951 Oxborough, K., Moore, C. M., Suggett, D. J., Lawson, T., Chan, H. G. & Geider, R. J. 2012.
952 Direct estimation of functional PSII reaction center concentration and PSII electron flux on a
953 volume basis: a new approach to the analysis of Fast Repetition Rate fluorometry (FRRf)
954 data. *Limnol. Oceanogr.: Methods* 10: 142-154.

955 Pakrasi, H. B., Riethman, H. C. & Sherman, L. A. 1985. Organization of pigment proteins in
956 the photosystem II complex of the cyanobacterium *Anacystis nidulans* R2. *Proc. Natl. Acad.*
957 *Sci. U. S. A*. 82: 6903-6907.

958 Pittera, J., Humily, F., Thorel, M., Grulois, D., Garczarek, L. & Six, C. 2014. Connecting
959 thermal physiology and latitudinal niche partitioning in marine *Synechococcus*. *ISME J*. 8:
960 1221-1236.

961 Platt, T., Gallegos, C. & Harrison, W. 1980. Photoinhibition of photosynthesis in natural
962 assemblages of marine phytoplankton. *J. Mar. Res*. 38: 103-111.

963 Quay, P., Peacock, C., Björkman, K. & Karl, D. 2010. Measuring primary production rates in
964 the ocean: Enigmatic results between incubation and non-incubation methods at Station
965 ALOHA. *Global Biogeochem. Cycles* 24: GB3014.

966 Quigg, A. & Beardall, J. 2003. Protein turnover in relation to maintenance metabolism at low
967 photon flux in two marine microalgae. *Plant, Cell Environ*. 26: 693-703.

968 Rattan, K. J., Taylor, W. D. & Smith, R. E. 2012. Nutrient status of phytoplankton across a
969 trophic gradient in Lake Erie: evidence from new fluorescence methods. *Can. J. Fish. Aquat.*
970 *Sci*. 69: 94-111.

971 Regaudie-De-Gioux, A., Lasternas, S., Agustí, S. & Duarte, C. M. 2014. Comparing marine
972 primary production estimates through different methods and development of conversion
973 equations. *Front. Mar. Sci*. 1: 19.

974 Roberty, S., Bailleul, B., Berne, N., Franck, F. & Cardol, P. 2014. PSI Mehler reaction is the
975 main alternative photosynthetic electron pathway in *Symbiodinium* sp., symbiotic
976 dinoflagellates of cnidarians. *New Phytol.* 204: 81-91.

977 Robinson, C., Suggett, D. J., Cherukuru, N., Ralph, P. J. & Doblin, M. A. 2014. Performance
978 of Fast Repetition Rate fluorometry based estimates of primary productivity in coastal waters.
979 *J. Mar. Syst.* 139: 299-310.

980 Roesler, C. S. 1998. Theoretical and experimental approaches to improve the accuracy of
981 particulate absorption coefficients derived from the quantitative filter technique. *Limnol.*
982 *Oceanogr.* 43: 1649-1660.

983 Ros, M., Camp, E.F., Hughes, D.J., Crosswell, J.R., Warner, M.E., Leggat, W.P. and Suggett,
984 D.J. 2020. Unlocking the black-box of inorganic carbon-uptake and utilization strategies
985 among coral endosymbionts (Symbiodiniaceae). *Limnol. Oceanogr*. doi: 10.1002/lno.11416

986 Saba, V. S., Friedrichs, M. A., Antoine, D., Armstrong, R. A., Asanuma, I., Behrenfeld, M.
987 J., Ciotti, A. M., Dowell, M., Hoepffner, N., Hyde, K. J. W. & Ishizaka, J. 2011. An
988 evaluation of ocean color model estimates of marine primary productivity in coastal and
989 pelagic regions across the globe. *Biogeosciences* 8: 489-503.

990 Schuback, N., Flecken, M., Maldonado, M. T. & Tortell, P. D. 2016. Diurnal variation in the
991 coupling of photosynthetic electron transport and carbon fixation in iron-limited
992 phytoplankton in the NE subarctic Pacific. *Biogeosciences* 13: 1019-1035.

