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10 **Adaptive responses of free-living and symbiotic microalgae to simulated future ocean**
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24 **Abstract**25 Marine microalgae are a diverse group of microscopic eukaryotic and prokaryotic organisms
26 capable of photosynthesis. They are important primary producers and carbon sinks but their

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27 physiology and persistence are severely affected by global climate change. Powerful
28 experimental evolution technologies are being used to examine the potential of microalgae to
29 respond adaptively to current and predicted future conditions, as well as to develop resources to
30 facilitate species conservation and restoration of ecosystem functions. This review synthesizes
31 findings and insights from experimental evolution studies of marine microalgae in response to
32 elevated temperature and/or $p\text{CO}_2$. Adaptation to these environmental conditions has been
33 observed in many studies of marine dinoflagellates, diatoms and coccolithophores. An
34 enhancement in traits such as growth and photo-physiological performance and an increase in
35 upper thermal limit have been shown to be possible, although the extent and rate of change differ
36 between microalgal taxa. Studies employing multiple monoclonal replicates showed variation in
37 responses among replicates and revealed the stochasticity of mutations. The work to date is
38 already providing valuable information on species' climate sensitivity or resilience to managers
39 and policy-makers but extrapolating these insights to ecosystem and community level impacts
40 continues to be a challenge. We recommend future work should include *in situ* experiments,
41 diurnal and seasonal fluctuations, multiple drivers and multiple starting genotypes. Fitness trade-
42 offs, stable *versus* plastic responses and the genetic bases of the changes also need investigating,
43 and the incorporation of genome resequencing into experimental designs will be invaluable.

44 **Introduction**

45 Marine microalgae are a diverse and polyphyletic group of unicellular, colonial and filamentous
46 eukaryotes and cyanobacteria that carry chlorophyll and (usually) conduct oxygenic
47 photosynthesis (Box 1). Atmospheric CO_2 levels have increased at a geologically unprecedented
48 rate in the Anthropocene (Hönisch et al., 2012). The atmospheric CO_2 level has increased from a
49 pre-industrial ~ 280 ppm to ~ 412 ppm (Tans, 2021) and is predicted to increase to >1000 ppm at
50 the end of the century under RCP 8.5 (IPCC, 2014). The resulting changes in seawater
51 temperatures and carbonate chemistry affect marine microalgae in many ways.

52

Box 1: Diversity of marine microalgae and their life cycles

Here we summarize the characteristics, habitats (Borowitzka, 2018; Matsunaga et al., 2005), species richness (Guiry & Guiry, 2020) and reproductive strategies (Borowitzka, 2018; Gastineau et al., 2016; Matsunaga et al., 2005) of several key marine microalgal phyla.

The **Bacillariophyta** (also known as Heterokontophyta, >16,000 known species) is one of the most diverse marine microalgal phyla. Different species inhabit marine, freshwater and terrestrial environments but diatoms (Bacillariophyceae) are among the major marine groups. *Thalassiosira pseudonana* is one of the most intensely studied diatom species. Diatoms account for about one-fifth of the world's photosynthesis (Nelson et al., 1995) and thus play an important role in marine food webs. Some are economically valuable owing to their use as industrial feedstocks (Baldisserotto et al., 2019). Unlike most microalgae, diatoms are diploid in all life stages (except the gametes). Diatoms predominantly reproduce asexually but short periods of sexual reproduction can be triggered when cells are reduced below a critical size or under certain light conditions.

The **Chlorophyta** (>6,700 known species), commonly known as green algae, mostly occur in freshwater but about 10% are marine. The marine groups consist of the green seaweeds Ulvophyceae and the planktonic prasinophytes. *Chlamydomonas* is one of the most studied unicellular freshwater genera and has been a key model in biofuel research (Scranton et al., 2015). The majority of Chlorophyta have both a vegetative and a sexual cycle, where sexual reproduction can be initiated by nitrogen starvation or a reduction in salinity. During sexual reproduction, the haploid cells of the opposite mating types fuse to form a diploid zygote, the zygospore, which later germinates to form and release the haploid cells.

Cyanobacteria (>4,700 known species) are gram-negative bacteria and the only bacteria capable of oxygenic photosynthesis. They inhabit marine, freshwater and extreme (e.g., polar, hot spring, desert) environments. The marine pelagic groups *Synechococcus* and *Prochlorococcus* are important carbon fixers and contributors to global oxygen production. *Trichodesmium* is one of the most studied marine cyanobacterial genera and it is also an important nitrogen fixer (Zehr et al., 2007). Cyanobacteria only reproduce asexually, but horizontal gene transfer plays an important role in their evolution (Bolhuis et al., 2010; Zhaxybayeva et al., 2006) (Box 2). Natural transformation, one mechanism of horizontal gene transfer, has recently been recorded in several cyanobacterial species (Wendt & Pakrasi, 2019).

Haptophyta (>750 known species) are mostly marine unicellular flagellates whose cells are commonly covered by calcium carbonate scales (coccoliths). They inhabit tropical, subtropical, temperate, subarctic, and subantarctic waters (Eikrem et al., 2017). Coccolithophorids, in particular *Emiliania huxleyi*, are among the best characterized. Coccolithophorids play an important role in marine primary production and carbon cycling. Their life cycle includes both a coccolith-bearing diploid and an organic scale-bearing flagellate haploid stage, but the factors triggering the stage change are unknown.

No sexual reproduction has been observed in *E. huxleyi* so far.

Miozoa (also known as Dinophyta, > 3,500 known species) are mostly marine and mostly represented by dinoflagellates. The majority are free-living, with 7% being parasitic and 1% mutualistic symbionts (Gómez, 2012). Some of the latter form symbioses with a wide range of cnidarians, other invertebrates and protists. Dinoflagellates are the second largest group of marine eukaryotic primary producers (second to diatoms), and many can produce toxins that are detrimental to fish and invertebrates during a bloom (i.e., “red tides”) (Zeigler & Parker, 2017). The dominant vegetative stage of Miozoa is haploid, but a diploid phase and sexual reproduction have been observed and can be triggered by nutrient depletion.

Rhodophyta (>7,000 known species), commonly known as red algae, are mostly macroalgae but a few microalgae (e.g., *Porphyridium* species) are also known. They mainly inhabit tropical and temperate water near the shore. The polysaccharides produced by *Porphyridium* have pharmaceutical (e.g., as an anti-viral and anti-allergic agent; Fleurence & Levine, 2018) and other commercial (e.g., as viscosity stabilizers) values. Only asexual reproduction has been observed in the microalgal taxa, and both haploidy (Sivan et al., 1995) and diploidy (Bhattacharya et al., 2013) have been reported in *Porphyridium* species.

53

54

Box 2. Glossary of terms

Horizontal gene transfer: the acquisition of foreign DNA that subsequently becomes fixed in an organism’s genetic material, through integration into its chromosome or replication as a plasmid.

Adaptation: change in phenotype frequencies within a population from generation to generation owing to allele frequency changes in response to natural selection acting on heritable genetic variation.

Plasticity: environmentally induced changes in phenotypes without underlying changes in the genetic composition of a population. Plastic responses can occur within a generation.

Epigenetic change: a form of plastic response that affects chromatin state, gene expression, and/or protein function via mechanisms such as DNA methylation, histone protein modification, and non-coding RNA-associated gene silencing (e.g., small RNAs), but without

underlying allele frequency change.

Wild type populations: the control populations in experimental evolution studies that are maintained under ambient conditions.

Evolved populations: the populations in experimental evolution studies that are being maintained under novel conditions (e.g., elevated temperatures) for many generations.

Ancestral populations: the ancestors of the wild type and evolved population, prior to the beginning of experimental evolution.

Generation time: for an asexual algal population, generation time is the doubling time, i.e., the time from when a cell is born to the time it divides into two identical daughter cells. For a sexual algal population, generation time refers to the time from when a cell is born to the time a zygote (e.g., planozygote, zygospor) is formed.

