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Supplementary Information for

Photosynthetic adaptation to low iron, light, and temperature in Southern Ocean phytoplankton

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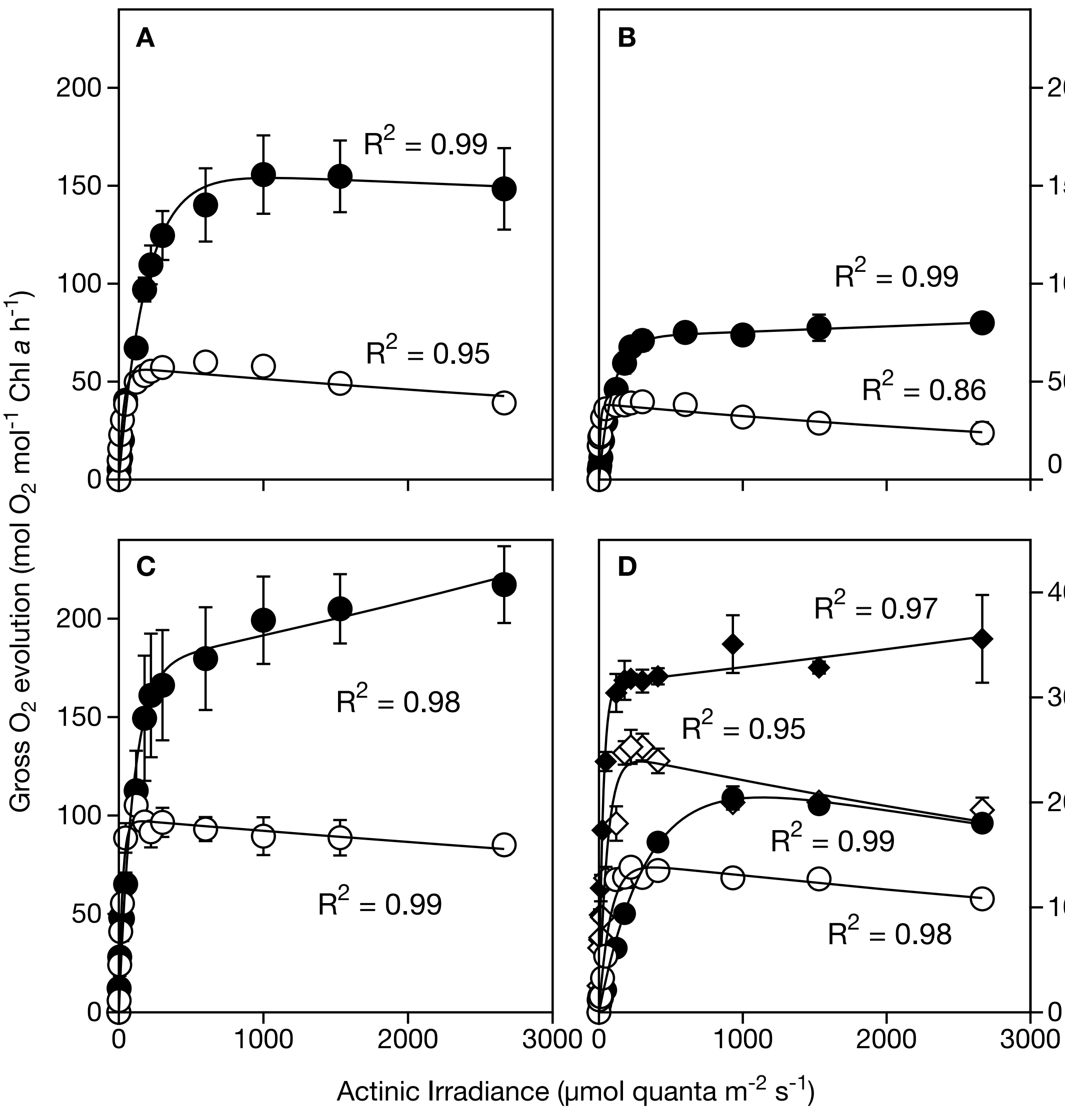

Fig. S1. Photosynthesis *versus* irradiance (PE) curves for the SO phytoplankton *P. inermis* (**A**), *E. antarctica* (**B**), *P. antarctica* (**C**), the temperate oceanic diatom *T. oceanica* (diamonds), and the temperate coastal diatom *T. weissflogii* (circles) (**D**). Note the different scale of the y-axis in panel D. Mean gross oxygen (O_2) evolution rates are plotted as a function of actinic irradiance for low iron (open symbols) and high iron (closed symbols) cultures. Error bars represent standard errors of replicate cultures and are smaller than the symbol when not visible (*n* = 3). Solid lines denote exponential model fits (1).

