Author/s:
Chan, WY;Oakeshott, JG;Buerger, P;Edwards, OR;van Oppen, MJH

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DR. WING YAN CHAN (Orcid ID : 0000-0001-9875-6903)
PROF. MADELEINE JOSEPHINE HENRIETTE VAN OPPEN (Orcid ID : 0000-0003-4607-0744)

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Adaptive responses of free-living and symbiotic microalgae to simulated future ocean conditions

Wing Yan Chan[1], John G. Oakeshott[2,3], Patrick Buerger[1, 2], Owain R. Edwards[2,3],
Madeleine J. H. van Oppen[1,4]

[1] School of BioSciences, University of Melbourne, VIC, Australia
[2] CSIRO Synthetic Biology Future Science Platform, Land & Water, ACT, Australia
[3]Applied Biosciences, Macquarie University, North Ryde, NSW, Australia
[4] Australian Institute of Marine Science, Townsville, QLD, Australia

Correspondence
Wing Yan Chan
Address: School of BioSciences, University of Melbourne, VIC, Australia
Email: w.chan@unimelb.edu.au

Abstract

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

Introduction

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

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.

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, zygospore) is formed.

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

Changes in the microalgal community (e.g., their size, lipid content, C:N:P ratio) caused by elevated temperatures can affect zooplankton grazers and change the biomass structure across trophic levels of food webs (Russell et al., 2013; Yvon-Durocher et al., 2011). A freshwater mesocosm experiment has shown that warming can result in a shift toward smaller-sized individuals with faster turnover, and an increase in zooplankton to phytoplankton biomass ratio (Yvon-Durocher et al., 2011). Further, the effects of global climate change on marine microalgae can extend to other species through symbiosis in organisms such as corals (Box 3).

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 (>10^6 cells per cm^2 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).

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

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

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

Experimental evolution studies

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

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

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

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

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

Experimental evolution under stable environmental conditions

Studies showing increased fitness following experiment evolution

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

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

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

Studies finding no fitness benefit from experimental evolution

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

Tracking the time course of adaptation

Time series analyses have rarely been undertaken in experimental evolution studies on marine microalgae propagated under high temperature and/or $p\mathrm{CO}_2$ conditions but those that have, have shown the importance of tracking the dynamics of the adaptation process. For instance, calcification rates of coccolithophores were found to be enhanced after ~200 generations of selection under high temperature and $p\mathrm{CO}_2$ conditions, yet this augmentation disappeared over the next ~500 generations (Benner et al., 2013). In contrast, improvement in coccolithophore growth rates was first observed after ~500 generations under elevated $p\mathrm{CO}_2$ conditions and this benefit was maintained, albeit without further enhancement, over the following ~500 generations (Lohbeck et al., 2012). Similarly, *C. goreaui* (Symbiodiniaceae) propagated at elevated temperature showed increased fitness *in vitro* at that temperature after ~80 generations of selection (Chakravarti et al., 2017) and had maintained that adaptation when assessed again at ~120 generations (Buerger et al., 2020).

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

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

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

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

Divergence among replicate populations

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

Upper thermal limits

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

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

Experimental evolution under fluctuating temperature and pCO_2 conditions

Diurnal and seasonal fluctuations in seawater temperature and *p*CO2/pH naturally occur in many marine environments (Shaw et al., 2012; Silverman et al., 2012). Physiological responses of organisms to fluctuating environmental conditions have been shown to differ from their responses to stable conditions in many species, including corals (Chan & Eggins, 2017; Oliver & Palumbi, 2011) and diatoms (Li et al., 2016). For example, Chan & Eggins (2017) found that corals under fluctuating pCO_2 conditions calcified more than those under stable conditions, despite the mean pCO_2 of the two conditions being the same. Fluctuating conditions may affect the strength, efficacy (Schaum et al., 2018) and direction of selection (Collins et al., 2020), and can influence phenotypic plasticity as well, which in turn interacts with the dynamics of selection (Ashander et al., 2016; Chevin et al., 2010).

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

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

The sporadic recurrence of extreme events (e.g., heatwaves, floods) is another form of environmental fluctuation, and climate change is increasing the frequency but reducing the predictability of these events (Botero et al., 2015; Haaland & Botero, 2019). Modelling predicts that such unpredictable extremes can also determine what mode of evolutionary response is favoured (Botero et al., 2015). Under some circumstances they can favour a response known as bet-hedging where genotypes that are less competitive under moderate conditions but superior at occasional extremes may be retained in the population, albeit at varying frequencies, over time (Botero et al., 2015; Haaland & Botero, 2019; Simons, 2002).

