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Quality–quantity trade-offs drive functional trait evolution in a model microalgal ‘climate change winner’

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1 Title: **Quality-quantity tradeoffs drive functional trait evolution in a model microalgal**
2 **“climate change winner”**

3

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8

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10

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28

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33

34 **Abstract**

35 Phytoplankton are the unicellular photosynthetic microbes that form the base of aquatic ecosystems,
36 and their responses to global change will impact everything from food web dynamics to global
37 nutrient cycles. Some taxa respond to environmental change by increasing population growth rates
38 in the short-term, and are projected to increase in frequency over decades. To gain insight into how
39 these projected “climate change winners” evolve, we grew populations of microalgae in ameliorated
40 environments for several hundred generations. Most populations evolved to allocate a smaller
41 proportion of carbon to growth while increasing their ability to tolerate and metabolise reactive
42 oxygen species (ROS). This tradeoff drives the evolution of traits that underlie the ecological and
43 biogeochemical roles of phytoplankton. This offers evolutionary and a metabolic frameworks for
44 understanding trait evolution in projected “climate change winners”, and suggests that short-term
45 population booms have the potential to be dampened or reversed when environmental amelioration
46 persists.

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54 Microbial primary producers (phytoplankton) form the base of aquatic food webs and fix
55 approximately 40% of global carbon annually (1). Photosynthetic microbes respond to increases in
56 carbon dioxide using plastic responses (short term change in phenotype with no underlying change
57 in genotype) (2, 3) and evolutionary responses (change in the genetic composition of a population
58 over time) (4, 5). Phytoplankton can be grouped based on their roles in biogeochemical cycles
59 (calcifiers, silicifiers, nitrogen fixers, other green), and there is variation in both plastic and
60 evolutionary responses to increased CO₂ availability within and between phytoplankton functional
61 groups (2, 3, 6, 7). This variation has led to the prediction that taxa that can respond to nutrient
62 enrichment (such as increased carbon availability) by increasing their population growth rate will
63 increase in frequency as these conditions persist (2, 8). These taxa are often called “winners” under
64 global change, and it is expected that their influence on food webs and nutrient cycles grow as
65 winners increase in frequency (9), and as eutrophication increases (10, 11). While predictions about
66 the composition and function of future microbial communities of primary producers are based on
67 the increasing importance of lineages that subjectively experience projected environmental changes
68 as improvements, our understanding of evolutionary processes is based predominantly on
69 experiments carried out in stressful, starvation, or toxic environments (10). A nuanced
70 understanding of within-lineage evolution under many aspects of global change thus requires
71 investigating how natural selection acts when organisms initially experience an environmental
72 change as a sustained improvement over their historical environment.

73
74 When short-term trait changes are used to project the characters of functional groups over longer
75 timescales (years or decades), it is assumed that plastic increases in lineage growth rate, alongside
76 changes in functional traits such as photosynthesis, respiration, and cell size, that initially occur in
77 the enriched environment be sustained indefinitely. However, theoretical (11, 12) and (admittedly
78 limited) empirical (13-16) support for this assumption is equivocal (but see (17)). We reason that
79 when populations are exposed to permanently ameliorated environments, they have probably
80 previously experienced those environmental improvements, or similar ones, episodically. For
81 environmental ameliorations such as increases in pCO₂ or other nutrient enrichment, cells may
82 express a plastic response to the new environment that appears (or is known to be) adaptive, given
83 that the environmental improvement is transient. For example, while increasing metabolic rates and
84 thus cell division rates over a few generations can be adaptive, maintaining high metabolic rates is
85 also associated with the increased production of toxic metabolic byproducts, such as reactive
86 oxygen species (ROS) (18) that can damage cellular components including proteins, nucleic acids
87 and membranes (19). Limiting ROS damage is thought to play a role major evolutionary transitions