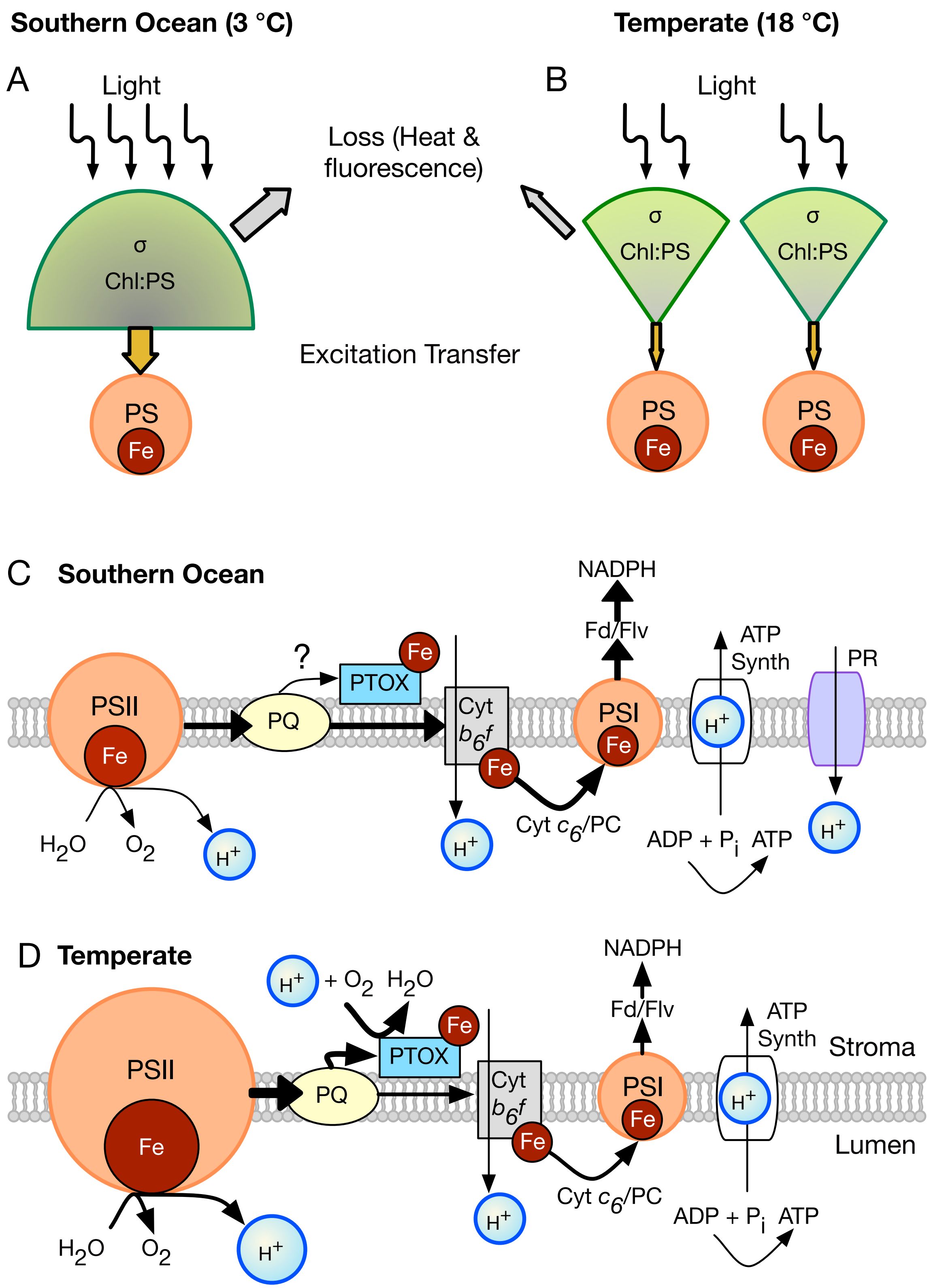
Southern Ocean (3 °C)
Temperate (18 °C)
A
Light
Loss (Heat &
fluorescence)
σ
Chl:PS
Excitation Transfer
PS
Fe
B
Light
σ
Chl:PS
σ
Chl:PS
PS
Fe
PS
Fe
C Southern Ocean
NADPH
Fd/Flv
?
PTOX
Fe
ATP
Synth
PR
PSII
Fe
PQ
Cyt
b_6f
Fe
PSI
Fe
H^+
H_2O
O_2
H^+
H^+
Cyt c_6/PC
ADP + P_i
ATP
H^+
D Temperate
H^+ + O_2 H_2O
NADPH
Fd/Flv
Fe
PTOX
ATP
Synth
Stroma
PSII
Fe
PQ
Cyt
b_6f
Fe
PSI
Fe
H^+
Lumen
H^+
Cyt c_6/PC
ADP + P_i
ATP
H_2O
O_2
H^+

Fig. S2. Schematic of the demonstrated and potential adaptations of SO phytoplankton to economize on the iron used for photosynthesis under low light conditions. **A** and **B**. The unexpectedly high rates of net C production per mol of photosynthetic iron in the SO species at their lower experimental temperature (3 vs 18 °C) and light intensity (30 vs 40 μmol quanta m^{-2} s^{-1}) (**A**) are associated with higher Chl:PS ratios and associated functional light absorption cross sections of PSII (σ_{PSII}) and resultant light absorption per photosystem than observed in the temperate species (**B**).