55

56 Modeling based on short-term responses of marine microalgae predicts that primary production
57 will decline by up to 20% of over the course of the 21st century under RCP 8.5, due to a
58 combination of thermal stress, increased grazing activities and nutrient limitation (Laufkötter et
59 al., 2015). Higher-than-usual seawater temperatures can negatively affect carbon fixation
60 (Murata et al., 2007), damage the structural integrity of chloroplast thylakoid membranes
61 (Titlyanov et al., 1996) and cause photoinhibition and photodamage of photosystem II (PSII)
62 (Warner et al., 1999). The excessive production of reactive oxygen species (ROS) in the
63 chloroplast under heat stress can damage DNA, proteins and lipids, subsequently inhibiting the
64 repair mechanisms of PSII (Murata et al., 2007). Elevated atmospheric $p\text{CO}_2$ increases the
65 availability of dissolved inorganic carbon and as such can facilitate photosynthesis by
66 microalgae, but at the same time causes ocean acidification that can be detrimental (Reusch &
67 Boyd, 2013). For example, coccolithophores produce calcite scales that sink to the deep ocean,
68 but ocean acidification reduces calcite and aragonite saturation states, limiting growth and
69 interfering with carbon cycling driven by these microalgae (Lohbeck et al., 2012).

70

71 Changes in the microalgal community (e.g., their size, lipid content, C:N:P ratio) caused by
72 elevated temperatures can affect zooplankton grazers and change the biomass structure across
73 trophic levels of food webs (Russell et al., 2013; Yvon-Durocher et al., 2011). A freshwater
74 mesocosm experiment has shown that warming can result in a shift toward smaller-sized

75 individuals with faster turnover, and an increase in zooplankton to phytoplankton biomass ratio
76 (Yvon-Durocher et al., 2011). Further, the effects of global climate change on marine microalgae
77 can extend to other species through symbiosis in organisms such as corals (Box 3).

78

Box 3: Symbiodiniaceae and their hosts

Dinoflagellates in the family Symbiodiniaceae can be free-living, facultative symbionts (i.e., with life stages both within and outside the host) or obligate symbionts. Most Symbiodiniaceae are facultative or obligate symbionts which form associations with many cnidarians (e.g., corals, anemones, jellyfish), other marine invertebrates (e.g., giant clams) and protists (e.g., foraminifera, ciliates). For example, the facultative symbiont *Cladocopium goreaui* has been found in >150 cnidarian host species on the Great Barrier Reef (Tonk et al., 2013). Symbiodiniaceae reside within the coral gastrodermal cells and occur in high density in healthy corals (>10⁶ cells per cm² of coral tissue) (Drew, 1972) (Box Figure 1). They supply the host with energy by translocating fixed organic carbon from photosynthesis and gain protection and inorganic waste metabolites in return (Muscatine, 1967). The leakage of excessive ROS by Symbiodiniaceae experiencing thermal stress is believed to be responsible for coral bleaching, where the coral host expels, digests or removes Symbiodiniaceae cells by apoptotic host cell death (Weis, 2008). The loss of Symbiodiniaceae has a significant impact on the health and survival of the coral host, as indicated by the extensive coral mortality that often follows mass bleaching events (Hughes et al., 2018).

79

80 While the short-term effects of global climate change on marine microalgae have been well
81 studied, less is known about the long-term evolutionary responses of marine microalgae. Those
82 responses will depend on their standing genetic variation, as well as their ability to generate
83 mutations and to recombine genetic variants. In nature, environmental heterogeneity prevents
84 uniform directional selection, avoiding dominance by a few clones and maintaining genetic
85 diversity of microalgal taxa (Bell, 1997; Ryneerson & Armbrust, 2005). High levels of standing
86 genetic variation have been reported in marine microalgae (e.g., Biller et al., 2015; Ryneerson &
87 Armbrust, 2005); for instance, field sampling of the diatom *Ditylum brightwellii* during a bloom
88 found 87% of the cells sampled each day were genetically distinct and at least 2400 genetically
89 distinct clonal lineages co-occurred (Ryneerson & Armbrust, 2005). Evolution may occur via
90 rapid sorting of these existing variants.

91

92 In addition, many microalgae are able to generate genetic variation relatively rapidly because
93 their large population sizes and fast cell division rates facilitate the appearance of novel
94 mutations in existing genes (Scheinin et al., 2015; Sunday et al., 2014). Certain cyanobacteria
95 can also access completely novel genes from exogenous DNA via natural transformation (Wendt
96 & Pakrasi, 2019). Moreover, ploidy state (i.e., the number of homologous chromosome sets per
97 cell) may affect the number and type of mutations that appear in eukaryotic microalgae. While
98 data are scant for microalgae, haploid yeasts have been shown to be more prone to single-
99 nucleotide mutations (especially for DNA replicated later in the cell cycle), whereas diploid
100 yeasts tend to exhibit large changes in genome structure (Sharp et al., 2018).

101
102 Once beneficial novel mutations or genes have appeared, recombination may shuffle them into
103 favourable combinations and further improve the fitness of the population (for review, see Crow,
104 1994). Recombination can be particularly important when the benefit of a certain mutation is
105 only realized in the presence of another mutation (i.e., epistasis). However, recombination
106 depends on sexual reproduction, the extent of which varies greatly among different microalgal
107 taxa (Box 1). While some diatoms, dinoflagellates and green algae periodically reproduce
108 sexually, often in response to environmental triggers such as nutrient depletion, many others,
109 such as cyanobacteria and the unicellular red algae, do not (Box 1). Hence, although many
110 microalgae may be able to generate new genetic variation relatively rapidly, their ability to
111 combine new variants into potentially favourable combinations may be limited by the relatively
112 rarity, or absence, of sexual reproduction.

113 114 **Experimental evolution studies**

115 The aims of experimental evolution studies generally involve understanding evolutionary
116 processes and/or enhancing traits of interest of the study organism(s). It involves the
117 maintenance of replicate populations in different controlled environments for many generations.
118 The great majority of studies relevant to this review have applied elevated temperatures and/or
119 $p\text{CO}_2$ conditions to cultures maintained in the laboratory. Most experiments were initiated with
120 monoclonal asexual cultures, in which case selection responses depended on the occurrence of
121 new mutations. Sexual reproduction was generally either not possible under laboratory
122 conditions or was deliberately avoided to better understand the mechanisms of adaptation.

123 Relatively few experiments were initiated with heterogenous cultures, allowing selection to
124 additionally act on standing genetic variation available from the beginning (Figure 1). Note that
125 the wild type populations used as controls in these studies (i.e., those maintained under ambient
126 conditions) are sometimes called ‘unevolved’ populations in the literature, but we prefer the
127 former terminology because these populations can also accumulate mutations over time and thus
128 their genetic constitutions are not unchanged (Figure 1) (Box 2).

129
130 In general, the assumption has been that the phenotypes of the wild type populations when tested
131 under the novel conditions represent plastic responses only (i.e., physiological adaptations
132 triggered by the environment) (Box 2). In contrast, responses from the ‘evolved’ populations that
133 have been maintained under the novel conditions for many generations include both these short-
134 term plastic responses and stably inherited genetic changes accumulated over generations (for
135 review, see Collins et al., 2020) (Box 2). However, it is increasingly recognized that the ability to
136 mount plastic responses can also be inherited and therefore evolve (Sgrò et al., 2016). Some
137 forms of plastic responses may thus be trans-generational epigenetic changes (Torda et al., 2017)
138 (Box 2). For example, changes in the methylation status of an esterase gene caused by an
139 insecticide treatment can be stable for one to four clonal generations in the aphid *Myzus persicae*
140 (Hick et al., 1996). There have not yet been any direct tests for epigenetic responses in
141 experimental evolution studies on marine microalgae but evidence for methylation exists for
142 various microalgal taxa such as diatoms (Rastogi et al., 2018; Veluchamy et al., 2013),
143 coccolithophores (Yusaff, 2016) and dinoflagellates (de Mendoza et al., 2018).