88 such as the evolution of dedicated germ lines and asymmetric cell division, and can limit the upper
89 rate of cell division possible in organisms without a dedicated germ line (11, 20-22). Similarly, we
90 have shown using simulations that the accumulation of toxic metabolic byproducts can limit
91 population growth rates of unicells with no dedicated germ lines in ameliorated environments, since
92 daughter cells can inherit parental damage and toxins. This can lead to reductions over time in
93 population growth rate if reallocating energy from growth and cell division to repairing damage
94 increases daughter cell survival enough to produce a higher number of viable offspring per unit time
95 than does simply dividing faster but passing along more damage to offspring. This is called
96 Prodigal Son dynamics – an adaptive decrease in population growth rate over time as a consequence
97 of the damage caused by toxic metabolic byproducts that accumulate during an initial period of
98 sustained increases in cell division rates (12). This explanation does not require that environmental
99 amelioration be limited to an increase in inorganic carbon. For simplicity, the experiment here uses
100 an increase in CO₂ only, but environmental amelioration could include any non-transient
101 environmental change that allows a substantial increase in metabolic rates, so could also apply to
102 mixotrophic and heterotrophic organisms. Based on this, we predict that in ameliorated
103 environments the rapidly growing types seen as a result of short term (plastic) responses to
104 environmental amelioration are eventually replaced by types that reallocate energy from growth to
105 repair to produce higher quality (less damaged) daughter cells, and that this succession lead to a
106 decrease in population growth rate over time in the ameliorated environment.

107
108 To investigate how lineage growth rate evolves in ameliorated environments, we evolved five
109 genotypes of the green alga *Chlamydomonas reinhardtii* in single genotype cultures for over ~900
110 rounds of binary fission in nutrient-rich, high pCO₂ environments. Evolved populations from the
111 high pCO₂ environment are called “High” (for High-evolved) populations, and those evolved in the
112 ambient pCO₂ environment are called “Amb” (for Ambient-evolved) populations. Our previous
113 empirical (13-15, 23, 24) and modelling (12) studies showed that decreases in population growth
114 rates can occur under these conditions, but did not provide a general mechanism driving trait
115 reversion in ameliorated environments. Here, we look for evidence of a quality-quantity tradeoff in
116 daughter cell production by measuring or calculating several traits associated with life history
117 (daughter cell production, cell cycle length) and daughter cell quality (ROS tolerance and heat
118 shock recovery). We show that this shift from producing many daughter cells to producing higher-
119 quality daughter cells is associated with shift in traits that are commonly used to understand the role
120 of microbial primary producers in aquatic ecosystems (photosynthesis and respiration rates, cell
121 size, biovolume production).

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Results and Discussion

Plastic responses to environmental enrichment. Because we were interested in the evolutionary consequences of sustained rapid growth exceeding that in a benign, nutrient-replete environment that populations were historically grown in, we used 2000ppm pCO₂ and continuous light in nutrient-replete media as our ameliorated environment. This results in a population growth rate increase of ~12% relative to the same media, temperature, and light conditions with ambient pCO₂, which is near the maximum reported for this species (25). These are not intended to be realistic environments, but rather ones that represent a standard, non-stressful control or ancestral environment (nutrient replete ambient pCO₂) and an improved environment that allows a substantial increase in population growth rate (nutrient replete high pCO₂). Further changes in nutrients, light and pCO₂, or changes in general culturing conditions, did not further increase population growth rates.

Plastic responses to environmental enrichment were measured by growing populations in the nutrient-rich, high pCO₂ environment for two transfers (~9 binary cell divisions). Plastic responses can be seen in Fig 1 by comparing the Amb populations growing in ambient pCO₂ (pink, left hand boxes of each plot) with the Amb populations growing in high pCO₂ (green, middle boxes of each plot). As expected, population growth rate and gross photosynthesis rates increase in the enriched environment in agreement with other studies on this and other microbial systems in marine and freshwater systems (2, 3, 7, 15)(effect of pCO₂ on population growth rate $t_{54}=8.18$, $p < 0.0001$ and gross photosynthesis rate per cell $t_{54}=9.54$, $p < 0.0001$). Plastic responses in respiration also occur in most genotypes, though the direction of response varies between genotypes. In phytoplankton, growth depends crucially on two metabolic fluxes, gross photosynthesis (P, the gross fixation of inorganic carbon (C)) and respiration (R, the gross remineralization of organic C) change (26); carbon use efficiency ($CUE = 1 - R/P$), is the fraction of C available for growth after the catabolic demands of the cell have been met. Carbon use efficiency, and thus the amount of C being allocated to growth relative to non-growth in cells, increases during plastic responses to increased pCO₂ in 4/5 genotypes. The single genotype (cc1691) that slightly decreases CUE under CO₂ enrichment has high CUE under both ambient and high pCO₂ relative to other genotypes. Further increasing CUE for cc1691 may not be possible in this experiment, since no genotype exceeds these CUE values under any conditions.