C and **D**. The iron- and light-limited SO species (**C**) had much lower ratios of PSII:PSI than the temperate oceanic species and the iron-limited temperate coastal species (**D**). The lower PSII:PSI ratios of the SO species (1.7 ± 0.2, ± SE) are consistent with primarily linear photosynthetic electron transport (PET) to produce the reducing equivalents (NADPH), and some but not all of the adenosine triphosphate (ATP) needed for carbon fixation, molecular biosynthesis and cell maintenance. The ATP is produced from adenosine diphosphate (ADP) and inorganic phosphate (P_i) by the membrane protein ATP synthase (ATP Synth) using the proton gradient across the thylakoid membrane generated by linear PET in all species, and in addition a proposed PSII/plastoquinol (PTOX) water/water cycle in *T. oceanica* (**D**), and potentially proteorhodopsin (PR) in the SO species (**C**).

In linear PET a photoreaction in PSII oxidizes water to molecular oxygen (O_2), which produces reducing electrons and releases hydrogen ions (H^+) into the lumen. The reducing electrons are then transferred along a potential gradient to plastoquinone (PQ) to form plastoquinol, and then to the iron-containing cytochrome b_6f (Cyt b_6f) complex, in a process that pumps more H^+ ions from the stroma into the lumen. The electrons are then transferred in the lumen from the Cyt b_6f complex (5 Fe) to photosystem I (PSI, 12 Fe) by soluble cytochrome c_6 (Cyt c_6, a protein containing a single Fe) or plastocyanin (PC, a copper protein). A photochemical reaction in PSI then transfers the electrons to a much higher reducing potential on the stromal side of the PSI complex, and allows for the stepwise reduction of ferredoxin (Fd, 2 Fe) or flavodoxin (Flv, no Fe) and then NADP to NADPH. This linear PET provides the reducing equivalents (NADPH) needed for carbon fixation in the Calvin cycle in the stroma, and some but not all of the ATP needed for carbon fixation and cell metabolism.

In *T. oceanica* (**D**), the extra needed ATP has been hypothesized (2) to be generated from a water-water cycle that involves photo-oxidation of water to O_2 by PSII followed by subsequent re-reduction of O_2 back to water in the stroma by reduced plastoquinol by the iron-containing enzyme plastoquinol terminal oxidase (PTOX). This water/water cycle transfers H^+ ions from the stroma to the lumen, and the resultant pH gradient across the thylakoid membrane is used to produce additional ATP. However, the process produces no reducing equivalents (NADPH). It requires additional photosynthetic iron, as PSII and PTOX each contain 2 Fe atoms. It also should increase the PSII concentration and PSII:PSI ratios (2) as observed in *T. oceanica*. In the SO species (**C**) the much lower PSII:PSI ratio (mean value 1.7) appears to be tuned primarily to linear PET. Here, the extra needed ATP is hypothesized to be generated by the non-iron protein proteorhodopsin (PR), which uses a photoreaction to pump H^+ ions across cellular membranes, and thereby create a pH gradient that can be used to generate ATP via ATP synthase (3, 4). Transcripts of PR have been identified in all three of our experimental SO species (5), but the location of this putative PR in cellular membranes is not known; i.e., whether it is present in the thylakoid membrane (as depicted in **C** above) or in some other cell membrane, such as the vacuolar membrane or the plasma membrane.

Table S1. Intracellular iron (Fe), cellular carbon (C), and chlorophyll *a* (Chl *a*) normalized per liter cell volume (L) and per m^2 cell surface area (SA); cellular Fe normalized to cell C; cellular iron use efficiencies (IUE). fL = femtoliters = 10^{-15} L. Temp. = temperate.

Species	Region	Iron level	Cell volume	Cell Fe	Cell C	Fe:C †	IUE	Cell Chl *a*	Chl *a* :SA
			(fL $cell^{-1}$)	(μmol L^{-1})	(mol L^{-1})	(µmol mol^{-1})	(kmol C mol Fe^{-1} d^{-1})	(mmol L^{-1})	(mmol m^{-2})
P. inermis	SO	Low	51,300 ± 300	0.20 ± 0.03	0.50 ± 0.04	0.40 ± 0.07*	770 ± 230	0.20 ± 0.00	0.85 ± 0.02
		High	35,300 ± 1500	1.90 ± 0.10	1.55 ± 0.04	0.86 ± 0.02*	520 ± 90	0.55 ± 0.00	1.96 ± 0.08
E. antarctica	SO	Low	11,100 ± 2900	2.20 ± 0.10	2.30 ± 0.10	0.94 ± 0.03	150 ± 8	0.85 ± 0.09	2.34 ± 0.66
		High	13,300 ± 300	5.30 ± 0.60	3.00 ± 0.50	1.8 ± 0.00	140 ± 20	0.89 ± 0.03	3.01 ± 0.12
P. antarctica	SO	Low	16.9 ± 2.4	31.0 ± 2.6	15.3 ± 2.0	2.0 ± 0.3	90 ± 10	2.83 ± 0.02	1.50 ± 0.21
		High	39.3 ± 4.7	63.9 ± 7.0	11.1 ± 3.4	6.0 ± 1.1	60 ± 10	4.62 ± 0.09	3.25 ± 0.40
T. oceanica	Temp.	Low	55.8 ± 2.1	66.3 ± 0.9	23.0 ± 1.6	2.8 ± 0.1	130 ± 10	4.0 ± 0.2	3.16 ± 0.20
	Oceanic	High	59.6 ± 0.5	450 ± 2	16.4 ± 1.0	32.0 ± 0.5	19 ± 1	6.4 ± 0.4	5.17 ± 0.33
T. weissflogii	Temp.	Low	653 ± 16	216 ± 14	10.3 ± 0.2	20.9 ± 0.2	16 ± 1	8.9 ± 0.5	16.0 ± 1.0
	Coastal	High	809 ± 38	620 ± 12	12.5 ± 0.7	49.5 ± 0.5	11 ± 0	9.9 ± 0.3	19.1 ± 1.1

