Temporal progression of photosynthetic strategy by phytoplankton in the Ross Sea, Antarctica

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25 Abstract

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27 The bioavailability of iron influences the distribution, biomass and productivity of 28 phytoplankton in the Ross Sea, one of the most productive regions in the Southern 29 Ocean. We mapped the spatial and temporal extent and severity of iron limitation of the native phytoplankton assemblage using long- (>48 h) and short-term (24 h) iron-30 31 addition experiments, along with physiological and molecular characterizations during 32 a cruise to the Ross Sea in December-February 2011-2012. Phytoplankton increased 33 their photosynthetic efficiency in response to iron addition, suggesting proximal iron 34 limitation throughout most of the Ross Sea during summer. Molecular and 35 physiological data further indicate that as nitrate is removed from the surface ocean there is a progression of the photosynthetic strategy of the extant phytoplankton, with 36 37 an increase in the size of the photochemical cross section of photosystem II (PSII) 38 (σ_{PSII}) and a decrease in the chlorophyll-normalized PSII abundance. These results suggest that phytoplankton with the ability to reduce their photosynthetic iron 39 40 requirements are selected as the growing season progresses, which may be a factor in the well-documented progression from Phaeocystis antarctica- to diatom-dominated 41 42 phytoplankton assemblages. Such a shift in the assemblage-level photosynthetic strategy potentially mediates further drawdown of nitrate following the development of 43 44 iron-deficient conditions in the Ross Sea.

46 Keywords

47 Iron, Phytoplankton, Photosynthetic proteins, Photosystem, Nutrient limitation,48 Ross Sea

50 1. Introduction

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The Ross Sea continental shelf is the most productive region in the Southern Ocean (Arrigo and van Dijken, 2004; Peloquin and Smith, 2007), with an annual productivity >200 g C m⁻² (Smith et al., 2006), which may account for as much as 27% of the estimated total Southern Ocean biological CO₂ uptake (Arrigo et al., 2008). An understanding of the controls on primary productivity is therefore needed given the potential for future changes in stratification (Boyd et al., 2008; Smith et al., 2014) and nutrient inputs to this region (Mahowald and Luo, 2003; Tagliabue et al., 2008).

59 A persistent polynya in the southern Ross Sea greatly increases in size in the 60 early austral spring (Arrigo and van Dijken, 2003; Reddy et al., 2007), and hosts large seasonal phytoplankton blooms, typically dominated by colonial haptophyte 61 62 *Phaeocystis antarctica* in spring through early summer (November – December), with an increase in abundance of diatoms in mid- to late summer (Arrigo and van Dijken, 63 2004; Arrigo et al., 1998; DiTullio and Smith, 1996; Goffart et al., 2000; Smith and 64 65 Gordon, 1997; Smith et al., 2003). Understanding the causes and consequences of this seasonal phytoplankton progression is important, as the spatial and temporal 66 distribution and abundance of P. antarctica and diatoms have significant 67 biogeochemical consequences on vertical export and elemental composition of 68 biogenic material (Arrigo et al., 1999; DeMaster et al., 1992; Smith and Dunbar, 1998; 69 Tagliabue and Arrigo, 2005). 70

71 Iron and irradiance are assumed to exert the major 'bottom-up' controls on 72 phytoplankton biogeography and productivity in the Ross Sea, given the incomplete 73 macronutrient removal at the end of the growing season (Arrigo and van Dijken, 2003; 74 Arrigo et al., 1998; Coale et al., 2003; Fitzwater et al., 2000; Sedwick et al., 2000; 75 Sedwick et al., 2007; Smith et al., 2003; Smith et al., 2000; Tagliabue and Arrigo, 76 2003). Light availability may limit spring phytoplankton growth when vertical mixing 77 is deep and daily integrated irradiance is low, this mixing will also supply dissolved 78 iron (DFe) to the euphotic zone (McGillicuddy et al., 2015). As the growing season 79 progresses and the water column stratifies, the flux of DFe from below is likely reduced 80 and may therefore become a more significant factor in limiting phytoplankton growth 81 rates. Indeed, shipboard iron-addition experiments have repeatedly demonstrated the 82 role of iron limitation in the Ross Sea (Bertrand et al., 2007; Coale et al., 2003; Cochlan et al., 2002; Martin et al., 1990; Olson et al., 2000; Sedwick and DiTullio, 1997;
Sedwick et al., 2000), consistent with other metrics of Fe stress including high levels
of flavodoxin (Maucher and DiTullio, 2003) and enhanced biological drawdown of
silicate relative to nitrate (Arrigo et al., 2000; Smith et al., 2006).