To understand the underlying life history strategies that led to plastic increases in overall population growth rate, we also measured the number of daughter cells produced per cell cycle, calculated the time needed for one complete cell cycle, and measured shifts in average cell size (Fig 1 panels E-G). Interestingly, both the strategy of increasing total cell cycle length and producing more daughter cells (increasing fecundity but reproducing less often) and decreasing total cell cycle length but producing fewer daughter cells (decreasing fecundity but reproducing more often) occur in response to CO₂ enrichment in *C.reinhardtii*, and strategies are genotype-specific. Both strategies increase population growth rate (μ). While cell size also responds to environmental enrichment, changes are not associated with any particular strategy or rate of population growth increase. Previous work with single genotypes of *C.reinhardtii* has found that the number of daughter cells produced per cell cycle was positively correlated with parent cell size within single populations of that genotype (27). In our study, differences in average cell size do not explain differences in the number of daughter cells produced between different genotypes.

Evolutionary responses to environmental enrichment. All High populations were grown in the high or ambient pCO₂ environments for 90 transfers, corresponding to ~900 rounds of binary fission in the High populations. Evolved trait values are shown as the trait values of the High populations grown at high pCO₂ (Fig 1, blue, right-hand boxes of each plot), so are a combination of evolutionary and sustained plastic responses. In all analyses, “selection environment” refers to the environment that the populations evolved in for 90 transfers, and “assay environment” refers to the environment they were grown in for the trait measurement.

By the end of the experiment, High populations had, on average, decreased their population growth rate relative to Amb populations (effect of selection environment $t_{82} = -4.11$, $p = 0.0001$, assay environment $t_{82} = 2.02$, $p = 0.046$, and interaction term $t_{82} = -2.28$, $p = 0.025$, on population growth rate). This is driven by the two genotypes that reproducibly reduced their growth rate at high pCO₂ relative to Amb populations of the same genotype. See Fig 1A. These data show that environmental amelioration by CO₂ enrichment can drive evolutionary responses, in line with previous studies in this and other microalgal systems (15, 17, 28, 29). There is intraspecific (between genotype) variation in both plastic and evolutionary responses to CO₂ enrichment in *C. reinhardtii*. Despite this variation, there is a pattern where the evolutionary responses can decrease, but not increase, population growth rates relative to the plastic trait value, such that High populations never grow faster, and sometimes grow more slowly than, Amb populations of the same genotype in high pCO₂. This partial or complete reversal of the plastic growth response to CO₂ enrichment is

189 consistent with decreased population growth rates being part of an adaptive phenotype in the
190 ameliorated environment when it occurs, presumably because growth is correlated with other traits
191 that determine cell quality.

192

193 **Quality-quantity tradeoffs in cell production in enriched environments.** Based on previous
194 simulations (11, 12, 20, 21), we hypothesized that the number of daughter cells produced can be
195 limited by their quality in the ameliorated environment. We further expected that quality be related
196 to the ability to tolerate or detoxify metabolic byproducts such as reactive oxygen species (ROS).
197 These data are summarized in Figure 1 and can be seen by comparing the plastic (green, middle
198 bars) trait values with evolved (blue, right hand bars) trait values for each genotype and trait.

199

200 To test whether or not there was evidence of a quality-quantity tradeoff in daughter cell production
201 driving decreased population growth rates in the High populations, we measured daughter cell
202 production (cell fecundity) as the average number of cells produced per mother cell per cell cycle
203 for the High and Amb populations (Figure 1E). Alongside the selection environment, the assay
204 environment, and the interaction between those two (statistics above), the number of daughter cells
205 modulates how the selection environment affects population growth rate (daughter cell x selection
206 environment $t_{82} = 4.00$, $p = 0.001$) and the growth responses of evolved populations to changes in
207 $p\text{CO}_2$ (daughter cell x selection environment x assay environment $t_{82} = -2.28$, $p = 0.025$). In 4/5
208 genotypes, daughter cell production per cell cycle in high $p\text{CO}_2$ decreased by ~1 cell after 90
209 transfers in the high $p\text{CO}_2$ environment. Regardless of whether the plastic response was to increase
210 or decrease fecundity in high $p\text{CO}_2$, the evolutionary response decreased fecundity relative to the
211 plastic trait value. In 4 genotypes, total cell cycle length and average cell volume also decreased
212 relative to the plastic trait values.