* Note that computed photosynthetic iron (Fe_{PH}, Table 1) was 2- to 4-fold higher for *P. inermis* than the cellular iron reported here. † Data from ref. 6.

Table S2. Photosynthetic parameters derived from modeled photosynthesis-irradiance (P-E) curves for oxygen-evolution versus light intensity.

Species	Region	Iron level	P^B_{max}	α^B	E_k	$1 / \tau$	R
P. inermis	SO	Low	57 ± 4	1.8 ± 0.2	33 ± 6	0.13 ± 0.02	11 ± 4
		High	158 ±22	0.8 ± 0.0	188 ± 25	0.35 ± 0.04	14 ± 0
E. antarctica	SO	Low	39 ± 4	4.0 ± 0.1	10 ± 1	0.14 ± 0.05	11 ± 1
		High	73 ± 4	0.8 ± 0.1	97 ± 14	0.13 ± 0.01	21 ± 3
P. antarctica	SO	Low	98 ±12	4.0 ± 0.2	25 ± 3	0.20 ± 0.01	34 ± 3
		High	174 ± 28	1.8 ± 0.4	97 ± 15	0.24 ± 0.01	59 ± 12
T. oceanica	Temperate	Low	249 ± 14	4.3 ± 0.3	58 ± 1	0.08 ± 0.00	52 ± 6
	Oceanic	High	312 ± 13	11.1 ± 0.4	28 ± 3	0.08 ± 0.01	34 ± 4
T. weissflogii	Temperate	Low	145 ± 21	1.8 ± 0.5	80 ± 23	0.09 ± 0.01	20 ± 2
	Coastal	High	242 ± 15	0.7 ± 0.0	346 ± 3	0.12 ± 0.01	15 ± 1

P^B_{max}: Gross maximum photosynthetic rate normalized to Chl *a* (mol O_2 [mol Chl *a*]$^{-1}$ h^{-1}). α^B: initial slope of the P-E curve (mol O_2 [mol Chl *a*]$^{-1}$ h^{-1} / μmol quanta m^{-2} s^{-1}). E_k: light-saturation parameter (μmol quanta m^{-2} s^{-1}). $1 / \tau$: maximum steady-state electron turnover rate from water to CO_2 (ms^{-1}). R: dark respiration rate normalized to Chl *a* (mol O_2 [mol Chl *a*]$^{-1}$ h^{-1}). P^B_{max}, α^B, and E_k derived from exponential model fits of P-E data (1).

Table S3. PSII and PSI use efficiencies (net mol C fixed or gross photosynthetic O_2 produced per mol photosystem per day).

Species	Iron Level	PSII UE (kmol C mol^{-1} PSII d^{-1})	PSI UE (kmol C mol^{-1} PSI d^{-1})	O_2 rate:PSII (kmol O_2 mol^{-1} PSII d^{-1})	O_2 rate:PSI (kmol O_2 mol^{-1} PSI d^{-1})
P. inermis	Low	1500±330	3000±260	1250±460	2260±350
	High	1790±110	2350±490	1600±180	1790±330
E. antarctica	Low	1340±390	1700±250	2640±640	4190±770
	High	1500±280	3550±780	870±80	2340±520
P. antarctica	Low	1500±200	2700±420	2860±470	5070±1000
	High	1018±390	1420±470	1600±400	2330±400
T. oceanica	Low	590±80	4370±1140	330±80	2710±1400
	High	380±60	4070±430	690±100	6870±840
T. weissflogii	Low	210±20	922±40	480±100	1990±380
	High	320±40	537±50	160±50	360±120

Net C use efficiencies are for the growth irradiances (30 and 40 µmol quanta m^{-2} s^{-1} for SO and temperate species, respectively), while the O_2 production use efficiencies were determined at an irradiance of 30 µmol quanta m^{-2} s^{-1}.