144
145 For the coral endosymbionts in the family Symbiodiniaceae, selection for increased temperature
146 tolerance via experimental evolution not only facilitates understanding of the algal responses to
147 global climate change, but might also generate evolved stocks that have the potential to enhance
148 climate resilience of the coral holobiont (Buerger et al., 2020; Chakravarti et al., 2017; van
149 Oppen et al., 2015). Given that the natural asexual generation times of Symbiodiniaceae (~3-74
150 days, Wilkerson et al., 1988) are much shorter than the sexual generation times of corals (~3-10
151 years, dela Cruz & Harrison, 2017; reviewed in Jones, 1973), and the growth rate of
152 Symbiodiniaceae can be accelerated further under laboratory culture conditions (Chakravarti et
153 al., 2017), response times should be much shorter for the symbionts compared with their hosts.

154 Cells of Symbiodiniaceae can be isolated from corals, evolved in response to elevated
155 temperature *in vitro* in the laboratory, and reintroduced into corals (Buerger et al., 2020;
156 Chakravarti et al., 2017; van Oppen et al., 2015). In theory, experimental evolution of the algal
157 symbiont could also be carried out *in hospite* but the coral host may limit the symbionts' growth
158 rates and thus evolutionary responses by digesting their cells (Titlyanov et al., 1996) and/or
159 restricting their nitrogen allocation (Rädecker et al., 2015). However, it would be relevant to
160 explore this approach, particularly for obligate symbionts.

161

162 **Experimental evolution under stable environmental conditions**

163 ***Studies showing increased fitness following experiment evolution***

164 Under stable environmental conditions (e.g., without diurnal or seasonal fluctuations in
165 temperature and/or $p\text{CO}_2$), experimental evolution studies of marine dinoflagellates, diatoms and
166 coccolithophores under high temperature and/or $p\text{CO}_2$ conditions have mostly resulted in
167 adaptation to the new environment (Table 1). Thus fitness gains have been demonstrated in traits
168 as diverse as cell growth (Buerger et al., 2020; Chakravarti et al., 2017; Chakravarti & van
169 Oppen, 2018; Huertas et al., 2011; Hutchins et al., 2015; Jin et al., 2013; Lohbeck et al., 2012;
170 Schaum et al., 2018; O'Donnell et al., 2018; Aranguren-Gassis et al., 2019; Benner et al., 2020),
171 polyunsaturated fatty acid content (O'Donnell et al., 2019), photo-physiological performance and
172 extracellular ROS level (Buerger et al., 2020; Chakravarti et al., 2017; Chakravarti & van Oppen,
173 2018). For example, populations of the marine diatom *Thalassiosira pseudonana* evolved under
174 elevated temperature (32°C) achieved similar growth rates at high temperature compared to wild
175 type cells grown under control temperature (22°C) after ~100 asexual generations (Schaum et al.,
176 2018). Selection under elevated temperature (31°C) also improved the ability of *T. pseudonana*
177 to sustain polyunsaturated fatty acid production under high temperature after ~500 asexual
178 generations (O'Donnell et al., 2019). In some cases, experimental evolution also resulted in an
179 increase in optimum temperature for growth (T_{opt}) (Listmann et al., 2016; O'Donnell et al., 2018)
180 and/or maximum persistence temperature (T_{max}) (Listmann et al., 2016).

181

182 Compared to the wild type populations, enhancement of upper temperature tolerance and
183 tolerance range, growth and photo-physiological performance have been observed in
184 Symbiodiniaceae after as little as ~40 asexual generations under elevated temperatures

185 (Chakravarti et al., 2017). Given that all these studies started with monoclonal asexual cultures,
186 the selection responses observed therefore depended on *de novo* mutations. These results suggest
187 that many marine microalgae can, to some extent at least, adapt to high temperature and/or $p\text{CO}_2$
188 conditions via new mutations and (presumably) in the absence of sexual recombination. These
189 findings also highlight the importance of incorporating some evolutionary responses into models
190 predicting microalgal population level changes under global climate change.

191

192 ***Studies finding no fitness benefit from experimental evolution***

193 Just a few experimental evolution studies of marine microalgae maintained under high
194 temperature and/or $p\text{CO}_2$ conditions did not find evidence of adaptation over time. Crawford et
195 al. (2011) and Müller et al. (2010) reported no enhancement in photo-physiological performance
196 and cell growth in diatoms and coccolithophores, respectively, following exposure to elevated
197 $p\text{CO}_2$ for ~65-150 generations. Fitness increases in the same species (*E. huxleyi* and *T.*
198 *pseudonana*) have only been observed after selection over hundreds of additional generations
199 (Table 1). However, caution should be applied when comparing between studies as they differ in
200 source populations, treatment conditions and maintenance routine. In addition, while Chakravarti
201 & van Oppen (2018) found enhancement in photo-physiological performance and growth in
202 strains from three genera of Symbiodiniaceae, a fourth strain of the genus *Durusdinium* did not
203 exhibit an adaptive response. More work is needed to determine if the latter result reflects a
204 general inability of this genus to adapt.

205

206 ***Tracking the time course of adaptation***

207 Time series analyses have rarely been undertaken in experimental evolution studies on marine
208 microalgae propagated under high temperature and/or $p\text{CO}_2$ conditions but those that have, have
209 shown the importance of tracking the dynamics of the adaptation process. For instance,
210 calcification rates of coccolithophores were found to be enhanced after ~200 generations of
211 selection under high temperature and $p\text{CO}_2$ conditions, yet this augmentation disappeared over
212 the next ~500 generations (Benner et al., 2013). In contrast, improvement in coccolithophore
213 growth rates was first observed after ~500 generations under elevated $p\text{CO}_2$ conditions and this
214 benefit was maintained, albeit without further enhancement, over the following ~500 generations
215 (Lohbeck et al., 2012). Similarly, *C. goreau* (Symbiodiniaceae) propagated at elevated

216 temperature showed increased fitness *in vitro* at that temperature after ~80 generations of
217 selection (Chakravarti et al., 2017) and had maintained that adaptation when assessed again at
218 ~120 generations (Buerger et al., 2020).

219
220 In another example, the cyanobacterium *Trichodesmium* showed an increase in growth rate after
221 only a few generations at high $p\text{CO}_2$ conditions, and long-term selection (i.e., another ~850
222 generations) did not result in further growth benefit (Hutchins et al., 2015). However, the
223 enhanced growth rates of these populations under ambient conditions were maintained even after
224 hundreds of subsequent generations growing under ambient conditions and were associated with
225 irreversible shifts in diurnal patterns of nitrogen fixation and enhanced activity of a potentially
226 regulatory DNA methyltransferase enzyme (Hutchins et al., 2015).

227
228 An increase in growth rate is also associated with an increase in cellular damage, which can
229 subsequently limit the maintenance of the enhanced growth rate due to selection for damage
230 avoidance or repair mechanisms (Collins, 2016). The Prodigal Son dynamics theory suggests that
231 the initial increase in growth rate in microbes can force a return to the ancestral growth rate,
232 unless selection has occurred to enhance tolerance to cellular damage, or to reduce the rate of
233 damage or the cost of repair (Collins, 2016). This may explain the variation in long-term
234 responses in the above studies.”

235
236 The extent of plastic responses within a generation is sometimes indicative of the extent (but not
237 direction) of evolutionary responses that can be expected in the longer term (Schaum & Collins,
238 2014). For instance, populations of the green alga *Ostreococcus* that displayed more plastic
239 responses also evolved more (in terms of oxygen evolution rate) under high $p\text{CO}_2$ conditions
240 than populations that had less plastic responses (Schaum & Collins, 2014). However, the
241 phenotypes resulting from short-term plastic responses are not necessarily indicative of the
242 phenotypes that would occur following long-term evolution. For example, exposure to elevated
243 $p\text{CO}_2$ conditions caused an increase in cell size and C:N ratio in the green alga *Ostreococcus*
244 prior to experimental evolution, but these phenotypes were not maintained when the population
245 had time to evolve under elevated $p\text{CO}_2$ and the responses began to reverse after 75-100
246 generations (Schaum et al., 2016).