213

214 The evolutionary response to high $p\text{CO}_2$ includes slowing down metabolism, as evidenced by lower
215 gross photosynthesis and (for 4/5 genotypes) lower rates of respiration per cell relative to the plastic
216 trait values (Fig 1B-C) (Effect of selection regime on gross photosynthesis rate per cell $t_{110} = -2.52$,
217 $p=0.013$). Gross photosynthesis and respiration do not slow proportionally during evolution in the
218 high CO_2 environment. This is reflected by differences in the plastic and evolutionary CUE values
219 (Fig 1D). Since photosynthesis unavoidably produces toxic metabolic byproducts such as ROS, and
220 higher photosynthetic rates are often associated with higher ROS production (30), lower gross
221 photosynthesis is consistent with decreasing photosystem II activity to limit ROS production. In 4/5
222 High genotypes, evolved CUE is also lower than the plastic trait value. This indicates that less C is

being used for growth, and more for catabolism, in High populations than expected based on the plastic response. The proportion of C allocated to growth decreases over time under persistent environmental amelioration, even if the external environment remains constant.

Along with reducing ROS production, a second possible way to respond to selection against ROS-induced damage is to increase ROS tolerance or detoxification. We tested whether High populations more tolerant to exogenous ROS than Amb populations when both were grown in high pCO₂, and found that they were (Figure 1I) ($t_{53}=11.07$, $p<0.001$). We also found that recovery from heat shock of High populations when grown at high pCO₂ was higher than recovery of Amb populations grown at high pCO₂ ($t_{53}=8.35$, $p<0.001$). The rationale for this measurement is that heat stress affects a wide range of cellular processes, such that low levels of damage with no phenotypic effect in benign environments may be revealed under heat stress (31). Together, these data show evidence of a quantity-quality tradeoff during evolution in an ameliorated environment. The data are consistent with the tradeoff being driven by selection against incurring damage from toxic metabolic byproducts, with ROS being one example of such a byproduct. Populations evolve to produce fewer, but higher-quality daughter cells per cell division cycle, than they do when they initially encounter the CO₂ enriched environment. High populations also recovered more rapidly from heat-shock indicating that daughter cell quality is not entirely environment-specific, as populations were never selected at high temperatures. Interestingly, short term exposure to high pCO₂ increases ROS tolerance (Figure 1I) but decreases recovery from heat shock (Figure 1H). This is consistent with ROS metabolism being stimulated, but cells being in worse overall condition or with energy being reallocated from other processes to ROS metabolism, even after only ~9 rounds of binary fission in high pCO₂.

Rose Bengal generates singlet oxygen, which can be scavenged by pigments, but which also activates an acclimation response that protects the cell from photooxidative damage (32). We also investigated whether high pCO₂-evolved cells showed evidence of increased protection against other reactive oxygen species, by measuring expression levels in key antioxidant genes, which is a strong indication that pathways that mitigate oxidative damage within the cell have been upregulated. To do this, we measured gene expression levels for the production of key enzymes *Chlamydomonas* ROS metabolism in three of the five genotypes in this experiment (cc-1691, cc-2931 and cc-2937) for three genes involved in ROS metabolism, covering both the mitochondria and the chloroplast. Specifically, we measured the expression of *Cat*, *Fsd1* and *Gpx* genes, encoding Catalase, Iron-Superoxide Dismutase and Glutathione Peroxidase, respectively, when

populations were grown in high pCO₂. Gene products are located in either the mitochondrion (CAT), the chloroplast (FSD1), or both (GPX) (33-35). See Figure 2. While expression levels vary with genotype and gene product, we find that in general, High populations have upregulated expression of the ROS metabolism genes measured relative to the Amb populations of the same genotype when grown at high pCO₂ (effects of selection on gene expression level *Cat*: $t_{14}=5.67$, $p=0.0001$, *Fsd1*: $t_{14}=2.05$, $p=0.05$, *Gpx*: $t_{14}=5.48$, $p=0.001$). Previous studies have shown that upregulating *Gpx* alone is sufficient to enhance singlet oxygen resistance in *C.reinhardtii* (18). Overall, better heat shock recovery, an increased ability to deal with both singlet oxygen and superoxide-peroxide-hydroxyl radicals is consistent with a lower CUE in High populations, where more carbon is being allocated to processes other than growth. This reallocation of energy from growth to an increased ability to mitigate damage is consistent with the driving mechanism of Prodigal Son dynamics proposed in our previous model (12).