Changes in phytoplankton composition from P. antarctica to diatom species 87 88 may be linked to the co-limitation and interaction between iron and light. Boyd (2002) 89 speculated that *P. antarctica* growth is limited by Fe availability from spring through 90 late summer. Sedwick et al. (2007) proposed an alternative scenario in which decreases 91 in iron availability through spring are mitigated by increases in irradiance, thereby 92 decreasing iron requirements, thus providing a mechanism by which diatoms can 93 outcompete P. antarctica during late summer due to their lower intracellular iron 94 requirements under elevated irradiance (Strzepek et al., 2012).

95 Phytoplankton that dominate in the Ross Sea may therefore need to be well adapted to low seasonal Fe availability (Sedwick et al., 2011) and/or low irradiance. 96 97 An antagonistic relationship between irradiance and photosynthetic Fe demand may be 98 predicted given that lower irradiances can increase Fe requirements associated with the 99 synthesis of the additional photosynthetic units required to increase light absorption 100 (Maldonado et al., 1999; Raven, 1990; Sunda and Huntsman, 1997). Each 101 photosynthetic electron transfer chain requires 22-23 Fe atoms, and the photosynthetic 102 apparatus can be the largest sink of Fe within a phytoplankton cell (Raven, 1990; Shi et al., 2007; Strzepek and Harrison, 2004). In contrast to the tight link between cellular 103 104 Fe requirements and light-harvesting capacity, studies on Southern Ocean diatoms and *P. antarctica* in culture suggest the Fe burden of photosynthesis may be significantly 105 106 reduced for these species through increases in the size rather than the number of 107 photosynthetic units (termed sigma-type acclimation) in response to iron and light 108 limitation (Strzepek et al., 2011; Strzepek et al., 2012). Effectively, these Southern 109 Ocean taxa appear to invest relatively more resources in the generation of a larger light-110 harvesting apparatus, rather than in the Fe-rich photosynthetic catalysts of photosystems I and II (Strzepek et al., 2012). This Fe-efficient strategy appears to be 111 most pronounced for Southern Ocean diatoms in culture, which have some of the largest 112 113 light-harvesting antennae reported (Strzepek et al., 2012), a phenotype which is more commonly associated with small cells (Suggett et al., 2009). The photosynthetic 114 115 strategy of Southern Ocean diatoms may therefore contribute to the apparently low Fe 116 requirement and cellular Fe:C ratio of these species (Coale et al., 2003; Kustka et al.,

2015; Sedwick et al., 2007; Strzepek et al., 2011; Strzepek et al., 2012), and as such
drive the seasonal progression from *P. antarctica* to diatoms, and associated
biogeochemical impacts in the Ross Sea.

In December 2011 – February 2012 a research cruise was conducted as part of 120 121 the multidisciplinary research project Processes Regulating Iron Supply at the Mesoscale - Ross Sea (PRISM-RS), in an effort to identify and quantify the major 122 123 sources of iron to the surface waters of the Ross Sea during the growing season. As part 124 of this study, physiological and molecular measurements were combined with shipboard incubation experiments in an effort to define the spatial and temporal extent 125 of phytoplankton iron limitation of phytoplankton and reveal the photosynthetic 126 strategy of the phytoplankton assemblages. 127

128 **2. Materials and Methods**

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130 2.1. Oceanographic Sampling

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132 The samples and data presented here were obtained during a cruise of the RVIB Nathaniel B. Palmer to the Ross Sea (cruise NBP12-01) from 24th December 2011 to 133 10th February 2012 (DOY 358 – 041). During the cruise, 29 short-term (24 h) and 3 134 long-term (>48 h) incubation experiments were performed (Figure 1). For the long-135 136 term incubation experiments, uncontaminated whole seawater was collected from ~5 m 137 depth whilst slowly underway, using a trace-metal clean towed fish system (Sedwick 138 et al., 2011). Uncontaminated whole seawater for the short-term incubation 139 experiments was collected from ~10 m depth in Teflon-lined, external closure 5 L 140 Niskin-X samplers (General Oceanics) deployed on a trace-metal clean CTD rosette 141 system (Marsay et al., 2014).