247

248 To the best of our knowledge only one marine microalgal experimental evolution study, Schaum
249 et al. (2018) discussed below, has carried out genome resequencing in conjunction with time
250 course analysis of the evolutionary process. However the added mechanistic insights generated
251 by such resequencing have been amply demonstrated in bacterial systems such as *Escherichia*
252 *coli* (Good et al., 2017; Lenski et al., 2015; Wisner et al., 2013). For example, Good et al. (2017)
253 conducted whole genome sequencing every 500 generations in 12 *E. coli* populations over
254 60,000 generations of evolution, finding that the genes targeted by selection changed over time
255 and the strength of selection on different genes was altered by epistasis and the cumulative
256 genetic history of the populations. Thus, while the rate of fitness gain decreased over time, new
257 genetic opportunities for adaptation that were not initially available opened up over time.

258

259 ***Divergence among replicate populations***

260 As noted, the responses of monoclonal asexual cultures of microalgae in experimental evolution
261 studies will depend on the timing and order of occurrence of different mutations contributing to
262 the adaptation. Due to the stochasticity of mutations, replicate cultures exposed to the same
263 treatment conditions might therefore be expected to follow different evolutionary trajectories.
264 This has indeed been observed among triplicate asexual populations of several, taxonomically
265 divergent microalgal strains (i.e., green algae, cyanobacteria, dinoflagellates, diatoms and
266 haptophytes) evolved to grow under elevated temperature (Huertas et al., 2011). It is also
267 possible that some replicates will show no fitness improvement as all the mutations required for
268 the trait of interest may not occur together in the one replicate (Huertas et al., 2011). As noted
269 above, variation has also been observed among ten strains derived from a monoclonal mother
270 culture of the Symbiodiniaceae species *C. goreau* and evolved under high temperature (Buerger
271 et al., 2020). While all ten strains were able to maintain positive growth and exhibited less
272 extracellular ROS under high temperature *in vitro* compared with the wild type strain, bioassays
273 *in hospite* showed only three conferred their gained thermal tolerance to the coral larvae
274 holobiont. Transcriptome analysis showed clear differences in gene expression patterns between
275 the tolerant and sensitive symbioses (and the wild type strain), confirming each monoclonal
276 replicate was behaving as an independent evolutionary unit (Buerger et al., 2020).

277

278 ***Upper thermal limits***

279 While field and laboratory studies have shown adaptation to warmer temperatures in many
280 organisms, it is generally accepted that individual species have upper thermal limits beyond
281 which basic physiological processes will be unable to cope. For example, Chakir et al. (2002)
282 studied various *Drosophila melanogaster* and *D. simulans* populations from Europe and North
283 America and found an upper thermal limit of male fertility of up to 30°C. Nevertheless, there is
284 variation between related species in their maximum thermal tolerances which suggests
285 significant scope for adaptation. For example, thermal tolerances in porcelain crabs range from
286 27 to 35°C for temperate species and 36 to 42°C for tropical species, depending on the
287 physiological limits of their heart and nerve functions (Stillman, 2002). In Symbiodiniaceae,
288 photosynthesis is generally impaired above 30°C and ceases completely at 34-36°C (Iglesias-
289 Prieto et al., 1992). Nevertheless, each functional trait (e.g., photosynthesis, carbon fixation,
290 phosphate uptake) of a marine microalga has a unique thermal performance curve (TPC) (Baker
291 et al., 2016), hence the upper thermal limit of a species can depend on the TPCs of these various
292 processes.

293
294 A ratchet design has been adopted in some microalgal experimental evolution studies (e.g.,
295 Chakravarti & van Oppen, 2018; Huertas et al., 2011) as a way of estimating how far an
296 organism's upper thermal limit can be pushed. In such studies, the temperature is increased in a
297 stepwise fashion ('ratcheting') once the algal growth rate is positive, or has reached or exceeded
298 that of a control population maintained at ambient temperature. Multiple genera of
299 Symbiodiniaceae (*Symbiodinium*, *Cladocopium*, *Durusdinium*, *Fugacium* and *Gerakladium*)
300 have been examined using this design but only *Cladocopium*, *Fugacium* and *Gerakladium* were
301 able to grow at 31°C (Chakravarti et al., 2017; Chakravarti & van Oppen, 2018). In a similar
302 vein, Huertas et al. (2011) studied 16 microalgal strains (chlorophytes, dinoflagellates,
303 prasinophytes, diatoms, haptophytes, and cyanobacteria) and found 12 evolved strains (covering
304 all the groups above) were able to grow at 30°C, seven strains at 35°C (covering all the groups
305 except dinoflagellates and diatoms) and only one strain at 40°C (a chlorophyte). In general, they
306 found that the upper thermal limits of the species tested were related to the long-term thermal
307 environment of their natural habitats, with species naturally experiencing larger thermal
308 fluctuations better able to adapt to increasing temperature (Huertas et al., 2011).

309

310 **Experimental evolution under fluctuating temperature and $p\text{CO}_2$ conditions**

311 Diurnal and seasonal fluctuations in seawater temperature and $p\text{CO}_2/\text{pH}$ naturally occur in many
312 marine environments (Shaw et al., 2012; Silverman et al., 2012). Physiological responses of
313 organisms to fluctuating environmental conditions have been shown to differ from their
314 responses to stable conditions in many species, including corals (Chan & Eggins, 2017; Oliver &
315 Palumbi, 2011) and diatoms (Li et al., 2016). For example, Chan & Eggins (2017) found that
316 corals under fluctuating $p\text{CO}_2$ conditions calcified more than those under stable conditions,
317 despite the mean $p\text{CO}_2$ of the two conditions being the same. Fluctuating conditions may affect
318 the strength, efficacy (Schaum et al., 2018) and direction of selection (Collins et al., 2020), and
319 can influence phenotypic plasticity as well, which in turn interacts with the dynamics of selection
320 (Ashander et al., 2016; Chevin et al., 2010).

321

322 The evolutionary outcomes under environmental fluctuations are heavily dependent on the time
323 scale over which the fluctuations occur in relation to the organisms' generation time, as well the
324 predictability of these fluctuations (Ashander et al., 2016; Botero et al., 2015).

325

326 Fluctuations within a generation may favour genotypes that are well adapted to all conditions
327 (e.g., high plasticity, good homeostasis). Fluctuations across a small number of generations can
328 result in rapid allelic frequency shifts within one phase but these may be reversed when the
329 conditions change. For example, natural selection favours earlier diapause in the copepod
330 *Diaptomus sanguineus* in years following high densities of a fish predator, and later diapause in
331 years after low predator densities (Hairston & Dillon, 1990). Longer term fluctuations over
332 hundreds of generations may lead to the fixation of genes and phenotypes which are only
333 reversed when new mutations arise (Bell, 2010).

334

335 The sporadic recurrence of extreme events (e.g., heatwaves, floods) is another form of
336 environmental fluctuation, and climate change is increasing the frequency but reducing the
337 predictability of these events (Botero et al., 2015; Haaland & Botero, 2019). Modelling predicts
338 that such unpredictable extremes can also determine what mode of evolutionary response is
339 favoured (Botero et al., 2015). Under some circumstances they can favour a response known as

340 bet-hedging where genotypes that are less competitive under moderate conditions but superior at
341 occasional extremes may be retained in the population, albeit at varying frequencies, over time
342 (Botero et al., 2015; Haaland & Botero, 2019; Simons, 2002).