The majority of our experimental populations showed evidence of Prodigal Son dynamics when evolving in high pCO₂, but there were exceptions. For example, cc125 High populations consistently maintain the same population growth rate and fecundity as the cc125 Amb populations in high pCO₂, despite the High populations having lowered gross photosynthesis rates and higher ROS tolerance. The cc125 High populations also show different directions of trait evolution than the other four genotypes. This indicates that evolving higher ROS tolerance without a decrease in fecundity is possible, at least in this environment.

The impact of Prodigal Son dynamics on projecting functional traits of future populations. To understand how the evolved strategies for growing in a persistent high pCO₂ environment can impact ecological function under environmental amelioration, we report our data as traits commonly used in aquatic ecosystem or biogeochemical models: cell size, biovolume (as a proxy for biomass) production per day, and chlorophyll a production per day (36, 37). See Figures 1G and 3. For four of the five genotypes, cell volume is smaller in High populations than in Amb populations grown at high pCO₂, which would affect both the sinking rate and grazability of the cells, with potential effects on nutrient cycling and trophic interactions. In general, the total biovolume produced per day by High populations is lower than the Amb populations grown at high pCO₂, which is consistent with most of the genotypes of High cells producing fewer, smaller daughter cells. One genotype, cc125, produces many large daughter cells. The total chlorophyll produced per day varies more over the 5 genotypes, illustrating that not only individual traits, but also trait correlations can evolve over time.

291

292 Many aspects of global change are detrimental to large numbers of organisms, but projections of
293 how future aquatic ecosystems will function are necessarily based on the traits of primary producers
294 predicted to survive and even thrive in a high CO₂ world. These projected winners are taxa that
295 respond to CO₂ enrichment by increasing their population growth rates, such as the cyanobacterium
296 *Synechococcus* (2), some nitrogen fixers (38), and some picoeukaryotes (39). Indeed, most
297 phytoplankton functional groups in both marine and freshwater systems have members with
298 positive growth rate responses to CO₂ enrichment (3, 40). Projections of the roles of these winners
299 in future ecosystems is currently based on the indefinite maintenance of increased population
300 growth rates in response to environmental amelioration. In contrast, we show that growth rate
301 responses can reverse in ameliorated environments, and that traits underlying life-history and
302 ecosystem function can shift substantially even in a constant, high-quality environment. Figure 4
303 shows examples of how evolutionary trait changes associated with Prodigal Son dynamics can
304 affect trait calculations used in projections. These examples are illustrative only, but highlight that
305 projections that rely on maintaining the plastic response (green) can differ from those that
306 incorporate trait reversions associated with Prodigal Son dynamics (blue). For population growth
307 rate (4A), the effect of trait reversion on the average trait value is ~5%, and well within the intra-
308 group variation used in projections of phytoplankton responses to increases in pCO₂ (2). In contrast,
309 the effect of trait reversion on biovolume production is ~30%. The magnitude of effects here are for
310 a laboratory study and cannot be extrapolated to natural populations, but our results show that trait
311 reversion has the potential to dramatically alter projections of functional traits of future populations
312 in ameliorated environments. We have used a freshwater laboratory study organism here, but stress
313 that the general mechanism underpinning the quality-quantity trade off in offspring production
314 found here is consistent with results from marine (29) and bacterial (41) studies, as well as general
315 models that do not rely on the specific biology of *Chlamydomonas*, or indeed on primary producers
316 at all (11, 12).