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2.2. Bioassay Incubation Experiments

145 Incubation experiments were performed using methods similar to those employed previously in the Southern Ocean (Moore et al., 2007; Nielsdóttir et al., 2012) and the 146 147 high-latitude North Atlantic (HLNA) (Moore et al., 2007; Nielsdóttir et al., 2009; Ryan-148 Keogh et al., 2013). Water for the experiments (see above) was transferred unscreened 149 into acid-washed 1.0 L polycarbonate bottles (Nalgene) for the short-term incubation 150 experiments and 4.5 L polycarbonate bottles for the long-term incubation experiments. 151 Incubation bottles were filled in a random order, with triplicate samples for initial measurements in the long-term incubation experiments collected at the beginning, 152 153 middle and end of the filling process. Initial samples for the short-term incubation 154 experiments were collected from the same Niskin-X sampling bottle. The short-term 155 experiments were run for 24 h and the long-term experiments were run for 168 h; both experiments consisted of two treatments: an unamended control treatment and 2.0 nmol 156 L^{-1} Fe treatment (hereafter, +Fe). All experimental incubations were conducted as 157 158 biological duplicates or triplicates.

All bottle tops were externally sealed with film (Parafilm[™]), and bottles were
 double-bagged with clear polyethylene bags to minimize risks of contamination during

161 the incubation. On-deck incubators were shaded using LEE "blue lagoon" filters to 162 provide light levels corresponding to ~35% of above-surface irradiance. Flowing 163 surface seawater was used to control the temperature in the incubators. Subsampling of 164 long-term incubations for measurements of chlorophyll *a*, dissolved macronutrient 165 concentrations and phytoplankton physiological parameters occurred after 24, 72, 120 166 and 168 h. Subsampling of short-term incubation experiments for the same parameters 167 occurred after 24 h.

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2.3. Chlorophyll *a* and Nutrient Analysis

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171 Samples for chlorophyll a (Chl) analysis (250 mL) were filtered onto GF/F filers and then extracted into 90% acetone for 24 h in the dark at 4°C, followed by analysis with 172 a fluorometer (TD70; Turner Designs) (Welschmeyer, 1994). Macronutrient samples 173 174 were drawn into 50 mL diluvials and refrigerated at 4°C until analysis, which typically commenced within 12 h of sampling. Nitrate plus nitrite (DIN), phosphate, ammonium 175 and silicate were determined shipboard on a five-channel Lachat Instruments 176 QuikChem FIA+ 8000s series AutoAnalyser (Armstrong et al., 1967; Atlas et al., 1971; 177 Bernhardt and Wilhelms, 1967; Patton, 1983). Dissolved iron (DFe) was determined 178 post-cruise using flow injection analysis modified from Measures et al. (1995), as 179 180 described by Sedwick et al. (2011); accuracy of the DFe method was verified by analysis of SAFe reference seawater samples (Johnson et al., 2007). 181

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2.4. Phytoplankton Photosynthetic Physiology

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Variable chlorophyll fluorescence was measured using a Chelsea Scientific Instruments 185 Fastracka[™] Mk II Fast Repetition Rate fluorometer (FRRf) integrated with a FastAct[™] 186 187 Laboratory system. All samples were acclimated in opaque bottles for 30 minutes at 4°C, and FRRf measurements were blank corrected using carefully prepared 0.2 µm 188 filtrates for all samples (Cullen and Davis, 2003). Blanks were typically around 1% and 189 190 always <10% of the maximum fluorescence signal. Protocols for FRRf measurements and data processing were similar to those detailed elsewhere (Moore et al., 2007). Data 191 192 from the FRRf were analyzed to derive values of the minimum and maximum fluorescence (F_o and F_m) and hence the photosynthetic energy conversion efficiency 193

194 (F_v/F_m) (where $F_v = F_m - F_o$), as well as the functional absorption cross-section of PSII 195 (σ_{PSII}) by fitting transients to the model of Kolber et al. (1998).