993 Schuback, N., Hoppe, C. J.¸ Tremblay, J. É., Maldonado, M. T. & Tortell, P. D. 2017.
994 Primary productivity and the coupling of photosynthetic electron transport and carbon
995 fixation in the Arctic Ocean. *Limnol. Oceanogr.* 62: 898-921.

996 Schuback, N., Schallenberg, C., Duckham, C., Maldonado, M. T. & Tortell, P. D. 2015.
997 Interacting effects of light and iron availability on the coupling of photosynthetic electron
998 transport and $CO_2$-assimilation in marine phytoplankton. *PLoS ONE* 10: e0133235.

999 Schuback, N. & Tortell, P. D. 2019. Diurnal regulation of photosynthetic light absorption,
1000 electron transport and carbon fixation in two contrasting oceanic environments.
1001 *Biogeosciences* 16: 1381-1399.

1002 Silsbe, G. M., Oxborough, K., Suggett, D. J., Forster, R. M., Ihnken, S., Komárek, O.,
1003 Lawrenz, E., Prášil, O., Röttgers, R., Šicner, M. & Simis, S. G. 2015. Toward autonomous
1004 measurements of photosynthetic electron transport rates: An evaluation of active

1005 fluorescence-based measurements of photochemistry. *Limnol. Oceanogr.: Methods* 13: 138-

1006 155.

1007 Steeman-Nielsen, E. 1952. The use of radioactive carbon ($C^{14}$) for measuring organic

1008 production in the sea. *ICES J. Mar. Sci.* 18: 117-140.

1009 Suggett, D. J., Goyen, S., Evenhuis, C., Szabó, M., Pettay, D. T., Warner, M. E. & Ralph, P.

1010 J. 2015. Functional diversity of photobiological traits within the genus *Symbiodinium* appears

1011 to be governed by the interaction of cell size with cladal designation. *New Phytol.* 208: 370-

1012 381.

1013 Suggett, D. J., Maberly, S. C. & Geider, R. J. 2006. Gross photosynthesis and lake

1014 community metabolism during the spring phytoplankton bloom. *Limnol. Oceanogr.* 51: 2064-

1015 2076.

1016 Suggett, D. J., Macintyre, H. L., Kana, T. M. & Geider, R. J. 2009a. Comparing electron

1017 transport with gas exchange: parameterising exchange rates between alternative

1018 photosynthetic currencies for eukaryotic phytoplankton. *Aquat. Microb. Ecol.* 56: 147-162.

1019 Suggett, D. J., Moore, C. M., Hickman, A. E. & Geider, R. J. 2009b. Interpretation of fast

1020 repetition rate (FRR) fluorescence: signatures of phytoplankton community structure versus

1021 physiological state. *Mar. Ecol.: Prog. Ser.* 376: 1-19.

1022 Sun, J. & Liu, D. 2003. Geometric models for calculating cell biovolume and surface area for

1023 phytoplankton. *J. Plankton Res.* 25: 1331-1346.

1024 Toepel, J., Langner, U. & Wilhelm, C. 2005. Combination of flow cytometry and single cell

1025 absorption spectroscopy to study the phytoplankton structure and to calculate the Chl A

1026 specific absorption coefficients at the taxon level. *J. Phycol.* 41: 1099-1109.

1027 Varkey, D., Mazard, S., Ostrowski, M., Tetu, S. G., Haynes, P. & Paulsen, I. T. 2016. Effects
1028 of low temperature on tropical and temperate isolates of marine *Synechococcus*. *ISME J.* 10:
1029 1252-1263.

1030 Verma, A., Hoppenrath, M., Harwood, T., Brett, S., Rhodes, L. & Murray, S. 2016.
1031 Molecular phylogeny, morphology and toxigenicity of *Ostreopsis* cf. *siamensis*
1032 (Dinophyceae) from temperate south-east Australia. *Phycol. Res.* 64: 146–159.