343
344 Running an experiment with fluctuating temperature and/or $p\text{CO}_2$ conditions is both time- and
345 resource-intensive and has seldom been undertaken on marine microalgae. However more
346 studies have been conducted in the bacterial field (e.g., Ketola et al., 2013; Saarinen et al., 2018)
347 and contrasting results under stable and fluctuating temperatures have been reported. After ~85
348 generations, bacterial populations evolved under fluctuating temperatures (2 h at 20, 30 then
349 40°C and repeat) had higher biomass yield than those that were evolved under stable
350 temperatures (20, 30 or 40°C) when assessed under fluctuating temperatures (Saarinen et al.,
351 2018). However, these populations did not exhibit difference in growth when assessed under
352 stable temperatures of 20, 30 or 40°C (Saarinen et al., 2018).

353
354 Few microalgal studies have utilized fluctuating temperature and/or $p\text{CO}_2$ conditions (Table 1).
355 While stable high $p\text{CO}_2$ (~1000 ppm) and fluctuating high $p\text{CO}_2$ conditions (~1000 ppm,
356 fluctuated to a random value between 700-1300 ppm every seven days) both drove the evolution
357 of new phenotypes of the green alga *Ostreococcus*, those evolved under fluctuating high $p\text{CO}_2$
358 conditions respired more, were smaller in size, and had lower C:N ratio and chlorophyll A
359 content than those evolved under stable high $p\text{CO}_2$ conditions (Schaum et al., 2016). Populations
360 evolved under fluctuating high $p\text{CO}_2$ conditions also exhibited an increase in plasticity compared
361 to the ancestral populations, whereas populations evolved under stable high $p\text{CO}_2$ conditions did
362 not (Schaum & Collins, 2014). These findings suggest that the phenotypes which evolve under
363 stable conditions cannot be used to predict those which evolve under fluctuating conditions
364 (Schaum et al., 2016).

365
366 Schaum et al. (2018) went on to show that populations of the marine diatom *Thalassiosira*
367 *pseudonana* maintained under both stable high temperature (32°C) and fluctuating temperatures
368 (22-32°C, fluctuated every 3-4 generations) had enhanced growth rates after ~300 generations of
369 selection compared to wild type control populations (Table 1). Notably the populations under
370 fluctuating temperatures maintained larger population sizes than those under stable high

371 temperature, which may have assisted their adaptation by reducing the likelihood of stochastic
372 increases in non-adaptive variants.

373

374 Importantly, Schaum et al. (2018) used genome resequencing to compare the genetic
375 composition of their different test populations. They found more divergence among replicate
376 populations in the fluctuating temperatures in terms of single-nucleotide variants in protein
377 coding regions than in either the ancestral populations or the populations evolved under stable 22
378 or 32°C, supporting theory that more complex environments lead to greater diversity (Replansky
379 & Bell, 2009). They also observed copy number variations among population across several
380 large genomic regions, suggesting large scale duplications and deletions are potentially important
381 mechanisms in microalgal adaptation responses. Copy number changes are important to
382 adaptation as each copy may evolve separately, resulting in functional diversity. The Schaum et
383 al. (2018) study is thus exemplary both for its incorporation of fluctuating environmental
384 conditions in its design and for its use of genome resequencing in its analysis.

385

386 In addition to environmental fluctuations, many marine microalgae have diurnal physiological
387 rhythms (Box 4) which could also determine both the nature of, and response to, the selection.
388 However, we are unaware of any experimental evolution studies of these organisms which have
389 investigated this issue.

390

Box 4: Diurnal cell cycle of microalgae

Diurnal light: dark fluctuations are inbuilt in the cell cycle of marine microalgae and a light: dark cycle is always used in microalgal experimental evolution studies. This inbuilt cell cycle involves three phases, G1(assimilation), S (DNA replication) and G2 + M (cell division and mitosis) and the proportion of cells in the different phases within a microalgal population varies during the day. Notwithstanding some differences between the various phyla, cell division and mitosis generally occur during the dark period (Müller et al., 2008; Yamashita & Koike, 2016). In diatoms, maximum photosynthetic capacity and electron transport rate are associated with the G1 phase (Claquin et al., 2004), whereas formation of their silica frustules is associated with the G2 + M phase (Claquin et al., 2002). In coccolithophores, calcification occurs only during the G1 cell cycle phase and ceases during the S and G2 + M phases (Müller et al., 2008). When *in vitro*, dinoflagellates in the family Symbiodiniaceae vary between the transient flagellated (motile) stage during the day and the

predominant non-flagellated spherical (coccolithophore) stage at night (Freudenthal, 1962; LaJeunesse, 2017). Hence, cell motility and division and other key functions such as photosynthesis, calcification and silicification vary during the diurnal cycle of marine microalgae, and adaptation to temperature and/or $p\text{CO}_2$ conditions will require modifications to a range of fundamental physiological processes. For the same reason, phenotypes are only comparable if they are measured at the same time of the day and researchers generally take great care to achieve this.

391

392 **Trade-offs**

393 Only a few of the microalgal experimental evolution studies that have found increased fitness in
394 response to selection at high temperature and/or $p\text{CO}_2$ conditions (either stable or fluctuating)
395 have found trade-offs in other components of fitness (Table 1). Buerger et al. (2020), O'Donnell
396 (2018) and Schaum et al. (2018) observed that their evolved populations with improved growth
397 rates at high temperatures had slower growth rates under ambient or low temperatures. In other
398 studies however, better performance of the evolved populations under novel conditions was not
399 linked to poorer performance than the wild type populations under ambient conditions (Lohbeck
400 et al., 2012; Scheinin et al., 2015). In fact in one case, the evolved population of the
401 coccolithophore *E. huxleyi* also had 4% faster growth under ambient conditions than the wild
402 type populations (Lohbeck et al., 2012).

403

404 However, trade-offs may also have existed in other components of fitness that were not
405 examined in these studies. Growth rate is the trait most commonly measured and cultures are
406 often only transferred to the next higher temperature when positive growth has been achieved
407 (Chakravarti et al., 2017; Huertas et al., 2011). An increase in growth corresponds to an increase
408 in cellular demand for resources (e.g., nutrients), which can result in trade-offs with traits
409 competing for the same resources (Hutchins et al., 2015). However, nutrients are made readily
410 available in most microalgal experimental evolution studies and may not be limiting for any trait
411 under those conditions.

412

413 Greater nutrient availability can improve species' capacity to cope with higher temperatures and
414 obscure underlying trade-offs (Thomas et al., 2017; for review, see Snell-Rood et al., 2015). For
415 example, the optimum growth temperature of the marine diatom *T. pseudonana* was 3-6°C lower
416 under low nitrogen and phosphorus conditions compared to those with replete nutrient supplies

417 (Thomas et al., 2017). While populations of the marine diatom *Chaetoceros simplex* evolved at
418 high temperature under both N-limited and N-replete conditions for 200 generations grew faster
419 than the control populations at 32°C, only the populations evolved under N-replete conditions
420 were able to survive in 75% of the trials and grow without delay under 34°C (Table 1)
421 (Aranguren-Gassis et al., 2019). In other words, nitrogen limitation may preclude thermal
422 adaptation. Given the limits on nutrient supply in nature and that nutrient limitation will likely
423 exacerbate the effects of warming, future studies testing for trade-offs in successfully evolved
424 populations would benefit from assessing effects under different levels of nutrient supply.

425

426 **Getting the most out of experimental evolution**

427 Experimental evolution studies to date have provided invaluable insights into the potential of
428 marine microalgae to adapt to a rapidly changing ocean. However, much of the potential of
429 experimental evolution studies has yet to be realized. We see opportunities to improve these
430 studies in three broad areas, namely in the choice of experimental populations and environments
431 and in the nature of the data collected (Figure 2).