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2.5. Phytoplankton Composition

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Samples for photosynthetic pigment analysis were collected and measured by high 199 200 performance liquid chromatography (HPLC). Samples (0.3–1.0 L) of sea-water were 201 filtered through GF/F filters, which were immediately flash frozen in liquid nitrogen 202 and stored at -80°C until analysis. Pigments were extracted into 90% acetone by sonification before quantification using a Waters Spherisorb ODSU C-18 HPLC 203 204 column and Waters HPLC system as described in Smith et al. (2006). Algal community 205 composition was then estimated from pigment concentrations following the method of Arrigo et al. (1999). 206

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2.6. Total Protein Extraction and Quantification

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210 Photosynthetic protein abundances were quantified using techniques similar to those 211 described elsewhere (Brown et al., 2008; Macey et al., 2014; Ryan-Keogh et al., 2012). Samples for protein extraction were collected by filtering 1.0-3.0 L of seawater onto 212 GF/F filters (Whatman) under low light for ~45 min to minimize changes in protein 213 abundance following sampling. Filters were flash frozen and stored at -80°C until 214 215 analysis. Proteins were extracted in the laboratory according to the protocol described 216 by Brown et al. (2008). Quantification was performed using custom Agrisera[™] primary 217 antibodies and peptide standards, which were designed against peptide tags conserved 218 across all oxygenic photosynthetic species for protein subunits that are representative 219 of the functional photosynthetic complex PsbA (PSII) (Campbell et al., 2003). Protein abundances were quantified using QuantityOne[™] and ImageLab[™] software; 220 221 quantification was performed within the unsaturated portion of the calibration curve. 222 The estimated protein abundances were comparable to those reported for natural 223 phytoplankton communities using similar methods (Hopkinson et al., 2010; Losh et al., 2013; Macey et al., 2014; Richier et al., 2012). 224

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3. Results and Discussion

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229 3.1. General Oceanography

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231 A range of oceanographically distinct regions was occupied on the Ross Sea continental shelf during the PRISM-RS cruise (Figure 1). These included areas close to the Ross 232 233 Ice Shelf, near and within pack ice, and over shallow bathymetric features, both of 234 which may provide important sources of DFe to the upper water column (McGillicuddy 235 et al., 2015). Highest chlorophyll *a* concentrations (Figure 2a) were associated with the ice-edge in the southwestern Ross Sea (24.6 ug Chl L⁻¹) and correlated with the lowest 236 237 DIN (dissolved inorganic nitrate + nitrite) concentrations (Figure 2b) and lowest 238 surface F_v/F_m values observed (Figure 2c). Surface DFe concentrations ranged from 239 0.067-0.787 nM (Figure 2d), were not correlated with chlorophyll or DIN 240 concentrations, and were elevated off the continental shelf in the northeast sector of the 241 Ross Sea where sea ice was still present.

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- 243 3.2. Mapping of Iron Limitation
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The response of phytoplankton to iron addition was assayed through a series of long-245 246 (>48 h) and short-term (24 h) iron-addition incubations (Figure 1). To compare iron-247 mediated changes in F_v/F_m , $\Delta(F_v/F_m)$ was calculated as defined in Ryan-Keogh et al. 248 (2013), as the difference in F_v/F_m between the Fe-amended and control treatments. 249 Values of $\Delta(F_v/F_m)$ were frequently positive following iron addition (ranging from 250 0.00-0.17) (Figure 3a). While no clear spatial pattern in iron stress could be observed 251 from this single cruise, these results confirm previous findings suggesting that, despite 252 being the most productive region in the Southern Ocean, phytoplankton growth in the 253 Ross Sea is limited by iron availability during summer (Bertrand et al., 2011; Bertrand 254 et al., 2007; Coale et al., 2003; Cochlan et al., 2002; Martin et al., 1990; Olson et al., 2000; Sedwick and DiTullio, 1997; Sedwick et al., 2000). 255