1033 Verma, A., Hughes, D. J., Harwood, D. T., Suggett, D. J., Ralph, P. J. & Murray, S. A. 2020.
1034 Functional significance of phylogeographic structure in a toxic benthic marine microbial
1035 eukaryote over a latitudinal gradient along the East Australian Current. *Ecol. Evol.* DOI:
1036 10.1002/ece3.6358

1037 Wagner, H., Jakob, T. & Wilhelm, C. 2006. Balancing the energy flow from captured light to
1038 biomass under fluctuating light conditions. *New Phytol.* 169: 95-108.

1039 Wei, Y., Zhao, X. & Sun, J. 2019. Fast repetition rate fluorometry (FRRF) derived
1040 phytoplankton primary productivity in the Bay of Bengal. *Front. Microbiol.* 10: 1164.

1041 Wood, A. M., Everroad, R. & Wingard, L. 2005. Measuring growth rates in microalgal
1042 cultures. In R. A. Andersen [Ed.] *Algal Culturing Techniques: A Book for All Phycologists*.
1043 Elsevier, pp. 269-285.

1044 Wu, Y., Jeans, J., Suggett, D. J., Finkel, Z. V. & Campbell, D. A. 2014. Large centric diatoms
1045 allocate more cellular nitrogen to photosynthesis to counter slower RUBISCO turnover rates.
1046 *Front. Mar. Sci.* 1: 68.

1047 Zhu, Y., Ishizaka, J., Tripathy, S. C., Wang, S., Mino, Y., Matsuno, T. & Suggett, D. J. 2016.
1048 Variation of the photosynthetic electron transfer rate and electron requirement for daily net
1049 carbon fixation in Ariake Bay, Japan. *J. Oceanogr.* 72: 761-776.

1050 Zhu, Y., Suggett, D., Liu, C., He, J., Lin, L., Le, F., Ishizaka, J., Goes, J. & Hao, Q. 2019.
1051 Primary productivity dynamics in the summer Arctic Ocean confirms broad regulation of the
1052 electron requirement for carbon fixation by light-phytoplankton community interaction.
1053 *Front. Mar. Sci.* 6: 275.

**Table 1** Summary of phytoplankton strains used in this study, indicating strain IDs and synonyms (where applicable) taxonomic class and cell volume ($\mu m^3$), together with growth conditions of temperature (°C) and photosynthetically active radiation, PAR ($\mu$mol photons $m^{-2}$ $s^{-1}$). The grey shaded area represents strains sampled opportunistically in addition to the main study (note the different growth conditions).

| Class | Species | Temperature (°C) | PAR (growth) | Media |
|---|---|---|---|---|
| Bacillariophyceae | *Ditylum brightwellii* CS-131 | 20 | 60 | f/2 |
| | *Nitzschia closterium* CS-5 | 20 | 60 | f/2 |
| | *Thalassiosira pseudonana* CS-173; CCMP1335; 3H | 20 | 60 | f/2 |
| | *Thalassiosira weissflogii* CS-871; CCMP1336 | 20 | 60 | f/2 |
| Chlorophyceae | *Dunaliella tertiolecta* CCMP1320, NEPCC1 | 20 | 60 | f/2 |
| | *Tetraselmis* sp. CS-91 | 20 | 60 | f/2 |
| | *Tetraselmis* sp. CS-352 | 20 | 60 | f/2 |
| Cyanophyceae | *Synechococcus* sp. CS-94, RRIMP N1 (S1) | 20 | 60 | f/2 |
| Eustigmatophyceae | *Nannochloropsis oculata* CS-179 | 20 | 60 | f/2 |
| Prymnesiophyceae | *Emiliania huxleyi* CS-370 | 20 | 60 | f/2 |
| | *Phaeocystis pouchetti* CS-165 | 20 | 60 | f/2 |
| Cryptophyceae | *Rhodomonas salina* CS-692; CRJB02 | 20 | 60 | f/2 |
| Dinophyceae | *Ostreopsis siamensis* F3; FR3 | 20 | 60 | f/10 |
| | *Ostreopsis siamensis* MW3 | 20 | 60 | f/10 |
| | *Durusdinium trenchii* SCF082 (D1a) | 26 | 180 | IMK |
| | *Claodocopium goreaui* SCF058-04 (C123) | 26 | 180 | IMK |
| | *Claodocopium goreaui* SCF055-06 (C124) | 26 | 180 | IMK |