432

433 ***Experimental populations***

434 The use of simplified populations, in particular monoclonal asexual starting cultures, has been
435 criticized as a major limitation of experimental evolution work on microalgae challenged with
436 elevated temperature and/or $p\text{CO}_2$ conditions (Collins et al., 2020). This approach provides
437 insight into the role of *de novo* mutations to adaptation (Sunday et al., 2014). Further, if used in
438 conjunction with genome resequencing, invaluable information on mutation rates as well as the
439 number and identity of the genes underpinning adaptive traits and trait values can be obtained.
440 However, the roles of selection on standing genetic variation and sexual recombination in
441 adaptation are overlooked in this case. As previously mentioned, recombination may shuffle
442 mutations and genes into favourable combinations and enable positive epistasis. The importance
443 of recombination in climate change adaptation of microalgae is yet to be explored.

444

445 The common use of long-term laboratory cultures is another issue of concern as these may have
446 lost traits critical for their existence in nature and no longer resemble their wild counterparts
447 (Willis et al., 2020). Research to understand the effects of laboratory domestication is largely

448 lacking in microalgae but the phenomenon has been intensively studied in other organisms,
449 particularly insects. Laboratory adaptation changes the population mean of many traits in insects
450 and can result in unusual selection responses and patterns of genetic correlations between traits,
451 including traits related to climate change tolerance (Hoffmann et al., 2001; Hoffmann & Ross,
452 2018; Sgrò & Partridge, 2000; Simões et al., 2008). For example, desiccation and starvation
453 resistance of *D. melanogaster* was rapidly lost over a three-year period in the laboratory
454 (Hoffmann et al., 2001). In contrast, significant increases in early female fecundity have been
455 found in *D. melanogaster* during laboratory adaptation (Sgrò & Partridge, 2000). In microalgae,
456 one study has shown differences in morphology, nitrogen fixation and toxin cell quota (i.e., total
457 toxin per cell) between daughter cultures of the cyanobacterium *Raphidiopsis raciborskii* after 23
458 years of laboratory domestication (Willis et al., 2020).

459
460 The issues associated with the use of both monoclonal and domesticated populations could be
461 addressed by starting the experiments with mixed cultures recently collected from diverse natural
462 communities (e.g., Scheinin et al., 2015). Allopatric populations that inhabit broad geographic
463 ranges may harbour adaptive genetic variation that facilitates adaptation to climate change
464 (Hoffmann et al., 2001; Kellermann et al., 2015).

465 466 ***Experimental environments***

467 Most experimental evolution studies to date have employed just a single driver in the laboratory
468 (Table 1). Natural populations live in very different and generally much more complex
469 environments and selection regimes than do their laboratory equivalents and the response to
470 selection due to a particular driver may be very different in the presence *versus* absence of other
471 drivers (Brennan et al., 2017; Hoffmann et al., 2001; Kellermann et al., 2015). This is because
472 the genetic correlations and evolutionary trade-offs influencing the response to each driver can
473 vary depending on the responses to others, potentially affecting the outcome of selection (Sunday
474 et al., 2014). For these reasons, extrapolating from the findings from laboratory evolution studies
475 using a single driver to natural populations needs to be done very cautiously (Hoffmann et al.,
476 2001; Hutchins et al., 2015; Kellermann et al., 2015; Scheinin et al., 2015).

477

478 Encouragingly, some laboratory evolution work on microalgae has already been conducted with
479 simultaneous exposure to multiple drivers (e.g., high temperature and low nutrient in marine
480 diatoms: Aranguren-Gassis et al., 2019; high temperature and $p\text{CO}_2$ in coccolithophores: Benner
481 et al., 2013; Listmann et al., 2016; low light, temperature and nutrient in diatoms and
482 haptophytes: Strzepek et al., 2019) (Table 1). One notable study on the freshwater microalga
483 *Chlamydomonas reinhardtii* has conducted experimental evolution under 96 unique
484 environments with combinations ranging from one to eight drivers (Brennan et al., 2017). The
485 study revealed that only a few dominant drivers (i.e., elevated $p\text{CO}_2$, elevated temperature,
486 reduced phosphate and herbicide addition) explained most of the evolutionary changes in
487 growth, but the populations adapted more when these dominant drivers occurred alongside other
488 drivers (Brennan et al., 2017). Since incorporating multiple drivers is not always feasible in a
489 single study, a suggestion has been to test populations with different drivers sequentially (Boyd
490 et al., 2018). This is a step in the right direction, although this approach would not address the
491 potential interactions between responses to the different drivers noted above.

492
493 One very promising approach that could overcome the limitations in the use of both simplified
494 populations and simplified environments in experimental evolution studies is to utilize natural
495 populations in *in situ* experiments. Scheinin et al. (2015) utilized marine diatoms (*Skeletonem
496 marinoi*) from a natural plankton community in an *in situ* $p\text{CO}_2$ -enriched mesocosm to study
497 experimental evolution in the field. Such a design allows the experimental driver (here: high
498 $p\text{CO}_2$) to interact with other natural drivers and biota in the ecosystem. After ~100 generations of
499 selection under 400 and 1100 ppm $p\text{CO}_2$, *S. marinoi* were isolated from the mesocosm to
500 establish monoclonal cultures for fitness assessment in the laboratory. Under elevated $p\text{CO}_2$
501 conditions in the laboratory, the growth rate of the field-evolved *S. marinoi* was found to be 1.3
502 times faster than those evolved under ambient conditions. The $p\text{CO}_2$ -evolved *S. marinoi* also had
503 similar growth rates to the control populations under ambient conditions in the laboratory.
504 Scheinin et al. (2015) acknowledged that they could not determine whether the responses
505 observed were driven directly or indirectly by elevated $p\text{CO}_2$, because the control and $p\text{CO}_2$
506 elevated mesocosms also differed in their phytoplankton communities (Brussaard et al., 2013)
507 and abiotic environments (Schulz et al., 2013).

508

509 Other than *in situ* experiments, the use of mixed populations enclosed in large-scale artificial
510 mesocosms (e.g., Biosphere 2; Atkinson et al., 1999) could also address the limitations caused by
511 simplified populations and environments. In addition, one possible way to further understand the
512 dynamics of adaptation in the wild is by characterizing trait values in natural populations with
513 known evolutionary histories and thermal regimes (Zhong et al., 2020).

514

515 ***The nature of the data collected***

516 Insights provided by experimental evolution studies on marine microalgae can be greatly
517 improved by measuring multiple traits and trait trade-offs, repeating measurements at multiple
518 timepoints, incorporating omics data and investigating the algae's mode of reproduction.
519 Measuring a series of traits and comparing trade-offs under, for instance, different nutrient levels
520 will achieve a more holistic understanding of an organism's evolutionary responses. Assessing
521 fitness at different generations reveals when an adaptation first appears and whether it is
522 maintained in subsequent generations.

523

524 Further, the incorporation of omics data provides deeper insights into the genetic mechanisms
525 underpinning the selection outcomes. For example, resequencing genomes of microalgal cultures
526 through time can be used to estimate mutation rates (both point mutations and copy number
527 variations) and genomic, transcriptomic, proteomic and metabolomic data can all assist
528 researchers in generating hypotheses about mechanisms underlying the observed phenotype, to
529 predict trade-offs, and in some cases, to develop management intervention tools. For instance,
530 transcriptome analysis in Symbiodiniaceae evolved under elevated temperature has revealed
531 lower expression of photosynthesis genes (possibly reducing the production of toxic ROS) in
532 parallel with higher expression of genes involved in carbon fixation and glyoxylate metabolic
533 processes (possibly compensating for the reduced photosynthetic rate) (Buerger et al., 2020).
534 Unveiling the metabolic and genetic basis of differences in key trait values can also be used to
535 direct future genetic engineering efforts aimed at trait enhancement.

536

537 We have seen that major differences in experimental evolution outcomes can occur under the
538 same thermal conditions between microalgal phyla and between species of the same phylum
539 (Chakravarti & van Oppen, 2018; Huertas et al., 2011), which could in part be related to their

540 modes of reproduction. Although not well characterized, the extent of sexual reproduction in
541 marine microalgae is limited and may be non-existent in some groups (Box 1). However, even
542 occasional recombination will create novel combinations of genotypes (Lobkovsky et al., 2016).
543 Some algae are known to switch from mitosis to meiosis under conditions causing physiological
544 stress (Box 1). Meiosis genes have been identified in the draft genome sequence of *S. goreauii*
545 (Liu et al., 2018) and some of these genes show increased expression under thermal stress (Levin
546 et al., 2016).