Table S4. Two-way Analysis of Variance (ANOVA) and multiple comparison tests (MCT) for Table 1 variables. Significant results highlighted in boldface. Level of significance for MCTs are low (*) to high (****). ns = not significant (Alpha = 0.05).

Variable	Statistical Test					
Growth rate (μ)	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	**3.47**	**0.0002**	***	**Yes**
		Region/Species	**54.6**	**< 0.0001**	****	**Yes**
		Iron	**31**	**< 0.0001**	****	**Yes**
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	***P. inermis***	**-0.14**	**-0.213 to -0.0673**	****	**Yes**
		E. antarctica	**-0.11**	**-0.183 to -0.0373**	***	**Yes**
		P. antarctica	**-0.18**	**-0.253 to -0.107**	****	**Yes**
		T. oceanica	**-0.29**	**-0.363 to -0.217**	****	**Yes**
		T. weissflogii	**-0.21**	**-0.283 to -0.137**	****	**Yes**
Fe_{PH} : C	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	**9.2**	**0.0193**	*	**Yes**
		Region/Species	**65.1**	**< 0.0001**	****	**Yes**
		Iron	**15.8**	**0.0009**	***	**Yes**
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	Southern Ocean (SO)	-1.6	-7.66 to 4.46	No	ns
		Temperate oceanic (TO)	-3.6	-9.66 to 2.46	No	ns
		Temperate coastal (TC)	**-11.4**	**-17.4 to -5.29**	**Yes**	***
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	SO vs. TO	-1.15	-6.96 to 4.66	No	ns
		SO vs. TC	**-7.91**	**-13.7 to -2.10**	**Yes**	**
		TO vs. TC	**-6.76**	**-12.6 to -0.946**	**Yes**	*
		High iron				
		SO vs. TO	-3.15	-8.96 to 2.66	No	ns
		SO vs. TC	**-17.7**	**-23.5 to -11.8**	**Yes**	****
		TO vs. TC	**-14.5**	**-20.3 to -8.70**	**Yes**	****
PS IUE	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	2.07	0.4421	ns	No
		Region/Species	**81.6**	**< 0.0001**	****	**Yes**
		Iron	2.13	0.2051	ns	No
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	Southern Ocean (SO)	-0.3	-49.4 to 48.8	No	ns
		Temperate oceanic (TO)	31.9	-17.2 to 81.0	No	ns
		Temperate coastal (TC)	9.4	-39.7 to 58.5	No	ns
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	SO vs. TO	-16.8	-63.9 to 30.3	No	ns
		SO vs. TC	**84.5**	**37.4 to 132**	**Yes**	**
		TO vs. TC	**101**	**54.2 to 148**	**Yes**	***
		High iron				
		SO vs. TO	15.4	-31.7 to 62.5	No	ns
		SO vs. TC	**94.2**	**47.1 to 141**	**Yes**	***
		TO vs. TC	**78.8**	**31.7 to 126**	**Yes**	**
PSII:PSI	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	10.4	0.0597	ns	No
		Region/Species	**72.2**	**< 0.0001**	****	**Yes**
		Iron	0.0762	0.8222	ns	No
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	Southern Ocean (SO)				
		Temperate oceanic (TO)				
		Temperate coastal (TC)				
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	**SO vs. TO**	**-5.78**	**-10.0 to -1.55**	**Yes**	**
		SO vs. TC	-2.68	-6.91 to 1.55	No	ns
		TO vs. TC	3.1	-1.13 to 7.33	No	ns
		High iron				
		SO vs. TO	**-9.05**	**-13.3 to -4.82**	**Yes**	***
		SO vs. TC	0.05	-4.18 to 4.28	No	ns
		TO vs. TC	**9.1**	**4.87 to 13.3**	**Yes**	***

Table S5. Two-way Analysis of Variance (ANOVA) and multiple comparison tests (MCT) for Figure 1 variables. Significant results highlighted in boldface. Level of significance for MCTs are low (*) to high (****). ns = not significant (Alpha = 0.05).