Data from long-term (>48 h) experiments (Table 1) enable a more detailed analysis of the response of phytoplankton to iron additions. Three experiments were initiated from (1) near the Ross Ice Shelf, (2) over the Ross Bank and (3) in an anticyclonic eddy (Figure 1). The three experiments revealed varying responses to iron 260 additions by the extant phytoplankton assemblage. Experiments 1 and 3 gave strong 261 and positive responses to iron additions, and provided evidence that phytoplankton 262 were iron limited. Short-term responses revealed elevated values of F_v/F_m (i.e., a 263 positive $\Delta(F_v/F_m)$) after 24 h (Figure 3b), with subsequent significant (ANOVA, 264 p < 0.05) increases in growth rates and nutrient removal observed after 72 h (Table 1). Experiment 2, initiated over Ross Bank, did not show an increase in photosynthetic 265 266 efficiency $\Delta(F_v/F_m)$ (Figure 3b). Moreover, growth rate and nutrient removal were not 267 significantly different between control and iron-addition conditions until after >168 h 268 (ANOVA, p > 0.05) (Table 1), which most likely reflects severe depletion of ambient 269 DFe in the control treatments by this time. Ross Bank has a shallow bathymetry (~150 270 m), and none of the iron-addition experiments in this region showed a significant 271 response (Figure 3). Benthic sources from Ross Bank may therefore provide significant and continuous DFe inputs to the euphotic zone, thereby ultimately stimulating 272 273 productivity.

274 The measurement of F_v/F_m is derived from analysis of the fluorescence kinetics 275 emitted from the photosynthetic reaction centre photosystem II (PSII) and its associated light-harvesting antenna (Kolber and Falkowski, 1993). Understanding the mechanism 276 277 of changes to F_v/F_m can provide information on the process by which phytoplankton 278 respond to iron limitation. Absolute changes in maximum fluorescence and variable 279 fluorescence normalized to chlorophyll *a* were calculated (Figure 3c and 3d), revealing a significant difference between the +Fe and control treatments in F_m Chl⁻¹ (t = 24 hr 280 (*t*-test, p < 0.05), whereas there was no significant difference for F_v Chl⁻¹ (t = 24 h) (*t*-281 282 test, p > 0.05). This suggests that changes in F_v/F_m reflect changes in the proportion of 283 chlorophyll that is photosynthetically coupled to active PSII reaction centres, rather 284 than changes in the activity of PSII per se (Behrenfeld et al., 2006; Macey et al., 2014). 285 A similar response was observed for all short-term iron-addition experiments that 286 exhibited positive changes in $\Delta(F_v/F_m)$.

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288 3.3 Temporal Development of Photosynthetic Strategy

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Given the high degree of spatial variability in response to iron additions, we placed all
 observations within a unified framework, hence producing a conceptualized model of
 temporal progression of phytoplankton within the Ross Sea (Figure 4). Total
 phytoplankton biomass accumulation is dependent on growth after the sampled regions

294 become ice-free (Arrigo and van Dijken, 2003) and the losses due to grazing, sinking 295 and physical removal. Spatial data therefore sample across a mosaic of different 296 temporal progressions that represent different stages of ecosystem development. We 297 utilized surface nitrate+nitrite (DIN) as a proxy to separate the temporal patterns from 298 any spatial differences (Figure 4). Such a framework assumed that surface DIN concentrations are relatively uniform throughout the Ross Sea at the start of the growing 299 300 period. McGillicuddy et al. (2015) estimated surface nitrate to range from 26-33 µM 301 nitrate as the Ross Sea becomes ice-free, thus this acknowledged assumption is minor 302 compared with the observed seasonal drawdown of DIN (33-6 µM; Figure 4). Thus, 303 adopting this framework we find a clear pattern within the collected data indicating that 304 as phytoplankton biomass (Chl) increased, nutrients were removed and F_v/F_m reduced 305 (Figure 4a). Pigment data further demonstrated that nutrient drawdown and Chl 306 increases in parallel with a shift in the phytoplankton assemblage from P. antarctica-307 dominated to diatom-dominated (Figure 4b). Within this conceptual framework, the relative severity of iron-stress ($\Delta F_v/F_m$) may be inferred from the iron-addition 308 309 incubation experiments. Two potential phases of Fe stress were identified (Figure 4c): 310 first, when DIN concentrations remain high (> 20 μ M) and P. antarctica is a major 311 component of the phytoplankton (labelled '1'), and secondly when DIN is further removed (to $< 20 \mu$ M) by diatom-dominated communities (labelled '2'; Figure 4c). 312 However, no systematic trend in $\Delta F_v/F_m$ is observed as iron stress is quite variable in 313 both regimes. 314