**Table 2** Mean (± SE) values of growth rate (μ, $d^{-1}$), particulate organic carbon to nitrogen ratio (POC:PON, dimensionless), maximum PSII photochemical efficiency ($F_v/F_m$, unitless), PSII functional absorption cross-section ($\sigma_{PSII}$, $nm^2$ $PSII^{-1}$), turnover time of PSII ($\tau_{PSII}$, ms), light saturation parameter ($E_k$, μmol photons $m^{-2}$ $s^{-1}$), incubation irradiance relative to $E_k$ ($E/E_k$, dimensionless), photosynthetic unit size (PSU size, mol Chl-*a* [mol RCII]$^{-1}$) and spectral correction factors (SCF). Grey area shows strains sampled opportunistically, i.e. where certain measurements were not performed (-).

| Species | μ | POC:PON | Cell Volume | $F_v/F_m$ | $\sigma_{PSII}$ | $\tau_{PSII}$ | $E_k$ | $E/E_k$ | PSU size | SCF |
|---|---|---|---|---|---|---|---|---|---|---|
| *D. tertiolecta* | 0.35 (0.01) | 6.43 | 293 (21) | 0.51 | 2.98 | 611.0 | 151.8 (7.1) | 0.27 | 565.6 (143.9) | 0.38 |
| *Tetraselmis* sp. (CS-91) | 0.57 (0.01) | 6.46 | 301 (17) | 0.56 | 2.99 | 696.3 | 157.5 (2.7) | 0.28 | 306.5 (7.5) | 0.49 |
| *Tetraselmis* sp. (CS-352) | 0.24 (0.01) | 4.97 | 1570 (45) | 0.60 | 1.82 | 701.6 | 354.8 (64.4) | 0.11 | 339.3 (23.6) | 0.40 |
| *N. oculata* | 0.28 (0.02) | 8.58 | 14 (1) | 0.53 | 3.52 | 689.6 | 144.1 (5.9) | 0.30 | 543.1 (58.6) | 0.45 |
| *T. weissflogii* | 0.76 (0.02) | 5.05 | 1718 (28) | 0.58 | 3.44 | 585.4 | 205.6 (13.2) | 0.27 | 642.3 (25.6) | 0.52 |
| *T. pseudonana* | 0.74 (0.01) | 5.18 | 132 (9) | 0.54 | 4.05 | 551.8 | 140.9 (12.5) | 0.31 | 447.1 (26.7) | 0.46 |
| *N. closterium* | 0.84 (0.05) | 5.77 | 206 (15) | 0.53 | 3.63 | 562.3 | 137.8 (9.53) | 0.32 | 501.9 (92.2) | 0.49 |
| *D. brightwellii* | 0.77 (0.03) | 6.62 | 54521 (225) | 0.49 | 3.71 | 571.8 | 159.9 (14.35) | 0.30 | 472.6 (79.3) | 0.56 |
| *Synechococcus* (CS-94) | 0.82 (0.05) | Lost | 1.35 | 0.49 | 1.79 | 619.1 | 132.8 (17.2) | 0.37 | 254.8 (11.0) | 0.63 |
| *E. huxleyi* | 0.71 (0.02) | 9.59 | 102.4 (12) | 0.47 | 6.86 | 521.9 | 58.8 (4.4) | 0.89 | 636.9 (30.1) | 0.41 |
| *P. pouchetti* | 0.51 (0.02) | 6.67 | 596.9 (41) | 0.46 | 5.06 | 594.9 | 65.03 (2.1) | 0.84 | 507.0 (9.1) | 0.44 |
| *R. salina* | 0.39 (0.01) | 5.11 | 168.5 (9) | 0.50 | 2.62 | 745.75 | 52.2 (7.8) | 1.11 | 457.6 (32.9) | 0.69 |
| *O. siamensis* (MW3) | - | - | 31556 | 0.44 | 3.73 | 589.6 | 131.9 (6.05) | 0.33 | 842.5 (60.1) | 0.44 |
| *O. siamensis* (F3) | - | - | 30752 | 0.46 | 3.54 | 611.3 | 124.78 (2.3) | 0.35 | 552.5 (6.6) | 0.44 |
| *D. trenchii* (D1a) | - | - | 1124 | 0.40 | 4.28 | 597.1 | 195.2 (9.9) | 0.92 | 350.5 (24.3) | 0.42 |
| *C. goreaui* (C123) | - | - | 1586 | 0.43 | 4.59 | 582.0 | 212.5 (8.6) | 0.85 | 267.7 (3.2) | 0.42 |
| *C. goreaui* (C124) | - | - | 1123 | 0.41 | 4.53 | 570.4 | 222.0 (6.5) | 0.81 | 338.7 (11.8) | 0.42 |