317

318 **Conclusions.** Projections of “climate change winners” are based on scenarios where future winners
319 currently exist in environments to which they are reasonably well adapted, and then experience
320 environmental change as amelioration. Since projected winners are lineages or functional groups
321 that increase in frequency, they must increase their relative growth rates; one way to do this is to
322 increase absolute population growth rates. We show that when the environment goes from good to
323 better and then remains improved over timescales long enough for evolution to occur, a tradeoff
324 between producing high quality offspring and high numbers of offspring can manifest. In the case

325 of this photosynthetic microbe, the decrease in offspring quality in the ameliorated environment is
326 linked to the production and metabolism of ROS, which is a necessary byproduct of photosynthesis
327 and respiration. Populations evolved in the ameliorated environment produce more robust but fewer
328 offspring per cell cycle, which is consistent with selection for tolerating and minimizing damage
329 associated with toxic metabolic byproducts such as ROS at the expense of allocating fixed C to
330 building new cells. Since the underlying metabolic changes involve conserved processes
331 (photosynthesis and ROS production), these dynamics are potentially general in phytoplankton
332 growing in persistently ameliorated environments.

333

334 Assessing how Prodigal Son dynamics can impact projections of traits in “climate change winners”
335 depends on the interplay between interlineage competition (genotype sorting) and intralinear
336 evolution. In this study, cc125 escapes Prodigal Son dynamics, but is not fast-growing relative to
337 the other genotypes. If its growth rate in single-genotype culture is indicative of its growth rate in
338 competition with other genotypes, it would fail to increase in frequency, despite having escaped
339 evolving lower lineage growth rates. If damage accumulation is slow, a genotype with a large
340 plastic response to amelioration, such as cc1952, could outcompete other genotypes, but then
341 experience a decrease in population size when selection against producing fragile offspring
342 intensifies. On the other hand, if Prodigal Son dynamics are rapid, genotypes with the largest plastic
343 growth responses could fail to overgrow competitors before having to reduce their lineage growth
344 rates, leading to the maintenance of more diverse, slower-growing populations in ameliorated
345 environments.

346

347 This study raises the question of which plastic trait changes will revert over time in ameliorated
348 environments. Ecosystem functions such as primary production are modelled using trait values (42),
349 so projections of functional traits in future environments requires predictions of which traits are
350 likely to evolve towards a new value, and which traits are likely to revert over hundreds or
351 thousands of generations. This study begins to answer this question. If the trends in our experiment
352 generalize, then it is reasonable to hypothesize that average cell division rates that far exceed
353 historical average cell division rates cannot be maintained indefinitely without affecting other
354 functional traits through emergent tradeoffs. Our rationale is that the pathways that clean up toxic
355 metabolic byproducts such as ROS have probably not been selected to maintain consistently high
356 activity levels, and we speculate that in most phytoplankton, the episodic nature of conditions
357 favourable to growth (the right combination of light, temperature and nutrients) means that cellular
358 repair could take place during periods of slow or arrested cell division. This would further decrease

359 selection on efficient repair during periods of rapid cell division. In ameliorated environments,
360 natural selection initially favours rapid population growth, it eventually shifts to favour lowering
361 the accumulation of and sensitivity to toxic metabolic byproducts, such as the reactive oxygen
362 generated by photosynthesis, at the expense of allocating fixed carbon to growth. While the
363 strategies used to accomplish this reduction in damage and ROS sensitivity vary between
364 genotypes, some decrease in gross photosynthesis, usually coupled to a decrease in fecundity and
365 population growth rate, is a general feature of the overall strategy for producing higher quality
366 daughter cells in enriched environments. Thus, we hypothesize that trait reversion is more likely for
367 metabolic traits involved in “dirty work” (11) – metabolic work that generates toxic byproducts. In
368 particular, we hypothesize that dirty work pathways that speed up substantially in response to
369 environmental amelioration are more likely to revert than are other traits that respond plastically to
370 the same environmental change.

371

372 This work provides both evolutionary and physiological mechanisms for understanding how
373 photosynthetic unicells respond to environmental amelioration. More broadly, it sheds light into
374 selection pressures that act during sustained, rapid growth in phytoplankton, such as algal blooms in
375 marine and freshwater systems. Taken together with observations that the production of the toxin
376 microcystin during algal blooms (43) may function to stabilize proteins during the late stages of
377 algal growth, this study points towards a general role of natural selection acting to mitigate the
378 effects of toxic metabolic byproducts on cells during rapid population growth. Here, instead of
379 producing compounds that mitigate the effects of toxic metabolic byproducts on proteins, our
380 populations evolved the ability to tolerate and metabolise ROS. Understanding how tradeoffs
381 between functional traits emerge during sustained rapid population growth in phytoplankton opens
382 up a new perspective on understanding how and why bloom-forming algae evolve, as well as how
383 natural selection acts on “climate change winners” over hundreds of generations.