315 We further considered phytoplankton photophysiological parameters within this framework. The relative size of the effective light-harvesting cross-section of PSII 316 (σ_{PSII}) (Figure 5a) is low (~1.6 nm⁻²) when DIN and F_v/F_m are high, and approximately 317 doubles to $\sim 3 \text{ nm}^{-2}$ as DIN is depleted and the assemblage becomes diatom-dominated. 318 319 Quantification of the photosynthetic catalyst PSII further characterizes the 320 photosynthetic strategy of phytoplankton in the Ross Sea. The abundance of the protein 321 target PsbA, which is taken to be indicative of the overall abundance of PSII (Brown et al., 2008), expressed as a ratio to chlorophyll (Chl:PsbA) can provide another 322 323 indication of the size of the light harvesting pigment antenna relative to the abundance 324 of the photosystems. Values of Chl:PsbA were lower at higher DIN concentrations and 325 increased as DIN and F_v/F_m decreased (Figure 5b). Combining the protein abundance data and the photophysiological measurements, the maximum fluorescent yield per 326

327 chlorophyll (F_m :Chl) (Figure 5c) and per PSII (F_m :PsbA) (Figure 5d) can also be 328 calculated. Both of these parameters increase with decreases in DIN and F_v/F_m .

329 Together, these photophysiological measurements and corresponding 330 environmental information at the time of sampling suggest a transition between two 331 physiologically distinct phytoplankton assemblages as biomass accumulates. In Figure 6 we functionally separate these into (a) assemblages where surface DIN is >20 μ M 332 333 and (b) those where surface DIN is $<20 \mu$ M. Whilst both groups exhibit signs of Fe 334 deficiency (Figure 4c), when DIN is high phytoplankton assemblages contain P. 335 antarctica, as opposed to being dominated by diatoms when DIN is low (Figure 4b). 336 Further, the photosynthetic strategy differs between these groups, when DIN is low the 337 phytoplankton assemblage has a significantly larger light-harvesting cross-section of 338 PSII (ANOVA, p < 0.05), a significantly higher Chl:PsbA ratio (ANOVA, p < 0.05), and increased maximal fluorescence yield (both per Chl and per PSII) i.e. Fm:Chl and 339 340 Fm:PsbA (ANOVA, p < 0.05). The analysis presented here thus demonstrates that a different photosynthetic strategy is apparent by phytoplankton responsible for the initial 341 342 DIN removal vs. those responsible for the later DIN removal.

343 Phytoplankton in the Ross Sea generally display a large, functional light-harvesting 344 cross-section for PSII (σ_{PSII}) compared to temperate species (Smith et al., 2011). As has 345 been proposed (Strzepek et al., 2012), this may reflect a strategy by which cells 346 acclimate through increasing the size of photosynthetic units rather than the number of photosynthetic units in a low Fe environment – thus escaping the typical antagonistic 347 348 relationship between iron demand and light capture. Our measurements of the 349 abundance of the photosynthetic catalyst PSII were also consistent with this strategy, 350 whereby the increase in the ratio of Chl:PSII mirrors the increase in σ_{PSII} (Figure 5a,b). 351 Such a strategy would significantly reduce the iron demand normally associated with 352 the photosynthetic apparatus (Strzepek et al., 2012), although presumably at some cost, potentially with regards to susceptibility to photodamage. Characterization of 353 354 phytoplankton from temperate regions would tend to suggest that relatively large 355 functional cross-sections would be unlikely to be associated with the large cell sizes typical of many Southern Ocean diatoms (Suggett et al., 2009). Large cells with large 356 357 functional cross-sections may either be susceptible to enhanced photodamage or may 358 have a requirement for rapidly inducible and significant non-photochemical quenching. 359 However, despite these potential negative consequences of a large σ_{PSII} , Antarctic 360 diatoms seem to have adopted such an adaptive response, likely underlining the