Variable	Statistical Test					
Chl : PSII	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	3.12	0.4568	ns	No
		Region/Species	**72**	**0.0002**	***	**Yes**
		Iron	2.53	0.2666	ns	No
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	Southern Ocean (SO)	750	-458 to 1958	ns	No
		Temperate oceanic (TO)	33	-1175 to 1241	ns	No
		Temperate coastal (TC)	94	-1114 to 1302	ns	No
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	**SO vs. TO**	**2119**	**960 to 3278**	**	**Yes**
		SO vs. TC	**1849**	**690 to 3008**	**	**Yes**
		TO vs. TC	-270	-1429 to 889	ns	No
		High iron				
		SO vs. TO	**1402**	**243 to 2561**	*	**Yes**
		SO vs. TC	**1193**	**33.7 to 2352**	*	**Yes**
		TO vs. TC	-209	-1368 to 950	ns	No
Chl : PSI	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	7.12	0.3948	ns	No
		Region/Species	**39.8**	**0.019**	*	**Yes**
		Iron	10.6	0.1093	ns	No
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	SO	1220	-948 to 3388	ns	No
		TO	-124	-2292 to 2044	ns	No
		TC	1241	-927 to 3409	ns	No
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	SO vs. TO	1803	-278 to 3884	ns	No
		SO vs. TC	1822	-259 to 3903	ns	No
		TO vs. TC	19	-2062 to 2100	ns	No
		High iron				
		SO vs. TO	459	-1622 to 2540	ns	No
		SO vs. TC	1843	-238 to 3924	ns	No
		TO vs. TC	1384	-697 to 3465	ns	No
PSII : C	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	**10.9**	**0.0088**	**	**Yes**
		Region/Species	**71.3**	**< 0.0001**	****	**Yes**
		Iron	**8.73**	**0.0053**	**	**Yes**
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	SO	-0.08	-0.706 to 0.546	ns	No
		TO	**-1.14**	**-1.77 to -0.513**	***	**Yes**
		TC	-0.105	-0.731 to 0.521	ns	No
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	SO vs. TO	-0.513	-1.11 to 0.0881	ns	No
		SO vs. TC	**-1.5**	**-2.10 to -0.895**	****	**Yes**
		TO vs. TC	**-0.983**	**-1.58 to -0.382**	**	**Yes**
		High iron				
		SO vs. TO	**-1.57**	**-2.17 to -0.971**	****	**Yes**
		SO vs. TC	**-1.52**	**-2.12 to -0.920**	****	**Yes**
		TO vs. TC	0.051	-0.550 to 0.652	ns	No
PSI : C	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	**15.9**	**< 0.0001**	****	**Yes**
		Region/Species	**63.6**	**< 0.0001**	****	**Yes**
		Iron	**16.2**	**< 0.0001**	****	**Yes**
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	SO	-0.085	-0.279 to 0.109	ns	No
		TO	-0.078	-0.272 to 0.116	ns	No
		TC	**-0.655**	**-0.849 to -0.461**	****	**Yes**
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	SO vs. TO	-0.008	-0.194 to 0.178	ns	No
		SO vs. TC	**-0.29**	**-0.476 to -0.104**	**	**Yes**
		TO vs. TC	**-0.282**	**-0.468 to -0.0957**	**	**Yes**
		High iron				
		SO vs. TO	-0.001	-0.187 to 0.185	ns	No
		SO vs. TC	**-0.86**	**-1.05 to -0.674**	****	**Yes**
		TO vs. TC	**-0.859**	**-1.05 to -0.673**	****	**Yes**

Table S6. Two-way Analysis of Variance (ANOVA) and multiple comparison tests (MCT) for Figure 2 and Table S2 variables. Significant results highlighted in boldface. Level of significance for MCTs are low (*) to high (****). ns = not significant (Alpha = 0.05).

Variable	Statistical Test					
P^{B}_{max}	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	0.636	0.6412	ns	No
		Region/Species	**64.9**	**< 0.0001**	****	**Yes**
		Iron	**17.6**	**< 0.0001**	****	**Yes**
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	**Southern Ocean (SO)**	**-70.3**	**-115 to -25.5**	**Yes**	**
		Temperate oceanic (TO)	-63	-141 to 14.6	No	ns
		Temperate coastal (TC)	**-97**	**-175 to -19.4**	**Yes**	*
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	**SO vs. TO**	**-184**	**-246 to -123**	**Yes**	****
		SO vs. TC	**-80.4**	**-142 to -18.9**	**Yes**	**
		TO vs. TC	**104**	**28.7 to 179**	**Yes**	**
		High iron				
		SO vs. TO	**-177**	**-239 to -116**	**Yes**	****
		SO vs. TC	**-107**	**-169 to -45.6**	**Yes**	***
		TO vs. TC	70	-5.32 to 145	No	ns
α^{B}	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	**31.3**	**< 0.0001**	****	**Yes**
		Region/Species	**63.5**	**< 0.0001**	****	**Yes**
		Iron	**2.77**	**< 0.0001**	****	**Yes**
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	**SO**	**2.12**	**1.46 to 2.78**	**Yes**	****
		TO	**-6.8**	**-7.94 to -5.66**	**Yes**	****
		TC	1.12	-0.0214 to 2.26	No	ns
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	**SO vs. TO**	**-1.05**	**-1.95 to -0.146**	**Yes**	*
		SO vs. TC	**1.43**	**0.526 to 2.33**	**Yes**	**
		TO vs. TC	**2.48**	**1.37 to 3.59**	**Yes**	****
		High iron				
		SO vs. TO	**-9.97**	**-10.9 to -9.07**	**Yes**	****
		SO vs. TC	0.43	-0.474 to 1.33	No	ns
		TO vs. TC	**10.4**	**9.29 to 11.5**	**Yes**	****
E_k	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	**27.1**	**< 0.0001**	****	**Yes**
		Region/Species	**40.3**	**< 0.0001**	****	**Yes**
		Iron	**23.9**	**< 0.0001**	****	**Yes**
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	**SO**	**-105**	**-146 to -64.0**	**Yes**	****
		TO	30	-41.0 to 101	No	ns
		TC	**-266**	**-337 to -195**	**Yes**	****
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	SO vs. TO	-36	-92.3 to 20.3	No	ns
		SO vs. TC	**-58**	**-114 to -1.73**	**Yes**	*
		TO vs. TC	-22	-90.9 to 46.9	No	ns
		High iron				
		SO vs. TO	**99**	**42.7 to 155**	**Yes**	***
		SO vs. TC	**-219**	**-275 to -163**	**Yes**	****
		TO vs. TC	**-318**	**-387 to -249**	**Yes**	****