547
548 Nor is sexual reproduction the only way to enhance genetic diversity. Microalgal taxa such as
549 dinoflagellates have high gene copy numbers (Lin, 2011) and hence can potentially generate high
550 genetic diversity even via mitosis. Asexually reproducing cyanobacteria can boost their
551 evolutionary potential by the acquisition of foreign DNA via horizontal gene transfer (Bolhuis et
552 al., 2010; Zhaxybayeva et al., 2006). Future studies incorporating genome resequencing of
553 cultures will enable us to tease apart the roles of point mutations, copy number changes, sexual
554 reproduction and recombination in their evolutionary outcomes.

555

556 **Conclusions and implications for management**

557 Marine microalgae are vital to their ecosystems as they are major primary producers, oxygen
558 producers, partners of symbiosis, and key members of carbon and nitrogen cycling systems in the
559 ocean. For free-living microalgal taxa, knowledge about their evolutionary responses and
560 adaptability to climate change will become increasingly important for their conservation and
561 management. For the coral endosymbionts, Symbiodiniaceae, experimentally adapted strains
562 may be harnessed to enhance the climate resilience of their host (Buerger et al. 2020). However
563 upscaling physiological and evolutionary responses observed in the laboratory to community and
564 ecosystem level impacts remains a major challenge and aspiration in ecology (Sutherland et al.,
565 2013). Species that exhibit resilience and adaptation in the laboratory may behave very
566 differently to the same driver via indirect effects that cannot be observed in the laboratory. These
567 indirect effects include modifications in habitat, availability of vital resources, competition
568 between species and trophic interactions, all of which can vastly alter the community and
569 ecosystem impacts of climate change (Boyd et al., 2018). Moving forward, *in situ* studies
570 utilizing natural microalgal communities and genome resequencing tools are one way of bridging

571 the gap between the laboratory and the field, providing vital information to managers and policy-
572 makers.

573

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577

578 **Data Availability Statement**

579 There is no data available.

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987 **Figures and figure legends**

988 **Box Figure 1.** Single polyp of the coral *Galaxea fascicularis* showing the symbiotic microalgae
989 Symbiodiniaceae. (a) fluorescence image with a green fluorescent protein filter where the
990 Symbiodiniaceae are red; (b) bright field image where the Symbiodiniaceae are brown.

991
992 **Figure 1.** Evolutionary responses of hypothetical monoclonal and heterogenous microalgal
993 populations under ambient condition or under selection over time. Symbols represent individual
994 algae, triangles represent new mutations, colours indicate genotypes and stars indicate resilient
995 genotypes.

996
997 **Figure 2.** Considerations in terms of population selection, experimental environment and data
998 collection recommended for future experimental evolution studies on marine microalgae. The
999 suitability of these considerations depends on the aims of the study.

Table 1. Summary of experimental evolution studies on marine microalgae maintained at elevated temperature and/or pCO₂. All studies listed in this table employed asexual cultures.

Organism	Stable/ fluctuating conditions	Selection conditions	Conditions tested post selection	Gene- ration/ time	Phenotypic effects	Trade-offs	Reference
Symbiodiniaceae: Cladocopium goreauii	Stable	<u>27</u> , 31°C	Same	~120, 4 years	Growth +ve Reactive oxygen species (less extracellular ROS) +ve Photo-physiological performance (8 out of 10 replicates) +ve	Slower growth than the wild type populations at ambient temperature	Buerger et al., 2020
Symbiodiniaceae: A3c, two types of D1, G3, F1	Stable	<u>27</u> , gradual increase to 34°C	27, 30, 31, 33, 34°C	~41-69, ~1 year	A3c, G3 and F1: Photo-physiological performance +ve Growth +ve D1: Photo-physiological performance -ve Growth -ve	Not observed in the traits measured	Chakravarti & van Oppen, 2018

Symbiodiniaceae: <i>C. goreauti</i>	Stable	<u>27</u> , 31°C	Same	~80, ~2.5 years	Photo-physiological performance +ve Growth +ve Reactive oxygen species (less extracellular ROS) +ve	Not observed in the traits measured. Photo-physiological performance and growth were similar to the wild type populations under ambient conditions	Chakravarti et al., 2017
Marine diatom: <i>Thalassiosira pseudonana</i>	Stable	16, 31°C ^α	10, 16, 26, 31°C	~500, ~2.5 years	Polyunsaturated fatty acid percentages +ve	Not assessed	O'Donnell et al., 2019
Marine diatom: <i>Chaetoceros simplex</i>	Stable	<u>25</u> , 31°C (under N- replete or N-limited conditions) ^β	10/12, 20, 25, 29, 31, 32, 34, 35°C	~100, ~200, time unknown	Growth under high temperatures [§] : after ~100 generations / after ~200 generations +ve	Slower growth at ambient temperature	Aranguren- Gassis et al., 2019

Marine diatom: T. pseudonana	Stable	16, 31°C ^α	10 temperatures across 3- 33°C	~350, time unknown	Optimum temperature for growth (T _{opt}) +ve Maximum growth rates (μ _{max}) +ve Maximum temperature possible for positive growth (CT _{max}) /	Slower growth at lower temperatures	O'Donnell et al., 2018
Marine diatom: T. pseudonana	Stable and fluctuating	22, 26, 32, 22-32°C ^θ	Same	~300, time unknown	Growth (after ~100 generations) +ve Cell size +	Slower growth rate at lower temperatures*	Schaum et al., 2018
Marine diatom: Skeletonema marinoi	Stable but with natural diurnal fluctuations	400, 1100 ppm pCO ₂ (in situ in mesocosms)	400, 1000 ppm pCO ₂ [@]	~100, 107 days	Growth +ve	Not observed in the traits measured. Growth between pCO ₂ evolved and wild type populations under ambient conditions were similar	Scheinin et al., 2015

Marine diatom: T. pseudonana	Stable	<u>380</u> , 760 ppm	Same	~100, 3 months	Photo-physiological performance / Functional cross section of PS II (i.e., wavelength dependent absorption character) /	N/A (due to no fitness gain)	Crawford et al., 2011
Coccolithophore: Emiliana huxleyi	Stable	<u>15</u> , 26.3°C, <u>400</u> , 1100, 2200 ppm (fully factorial)	15, 18, 22, 24, 26, 27°C	~1200, 2.5 years	Optimum temperature for growth (T_{opt}) +ve Maximum persistence temperature (T_{max}) +ve	Not observed in the traits measured	Listmann et al., 2016
Coccolithophore: E. huxleyi	Stable	<u>20°C</u> and <u>400 ppm</u> , 24°C and 900 ppm	Same	~200, ~400, ~700, time unknown	Calcification % +ve Primary production / Inorganic and organic carbon content results varied depending on the generation accessed	Not observed in the traits measured	Benner et al., 2013
Coccolithophore: E. huxleyi	Stable	<u>400</u> , 2200 ppm	Same	~500, 1 year,	Growth +ve	Not observed in the traits measured.	Lohbeck et al., 2013

				~750 and ~1000, 2 years	(first appeared at ~500 generations, and no additional benefit after ~750 and ~1000 generations)	Evolved populations showed 4% increase in growth rates under ambient conditions compared to the wild type populations	
Coccolithophore: <i>Gephyrocapsa oceanica</i>	Stable	<u>390</u> , 1000 ppm	Same	~670, 1.3 years	Growth +ve Photosynthetic carbon fixation +ve C:N ratio -	Not observed in the traits measured	Jin et al., 2013
Coccolithophore: <i>E. huxleyi</i>	Stable	<u>260</u> , 1150 ppm	Same	~150, 98 days	Growth -ve Cellular particulate inorganic carbon: total	N/A (due to no fitness again)	Müller et al., 2010
Coccolithus <i>braarudii</i>		<u>260</u> , 930 ppm		~65, 66 days	particulate nitrogen - Particulate inorganic carbon: particulate organic carbon /		