384

385

386 **Methods summary**

387 Detailed methods, calculations, and statistical models are in the supplementary material.

388

389 **Culturing methods and selection experiment.** All populations were started from single cells of
390 *Chlamydomonas reinhardtii* provided by N.Colegrave (University of Edinburgh). Genotypes are
391 catalogued as cc125, cc1691, cc1952 and cc2937 in the Chlamydomonas Resource Collection. Six
392 independent replicate populations of each genotype in each environment were grown, for a total of
393 60 populations in the experiment. All populations were grown in sterile microwell plates in 2mL of
394 Tris-Acetate-Phosphate (TAP) media (44). pCO₂ levels in media were manipulated by pre-bubbling
395 media with air of the desired pCO₂ and then keeping growing plates in CO₂ controlled incubators
396 (13). Every two days the 1% of each population was transferred to a fresh well with pre-bubbled
397 TAP media. Transfer population sizes ranged from ~30 000 to ~65 000 cells over the entire
398 experiment. All populations, regardless of pCO₂ level, were grown at 25°C under continuous light
399 at 170 $\mu\text{mol photons/sec/m}^2$ and shaking at 100rpm. The high and ambient environments differed
400 only in their pCO₂ levels, and were otherwise identical. The entire selection experiment lasted 90
401 transfers, which corresponds to approximately 900 rounds of binary fission in the High populations.
402 Since multiple rounds of binary fission occur per cell cycle, this will correspond to < 900 total cell
403 cycles in most genotypes and treatments.

404

405 **Plastic and evolutionary trait responses.** For all trait assays, populations were acclimated to the
406 assay environment for one growth cycle. For all traits measured, the plastic response is calculated
407 by comparing the trait values of a given population in ambient and high pCO₂. For example, the
408 plastic growth response of a population, regardless of the regime where it evolved, would be
409 calculated by comparing growth at high pCO₂ and growth at ambient pCO₂. The evolutionary
410 response is calculated by trait values of populations evolved in the high pCO₂ environment with the
411 trait values of the same genotype evolved in the ambient pCO₂ environment when grown in a
412 common environment. For example, the evolutionary growth response to high pCO₂ is calculated
413 using the growth rates of High and Amb populations of the same genotype in the high pCO₂
414 environment.

415

416 **Population growth rates.** For all trait assays, populations were acclimated to the assay
417 environment for one growth cycle. For population growth rate measurements, populations were
418 diluted after acclimate in the assay environment and an inoculum of ~40 000 cells were transferred
419 to fresh media at the assay pCO₂. Population growth was calculated as in (45), after 48 hours, as

420 growth = $\ln(N_{48h}/N_{0h})$. Cell number was quantified by measuring absorbance at 750nm and
421 converting to cell number using genotype and environment-specific standard curves. Note that
422 because *C. reinhardtii* can undergo multiple rounds of binary fission per cell cycle, fecundity must
423 be taken into account when calculating how the number of cell cycles is related to the rate of
424 population increase. Since fecundity varied between genotypes and treatments in this experiment,
425 so we report the total number of binary fission events over time to allow comparison between
426 treatments and genotypes, to provide an intuitive link between cell division events and population
427 growth rates, and to allow comparison with other experiments that use *C.reinhardtii*, as this is the
428 usual way of reporting and calculating the length of evolution experiments.

429
430 **Cell fecundity.** *C. reinhardtii* divides by multiple rounds of binary fission (46). The number of
431 daughter cells per mother cell was measured by fixing cells on agar and incubating them in the
432 dark, which arrests cell growth and initiates binary fission (27). Populations were grown to mid-
433 exponential phase in the relevant environments, diluted 10-fold, and plated onto 1.5% TAP agar
434 plates. The plates were incubated in the dark for 12 hours at 25°C. The number of daughter cells per
435 mother cell was scored under a microscope. Time needed for a single cell cycle was calculated from
436 population growth rates and average fecundity.

437
438 **ROS tolerance.** ROS tolerance was assayed on a gradient of Rose Bengal (RB) ranging from 0 to
439 4.5µM. Growing populations were inoculated into fresh growth wells containing TAP and RB and
440 growth was measured over one growth cycle. RB resistance as the amount of RB needed to reduce
441 population growth by 50% was then calculated using a sigmoid decay function.