*Figure Legends*

**Figure 1.** Measured values of the electron requirement for carbon fixation (mol e- [mol C]-1), $\Phi_{e,C}$ for the 17 phytoplankton strains (14 species) examined in this study. Values were determined using Fast Repetition Rate fluorometry (FRRf) and small volume $^{14}C$ incubations using a "dual incubation" approach (see Supplementary Fig. S1). Vertical labels indicate phytoplankton taxonomic class. Error bars indicate standard errors and letters indicate means that are statistically indistinguishable ($\alpha = 0.05$) (ANOVA).

**Figure 2**. Boxplot of the electron requirement for carbon fixation, $\Phi_{e,C}$ (mol e- [mol C]-1) for the 17 phytoplankton strains examined in this study, grouped by a) taxonomic class – abbreviated by the first 3 letters of the class name: Chlorophyceae, Eustigmatophyceae, Bacillariophyceae, Dinophyceae, Cyanophyceae, Prymnesiophyceae and Cryptophyceae, and b) arbitrary bins of cell volume ($\mu m^3$). The length of the box corresponds to the inter-quartile range, solid line denotes the mean, whiskers represent the minimum and maximum values within 1.5 times the inter-quartile range and open circles denote outliers (not excluded from statistical analyses). The letters *n* denotes the number of strains within each sample group. Although a Kruskal-Wallis failed to find significant differences in $\Phi_{e,C}$ across taxonomic classes, a comparison of diatoms and chlorophytes in isolation (representing a subset of the data excluding dinoflagellates due to different growth conditions and other sample groups with a sample size, $n <= 2$) detected a difference in $\Phi_{e,C}$ (t-test, $p < 0.05$, denoted by asterix).

**Figure 3.** Relationship between the electron requirement for carbon fixation, $\Phi_{e,C}$ (mol e- [mol C]-1), and corresponding measures of a) phytoplankton growth rate ($d^{-1}$), b) log-normalised cell volume (V[log]) and c) non-photochemical quenching, estimated as the normalised Stern-Volmer coefficient (McKew et al. 2013), denoted here as $NPQ_{NSV}$

(dimensionless) for all strains grown in the main study (i.e. excluding strains sampled "opportunistically" where growth data was unavailable. The relationship between growth rate and $\Phi_{e,C}$ is described by $\Phi_{e,C} = -3.73* \mu + 7.31$. Symbols represent the different phytoplankton classes (see inset key). The cryptophyte, *R. salina* (denoted by square symbol) has been excluded from the regression in panel a – if included the relationship weakens ($R^2 = 0.49$, $p <$ 0.05).

**Figure 4**. Distance-based redundancy (dbRDA) plot illustrating the DistLM model with growth rate as the response variable, showing selected predictive variables of cell volume, POC:PON ratio and the electron requirement for carbon fixation ($\Phi_{e,C}$) for 11 strains of phytoplankton grown in the main study (note: *R. salina* is excluded from this analysis, see results section, together with strains sampled opportunistically for which POC:PON and growth rates were not measured – see materials and methods section). Symbols represent the different phytoplankton classes (see inset key).