361 relevance of iron availability as a major control factor in the system, alongside providing some explanation for the low Fe:C ratios in some of these species (Strzepek 362 363 et al., 2012). These observations may also support recent findings that Southern Ocean 364 diatoms may both acquire (Kustka et al., 2015) and utilize (Strzepek et al., 2012) iron 365 more effectively than *P. antarctica*, and that the observed progression in phytoplankton 366 species enables further drawdown of nitrate from the surface of the Ross Sea (Figure 367 4b).While there can be an array of reasons for diatoms being better at acquiring and utilizing available DFe as it becomes limiting during summer in the Ross Sea, 368 369 differences in photosynthetic strategy have the potential to be a significant factor, given 370 that the photosynthetic apparatus represents the dominant sink for Fe in a phytoplankton cell (Raven, 1990; Aspinwall et al., 2004; Strzepek and Harrison, 2004). 371

372 While the observations in this study were restricted to the summer season, they 373 do include some areas that had only recently become ice-free, containing DIN 374 concentrations similar to those estimated for the winter mixed layer (McGillicuddy et 375 al., 2015). Thus, the observed conditions are analogous to a broader seasonal progression in phytoplankton composition in the Ross Sea from *P. antarctica* early in 376 377 the growing season to diatom-dominance later in summer (Smith et al., 2010). The 378 current study therefore provides evidence for underpinning mechanisms for the 379 seasonal progression, while also reflecting the large degree of spatial heterogeneity in 380 physical and biological processes throughout the growing season in the Ross Sea (Smith 381 and Jones, 2015).

382 The data presented here also provide insights into the mechanism of the ironstress response of phytoplankton. Increases in F_v/F_m are commonly reported as a 383 384 response to iron addition (Boyd et al., 2008; Feng et al., 2010). Results from 385 experiments and observations show that increases in F_v/F_m in response to iron addition 386 and elevated F_v/F_m values in regions with modest DIN removals are as a result of 387 reductions in the ratio of Fm:Chl (or Fm:PSII) rather than changes in Fv:Chl. This is in agreement with similar observations from the HLNA and Equatorial Pacific 388 (Behrenfeld et al., 2006; Macey et al., 2014) and implies that low F_v/F_m results from 389 changes in the coupling of light-harvesting chlorophyll-binding proteins to 390 391 photosynthesis rather than accumulation of damaged photosystems. Accumulation of 392 non-photosynthetically active chlorophyll-binding proteins in Fe-limited oceanic 393 regions can subsequently have consequences for estimates of productivity in these 394 regions (Behrenfeld et al., 2006).

395 **4. Conclusions**

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397 The current study represents an analysis of the summer photosynthetic strategies of phytoplankton in the Ross Sea and highlights how different iron-efficient 398 399 photosynthetic strategies occur in phytoplankton as Fe becomes limiting and irradiance presumably increases. These strategies enable a more complete drawdown of nitrate as 400 401 the growing season progresses. Such observations are important for understanding Fe usage efficiency and modelling primary productivity in the region. Climate-mediated 402 403 changes to the mixed layer depth, sea-ice cover and dust inputs could change iron-404 limitation strategies and phytoplankton phenology (Boyd et al., 2012, Mackie et al., 405 2008). An understanding of the role of iron limitation in this highly dynamic 406 environment is thus particularly important as climatically mediated variability is 407 expected to increase.

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- 669 **Tables and Figure Legends**
- 670

Table 1 Locations for long-term experiments conducted during NBP12-01, along with values

- 672 of initial F_v/F_m , $\Delta(F_v/F_m)$, net growth rates estimated from chlorophyll accumulation and nitrate 673 drawdown over 168 h. Shown are averages \pm standard errors (n = 3 or 5); * indicates significant 674 differences from control.
- 675

Figure 1 Composite map of Southern Ocean MODIS chlorophyll *a* for December 2011 –
February 2012. Inset: Long-term (blue dots) and short-term (red dots) experimental locations
conducted on cruise NBP12-01 in the Ross Sea with 250 m bathymetric contours. Surface *in situ* samples were also collected at these locations and at those marked CTD-station (black
dots).