442
443 **Other trait measurements.** Cells were counted and cell size was measured by flow cytometry
444 using size-bead calibrated forward scatter. Photosynthesis and respiration rates were measured for
445 each population using 2-mL oxygen optodes (PreSens) in pre-bubbled media at the relevant pCO₂
446 level.

447
448 **ROS gene expression.** Gene expression for *Cat*, *Gpx* and *FsdI* were measured using quantitative
449 PCR (qPCR) using previously developed primers(33-35). Primer information is in SI. RNA from
450 independent triplicate acclimated cultures for each population was extracted as per(47). cDNA was
451 synthesized using Bioline's Tetro cDNA kit and cDNA libraries were stored at -20C°. qPCR
452 reactions were prepared using a GoTaq qPRC kit (Promega).

453

454 **Statistical analyses**

455 All statistical analyses, including the models used to produce Figure 4, are detailed in the SI and
456 were carried out in the R environment (final analyses carried out in version 3.5.0, R Core Team
457 (2018). All code will be made available at the time of manuscript acceptance.

458

459

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581 Figure 1

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584 Control, plastic, and evolved trait values for functional traits. Trait values in pink correspond to the
585 Amb populations growing in ambient pCO₂, and correspond to the baseline trait value prior to
586 environmental amelioration. Trait values in green show Amb populations growing in high pCO₂,
587 which corresponds to the plastic response to environmental amelioration. Trait values in blue show
588 High populations growing in high pCO₂, and are the evolved phenotype after ~900 rounds of binary
589 fission in the ameliorated environment. Each point represents the average trait value for a single
590 replicate population, boxes show means for each genotype.

591

592

Figure 2

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597

598 Gene expression levels of three antioxidant genes at high pCO₂. CAT = Catalase, found in the
599 mitochondria; FSD= Iron-Superoxide Dismutase, found in the chloroplast; GPX = Glutathione
600 Peroxidase, found in both locations. Pink dots are for Amb populations growing at high pCO₂, blue
601 dots are for High populations growing at high pCO₂. Each dot is a single independently evolved
602 population, bars show SEM for independent replicate cultures and RNA extractions for each
603 population. For each genotype and gene combination, the average value of the Amb population
604 expression levels is set to 1.0; values shown are relative to this, and so are fold-change in
605 expression level relative to Amb of the same genotype.

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608 Figure 3
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612 Measures of two proxies of primary production. Trait values in pink correspond to the Amb
613 populations growing in ambient pCO₂, and correspond to the baseline trait prior to environmental
614 amelioration. Trait values in green show Amb populations growing in high pCO₂, which
615 corresponds to the plastic response to environmental amelioration. Trait values in blue show High
616 populations growing in high pCO₂, and are the evolved phenotype after ~900 rounds of binary
617 fission in the ameliorated environment. Each point represents the average trait value for a single
618 replicate population, boxes show means for each genotype. For both traits, values are calculated as
619 the amount of either biovolume (A) or chlorophyll a (B) that would be produced starting from a
620 single ancestral cell, through growth and cell division, over a 24 hour period.

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622

623 Figure 4
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627 Visualization of statistical models of trait values, illustrating how projections can be affected by
628 evolutionary responses. Lines indicate change in relative population growth (A) and relative
629 biovolume production per unit time (B-C) explained by pCO₂. Models fit on Amb populations are
630 in green; models fit on High populations are in blue. Shading represents variation in model
631 explained by genotype effects and is constant over pCO₂ levels. Data are as in previous figures,
632 details of statistical models are in SI. In all cases, the average trait value over all strains for the Amb
633 populations at ambient pCO₂ has been set to 1.0; other values are relative to this. In (A),
634 evolutionary responses to high pCO₂ reduce growth rate over all levels of pCO₂, but slopes are
635 similar. All five genotypes are included in this model. In (B) all five genotypes are also included in
636 the model. Strain cc125 did not reduce fecundity or cell size, in contrast to other strains, after
637 evolving under high pCO₂. The evolutionary response to high pCO₂ increases the variance in
638 projections, but does not change the average response relative to projections based on plastic

639 responses alone. In (C), genotype cc125 is excluded; all other genotypes show evidence of Prodigal
640 Son dynamics. Here, evolutionary responses decrease biovolume projections relative to using
641 plastic responses to changes in pCO₂ alone.