Green alga: Micromonas polaris	Stable	2, <u>6</u> , 13°C ¶	Same	~170- 260, time unknown	Growth +ve	Not observed in the traits measured	Benner et al. 2020
Green alga: Ostreococcus tauri	Stable and fluctuating	<u>430</u> , 430-630, 1000, 700-1300 ppm	Same and 2000 ppm	~100, 100 days ~400, 400 days	Stable conditions &: Lipid content +ve Oxygen evolution - oxygen consumption - R:P ratio - C:N ratio -	Not observed in the traits measured	Schaum et al. 2016
Green alga: Chlamydomonas	Stable	<u>430</u> , 1050 ppm	Same	~1000, time unknown	Growth / Some replicates showed higher photosynthetic and respiration rates and reduced growth under ambient condition.	N/A (due to no fitness again)	Collins & Bell, 2004
Cyanobacterium Trichodesmium	Stable	<u>380</u> , 750 ppm	Same	~850, 4.5 years	Growth +ve Nitrogen fixation +ve (both occurred within a few generations, but no	Not observed in the traits measured	Hutchins et al., 2015

					further benefit after another ~850 generations of selection)		
Twelve phytoplankton species (16 strains)	Stable	22, 30, 35°C ‡	Same	Varies	Growth # +ve	Not assessed	Huertas et al., 2011

§ The ambient condition is underlined, and the optimal growth condition is reported individually if the information is available.

+ve an increase in fitness compared to the wild type populations. A +ve sign indicates an increase in trait value (e.g. more growth, high percentage, bigger cells) in all cases, except for ROS (where a decrease in trait value equals to higher fitness).

+ an increase in trait value compared to the wild type populations. Its fitness consequence is dependent on the context.

-ve a decrease in fitness compared to the wild type populations. A -ve sign indicates a decrease in trait value in all cases.

- a decrease in trait value compared to the wild type populations. Its fitness consequence is dependent on the context.

/ no change compared to the wild type populations.

^α the temperatures for selection are below or above ancestral thermal optimum of 24-26°C.

^β the ambient condition of which the culture was maintained was 25°C and the native monthly temperature range at the collection site was 19-31°C.

[§] only the evolved populations under N-replete condition were able to survive in 75% of the trials and to grow without delay under 34°C.

^θ the optimum growth temperature was ~27°C.

* for populations grown under fluctuating temperatures only.

@ diatom populations were evolved under treatments in in situ mesocosms for ~107 days, then isolated from the mesocosms and had their growth assessed in the laboratory.

% significant positive effect was observed at ~200 generations only, not at ~700 generations. Calcification was not measured at ~400 generations.

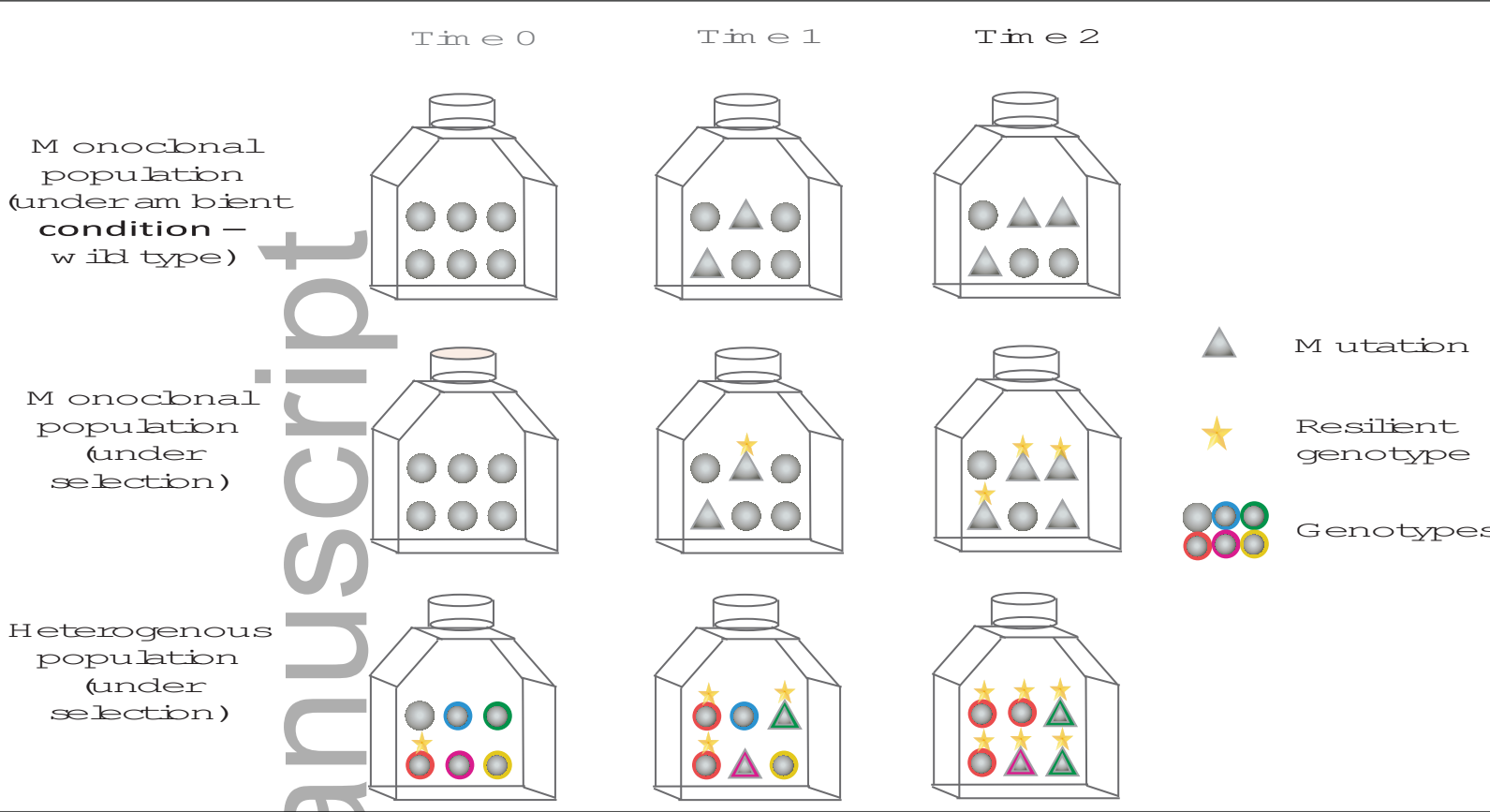
& The phenotypic effects listed are based on the comparison between wild-type population and those evolved under stable high pCO₂ conditions. Population evolved under fluctuation high pCO₂ conditions respired more, were smaller in size, and had lower C:N ratio and chlorophyll a content than those evolved under stable high pCO₂ conditions.

¶ All cultures were under $110 \mu\text{mol photos m}^{-2} \text{ s}^{-1}$ of light, except the 13°C treatment had an addition of high light treatment of $220 \mu\text{mol photos m}^{-2} \text{ s}^{-1}$.

& The phenotypic effects listed are based on the comparison between wild-type population and those evolved under stable high pCO_2 conditions. Population evolved under fluctuation high pCO_2 conditions respired more, were smaller in size, and had lower C:N ratio and chlorophyll a content than those evolved under stable high pCO_2 conditions.

A total of 12 out of 16 evolved strains were able to grow at 30°C (compared to 7 out of 16 of the wild type strains). A total of 7 out of 16 evolved strains were able to grow at 35°C , while only 1 wild type strain was able to grow at that temperature.

‡ The ambient temperature when the strains were isolated ranged from $12\text{-}23^\circ\text{C}$. Refer to Table 1 in Huertas et al. (2011) for details of each strain.



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