Table S7. Two-way Analysis of Variance (ANOVA) and multiple comparison tests (MCT) for Figure 3 variables. Significant results highlighted in boldface. Level of significance for MCTs are low (*) to high (****). ns = not significant (Alpha = 0.05).

Variable	Statistical Test					
PSII UE	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	3.35	0.2578	ns	No
		Region/Species	**83**	**< 0.0001**	********	**Yes**
		Iron	0.489	0.5175	ns	No
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	Southern Ocean (SO)	-381	-1050 to 288	No	ns
		Temperate oceanic (TO)	212	-457 to 881	No	ns
		Temperate coastal (TC)	-109	-778 to 560	No	ns
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	**SO vs. TO**	**860**	**218 to 1502**	**Yes**	******
		SO vs. TC	**1239**	**597 to 1881**	**Yes**	*******
		TO vs. TC	379	-263 to 1021	No	ns
		High iron				
		SO vs. TO	**1453**	**811 to 2095**	**Yes**	*******
		SO vs. TC	**1511**	**869 to 2153**	**Yes**	*******
		TO vs. TC	58	-584 to 700	No	ns
PSI UE	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	0.218	0.9468	ns	No
		Region/Species	**75.4**	**0.0002**	*******	**Yes**
		Iron	0.522	0.6176	ns	No
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	SO	26	-2200 to 2252	No	ns
		TO	300	-1926 to 2526	No	ns
		TC	385	-1841 to 2611	No	ns
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	SO vs. TO	-1905	-4042 to 232	No	ns
		SO vs. TC	1543	-594 to 3680	No	ns
		TO vs. TC	**3448**	**1311 to 5585**	**Yes**	******
		High iron				
		SO vs. TO	-1631	-3768 to 506	No	ns
		SO vs. TC	1902	-235 to 4039	No	ns
		TO vs. TC	**3533**	**1396 to 5670**	**Yes**	******
O_2 rate : PSII	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	7.83	0.3279	ns	No
		Region/Species	**51.4**	**0.0061**	******	**Yes**
		Iron	2.37	0.4061	ns	No
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	SO	892	-688 to 2472	No	ns
		TO	-365	-1945 to 1215	No	ns
		TC	321	-1259 to 1901	No	ns
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	**SO vs. TO**	**1921**	**404 to 3438**	**Yes**	*****
		SO vs. TC	**1767**	**250 to 3284**	**Yes**	*****
		TO vs. TC	-154	-1671 to 1363	No	ns
		High iron				
		SO vs. TO	664	-853 to 2181	No	ns
		SO vs. TC	1196	-321 to 2713	No	ns
		TO vs. TC	532	-985 to 2049	No	ns

Table S7. (cont.)

Variable	Statistical Test					
O_2 rate : PSI	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	**32.9**	**0.0099**	**	**Yes**
		Region/Species	**38.2**	**0.0061**	**	**Yes**
		Iron	0.345	0.7096	ns	No
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	SO	1683	-1856 to 5222	No	ns
		TO	**-4154**	**-7693 to -615**	**Yes**	*
		TC	1630	-1909 to 5169	No	ns
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	SO vs. TO	1125	-2272 to 4522	No	ns
		SO vs. TC	1847	-1550 to 5244	No	ns
		TO vs. TC	722	-2675 to 4119	No	ns
		High iron				
		SO vs. TO	**-4712**	**-8109 to -1315**	**Yes**	**
		SO vs. TC	1794	-1603 to 5191	No	ns
		TO vs. TC	**6506**	**3109 to 9903**	**Yes**	***
1/ τ	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	7.23	0.0691	ns	No
		Region/Species	**71**	**< 0.0001**	****	**Yes**
		Iron	**8.93**	**0.0138**	*	**Yes**
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	**SO**	**-0.08**	**-0.141 to -0.0188**	**Yes**	*
		TO	0	-0.0612 to 0.0612	No	ns
		TC	-0.03	-0.0912 to 0.0312	No	ns
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	**SO vs. TO**	**0.08**	**0.0212 to 0.139**	**Yes**	**
		SO vs. TC	**0.07**	**0.0112 to 0.129**	**Yes**	*
		TO vs. TC	-0.01	-0.0688 to 0.0488	No	ns
		High iron				
		SO vs. TO	**0.16**	**0.101 to 0.219**	**Yes**	****
		SO vs. TC	**0.12**	**0.0612 to 0.179**	**Yes**	***
		TO vs. TC	-0.04	-0.0988 to 0.0188	No	ns
Dark Respiration C normalized	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	**18.3**	**0.0357**	*	**Yes**
		Region/Species	**57**	**0.0007**	***	**Yes**
		Iron	0.118	0.8144	ns	No
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	SO	-0.053	-0.213 to 0.107	No	ns
		TO	-0.101	-0.261 to 0.0595	No	ns
		TC	0.13	-0.0305 to 0.290	No	ns
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	SO vs. TO	-0.107	-0.261 to 0.0470	No	ns
		SO vs. TC	**-0.305**	**-0.459 to -0.151**	**Yes**	***
		TO vs. TC	**-0.198**	**-0.352 to -0.0440**	**Yes**	*
		High iron				
		SO vs. TO	**-0.155**	**0.309 to -0.00097**	**Yes**	*
		SO vs. TC	-0.122	-0.276 to 0.0320	No	ns
		TO vs. TC	0.033	-0.121 to 0.187	No	ns