**Figure 5**. Boxplot of the electron requirement for carbon fixation (mol $e^-$ [mol C]$^{-1}$), $\Phi_{e,C}$ including data from meta-analysis together with all strains measured during this study, ($n$ = 25), grouped by a) taxonomic class – abbreviated by the first three letters of the class name as outlined in Fig. 3 with an additional class, Pelagophyceae, and b) arbitrary size-class bins, based upon cell volume (μm3). The length of the box corresponds to the inter-quartile range, solid line denotes the mean, whiskers represent the minimum and maximum values within 1.5 times the inter-quartile range and open circles denote outliers (not excluded from statistical analyses). The letters *n* denotes the number of strains within each sample group.

**Figure 6**. Relationship between the electron requirement for carbon fixation, $\Phi_{e,C}$ (mol $e^-$ [mol C]$^{-1}$), and corresponding measures of a) phytoplankton growth rate (d-1), b) log-normalised cell volume (V[log]) and c) non-photochemical quenching, estimated as the normalised Stern-Volmer coefficient (McKew et al. 2013), denoted here as $NPQ_{NSV}$ (dimensionless) for all strains grown in the main study (i.e. excluding strains sampled "opportunistically" where growth data was unavailable, together with meta-analysis data. Symbols represent the different phytoplankton classes (see figure key).

*Supplementary Figure Legends*

**Supplementary Figure S1.** Schematic of a "dual" incubation, used to measure $ETR_{PSII}$ and $^{14}C$- incorporation for a sample simultaneously in order to derive the electron requirement for carbon fixation, $\Phi_{e,C}$ (mol $e^-$ [mol C]$^{-1}$). Three mL of phytoplankton culture or seawater sample is radio-labelled inside the FRRf-specific test tube, which is then loaded into the FRRf optical head where the cool-white LED (see actinic LED spectra) provides a pre-determined irradiance level. $ETR_{PSII}$ is continuously measured via the 450 nm blue excitation LED (spectral output shown). Upon completion of the incubation, the sample is removed and processed to measure $^{14}C$-incorporation – i.e. acidified and vented to remove unincorporated 14C, then counted using a liquid scintillation counter. Sample temperature is maintained via a water jacket inside the optical head which is plumbed to a heater-chiller (in this example set to 20 ºC, but during experimentation was set to match that of the strain's growth temperature).

**Supplementary Figure S2.** Principle component analysis (PCA) of metadata, showing the variability in $\Phi_{e,C}$ explained by experimental growth conditions. The colour of each point represents $\Phi_{e,C}$ value as a scale from lowest (yellow) to highest (red). The shape of the symbol depicts the approach used to quantify/estimate PSII reaction centre content [RCII], triangle = direct measurement via $O_2$ flash-yield, circle = fluorometric estimate of RCII ($[RCII]_{(FRRf)}$) according to

Oxborough et al. (2012), square = assumed constant value ($n_{PSII}$). Groupings of high $\Phi_{e,C}$ values appears to correspond to $O_2$ flash-yield assessment, with low values predominately associated with $n_{PSII}$ and $[RCII]_{FRRf}$.

**Supplementary Figure S3.** Fast Repetition Rate fluorometry (FRRf) measurements of PSII maximum photochemical efficiency ($F_v/F_m$, dimensionless) and the functional absorption cross-section of PSII ($\sigma_{PSII}$, $nm^2$ $PSII^{-1}$) using the blue excitation LED (450 nm) for all strains examined during this study with the exception of *Synechococcus* (excluded due to having a unique LED protocol [blue + orange LEDs]). The equation for the generated regression line was y=-11.11x + 9.33 with an $R^2$ of 0.35. Values shown represent means from triplicate measurements prior to measurement of the electron requirement for carbon fixation ($\Phi_{e,C}$).