681

Figure 2 (a) Surface chlorophyll concentrations (μ g L⁻¹) from CTD stations. (**b**) Surface DIN concentrations (μ M). (**c**) Surface F_v/F_m. (**d**) Surface DFe concentrations (nM). Chlorophyll, DIN and F_v/F_m from samples collected at 1-5 m depth, DFe from samples collected at ~10 m depth.

686

Figure 3 (a) Spatial distribution of $\Delta(F_v/F_m)$ calculated from all iron-addition incubation 687 experiments. (b) $\Delta(F_v/F_m)$ calculated from three long-term experiments: (1) near the Ross Ice 688 Shelf, (2) over the Ross Bank and (3) within an anti-cyclonic eddy. (c) The change in 689 chlorophyll normalized maximum fluorescence, (ΔF_m Chl⁻¹) from the three long-term iron-690 addition incubation experiments. (d) The change in chlorophyll normalized variable 691 fluorescence $\Delta(F_v \text{ Chl}^{-1})$ from the three long-term iron-addition incubation experiments. Shown 692 are averages with \pm standard errors (n = 4 or 5). * represent statistically significant differences 693 694 (*t*-*test*, *p*<0.05).

695

Figure 4 Relationship of DIN (μ M) and photosynthetic efficiency (F_v/F_m) throughout the Ross Sea as a function of (**a**) chlorophyll concentrations (μ g L⁻¹), (**b**) phytoplankton composition (%), and (**c**) the relative degree of Fe stress $\Delta(F_v/F_m)$. Grey dots represent stations where DIN and F_v/F_m were measured but no corresponding additional variables were measured.

Figure 5 Relationship of DIN (μ M) and photosynthetic efficiency (F_v/F_m) throughout the Ross Sea as a function of (**a**) photosystem II light-harvesting cross-section (σ_{PSII}) (nm⁻²), (**b**) the ratio

- of chlorophyll to PsbA (a core subunit of PSII) (mmol mol⁻¹), (c) the ratio of the maximum fluorescence yield to chlorophyll (F_m :Chl), and (d) the ratio of the maximum fluorescence yield to PsbA (F_m :PsbA). Grey dots represent stations where DIN and F_v/F_m were measured but no corresponding additional variables were measured.
- 707

Figure 6 Characterization of phytoplankton composition from populations sampled at > 20 μ M DIN or <20 μ M DIN. (a) Chl (μ g L⁻¹), (b) DFe (nM) (c) F_v/F_m (d) σ_{PSII} (nm⁻²) (e) Chl:PsbA(mmol mol⁻¹) (f) Δ (F_v/F_m) (g) (g) F_m:Chl (h) Fm:PsbA. All parameters are significantly different between phytoplankton in different DIN environments, one-way ANOVA (*p* <0.05).

Table 1

Experiment	1	2	3
Lat (°S)	75.72	76.72	77.55
Long (°W)	183.40	179.08	175.97
Fv/Fm Initial	0.26 ± 0.01	0.29 ± 0.00	0.21 ±0.00
Δ (F _v /F _m), 24 h	0.04 ± 0.01	0.00 ± 0.00	0.01 ±0.00
μ ^{Chl} _{Control} (d ⁻¹), 0 -168 h	0.11 ±0.02	0.25 ± 0.01	0.13 ±0.01
μ_{Fe}^{Chl} (d ⁻¹), 0 -168 h	$0.17* \pm 0.02$	$0.29* \pm 0.00$	0.19* ±0.01
ΔNO_3^- control (μ M d ⁻¹), 0 – 168 h	1.61 ±0.33	1.50 ± 0.04	2.43 ±0.08
ΔNO_{3}^{-} Fe (μ M d ⁻¹), 0 – 168 h	2.53* ±0.13	1.57 ± 0.05	2.93* ±0.07



Figure 2



Figure 3





Figure 5