Table S8. Two-way Analysis of Variance (ANOVA) and multiple comparison tests (MCT) for Figure 4 variables. Significant results highlighted in boldface. Level of significance for MCTs are low (*) to high (****). ns = not significant (Alpha = 0.05).

Variable	Statistical Test					
F_v / F_m	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	**1.99**	**0.009**	**	**Yes**
		Region/Species	**81.9**	**< 0.0001**	****	**Yes**
		Iron	**14.5**	**< 0.0001**	****	**Yes**
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	**Southern Ocean (SO)**	**-0.11**	**-0.149 to -0.0707**	****	**Yes**
		Temperate oceanic (TO)	**-0.04**	**-0.0793 to -0.000693**	*	**Yes**
		Temperate coastal (TC)	**-0.1**	**-0.139 to -0.0607**	****	**Yes**
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	**SO vs. TO**	**-0.24**	**-0.278 to -0.202**	****	**Yes**
		SO vs. TC	**-0.22**	**-0.258 to -0.182**	****	**Yes**
		TO vs. TC	0.02	-0.0177 to 0.0577	ns	No
		High iron				
		SO vs. TO	**-0.17**	**-0.208 to -0.132**	****	**Yes**
		SO vs. TC	**-0.21**	**-0.248 to -0.172**	****	**Yes**
		TO vs. TC	**-0.04**	**-0.0777 to -0.00227**	*	**Yes**
σ_{PSII}	ANOVA	Source of Variation	% of total variation	P value	P value summary	Significant?
		Interaction	**5.26**	**0.0293**	*	**Yes**
		Region/Species	**83.2**	**< 0.0001**	****	**Yes**
		Iron	**4.94**	**0.011**	*	**Yes**
	Bonferroni's MCT	Low iron vs. High iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Iron effect	**SO**	**4.7**	**1.62 to 7.78**	**	**Yes**
		TO	0.84	-2.24 to 3.92	ns	No
		TC	0.22	-2.86 to 3.30	ns	No
	Tukey's MCT	Low iron	Mean Diff.	95% CI of diff.	Summary	Significant?
	Region effect	**SO vs. TO**	**9.47**	**6.52 to 12.4**	****	**Yes**
		SO vs. TC	**11.2**	**8.28 to 14.2**	****	**Yes**
		TO vs. TC	1.76	-1.19 to 4.71	ns	No
		High iron				
		SO vs. TO	**5.61**	**2.66 to 8.56**	***	**Yes**
		SO vs. TC	**6.75**	**3.80 to 9.70**	***	**Yes**
		TO vs. TC	1.14	-1.81 to 4.09	ns	No

References

1. Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J Mar Res* 28:687-701.
2. Behrenfeld MJ, Milligan AJ (2013) Photophysiological expressions of iron stress in phytoplankton. *Annu Rev Mar Sci* 5:217–46.
3. Marchetti A, Catlett D, Hopkinson BM, Ellis K, Cassar N (2015) Marine diatom proteorhodopsins and their potential role in coping with low iron availability. *The ISME Journal* 9:2745–2748.
4. Marchetti A, Schruth DM, Durkin CA, Parker MS, Kodner RB, Berthiaume CT, et al. (2012) Comparative metatranscriptomics identifies molecular bases for the physiological responses of phytoplankton to varying iron availability. *Proc Natl Acad Sci USA* 109:E317-E325
5. Moreno CM, Lin Y, Davies S, Monbureau E, Cassar N, Marchetti A (2018) Examination of gene repertoires and physiological responses to iron and light limitation in Southern Ocean diatoms. *Polar Biol* 41: 679. https://doi.org/10.1007/s00300-017-2228-7
6. Strzepek RF, Hunter KA, Frew RD, Harrison PJ, Boyd PW (2012), Iron–light interactions differ in Southern Ocean phytoplankton. *Limnol Oceanogr* 57:1182–1200.