Running an experiment with fluctuating temperature and/or $p\mathrm{CO_2}$ conditions is both time- and resource-intensive and has seldom been undertaken on marine microalgae. However more studies have been conducted in the bacterial field (e.g., Ketola et al., 2013; Saarinen et al., 2018) and contrasting results under stable and fluctuating temperatures have been reported. After ~85 generations, bacterial populations evolved under fluctuating temperatures (2 h at 20, 30 then 40°C and repeat) had higher biomass yield than those that were evolved under stable temperatures (20, 30 or 40°C) when assessed under fluctuating temperatures (Saarinen et al., 2018). However, these populations did not exhibit difference in growth when assessed under stable temperatures of 20, 30 or 40°C (Saarinen et al., 2018).

Few microalgal studies have utilized fluctuating temperature and/or $p\mathrm{CO_2}$ conditions (Table 1). While stable high $p\mathrm{CO_2}$ (~1000 ppm) and fluctuating high $p\mathrm{CO_2}$ conditions (~1000 ppm, fluctuated to a random value between 700-1300 ppm every seven days) both drove the evolution of new phenotypes of the green alga *Ostreococcus*, those evolved under fluctuating high $p\mathrm{CO_2}$ conditions respired more, were smaller in size, and had lower C:N ratio and chlorophyll A content than those evolved under stable high $p\mathrm{CO_2}$ conditions (Schaum et al., 2016). Populations evolved under fluctuating high $p\mathrm{CO_2}$ conditions also exhibited an increase in plasticity compared to the ancestral populations, whereas populations evolved under stable high $p\mathrm{CO_2}$ conditions did not (Schaum & Collins, 2014). These findings suggest that the phenotypes which evolve under stable conditions cannot be used to predict those which evolve under fluctuating conditions (Schaum et al., 2016).

Schaum et al. (2018) went on to show that populations of the marine diatom *Thalassiosira pseudonana* maintained under both stable high temperature (32°C) and fluctuating temperatures (22-32°C, fluctuated every 3-4 generations) had enhanced growth rates after ~300 generations of selection compared to wild type control populations (Table 1). Notably the populations under fluctuating temperatures maintained larger population sizes than those under stable high temperature, which may have assisted their adaptation by reducing the likelihood of stochastic increases in non-adaptive variants.

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

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

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 (coccoid) stage at night (Freudenthal, 1962; LaJeunesse, 2017). Hence, cell motility and division and other key functions such as photosynthesis, calcification and silication vary during the diurnal cycle of marine microalgae, and adaptation to temperature and/or $p\mathrm{CO}_2$ 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.

Trade-offs

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

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

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

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

Getting the most out of experimental evolution

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

Experimental populations

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

The common use of long-term laboratory cultures is another issue of concern as these may have lost traits critical for their existence in nature and no longer resemble their wild counterparts (Willis et al., 2020). Research to understand the effects of laboratory domestication is largely lacking in microalgae but the phenomenon has been intensively studied in other organisms, particularly insects. Laboratory adaptation changes the population mean of many traits in insects and can result in unusual selection responses and patterns of genetic correlations between traits, including traits related to climate change tolerance (Hoffmann et al., 2001; Hoffmann & Ross, 2018; Sgrò & Partridge, 2000; Simões et al., 2008). For example, desiccation and starvation resistance of *D. melanogaster* was rapidly lost over a three-year period in the laboratory (Hoffmann et al., 2001). In contrast, significant increases in early female fecundity have been found in *D. melanogaster* during laboratory adaptation (Sgrò & Partridge, 2000). In microalgae, one study has shown differences in morphology, nitrogen fixation and toxin cell quota (i.e., total toxin per cell) between daughter cultures of the cyanobacterium *Raphidiopsis raciborskii* after 23 years of laboratory domestication (Willis et al., 2020).

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

Experimental environments

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

Encouragingly, some laboratory evolution work on microalgae has already been conducted with simultaneous exposure to multiple drivers (e.g., high temperature and low nutrient in marine diatoms: Aranguren-Gassis et al., 2019; high temperature and *p*CO2 in coccolithophores: Benner et al., 2013; Listmann et al., 2016; low light, temperature and nutrient in diatoms and haptophytes: Strzepek et al., 2019) (Table 1). One notable study on the freshwater microalga *Chlamydomonas reinhardtii* has conducted experimental evolution under 96 unique environments with combinations ranging from one to eight drivers (Brennan et al., 2017). The study revealed that only a few dominant drivers (i.e., elevated $p\mathrm{CO_2}$, elevated temperature, reduced phosphate and herbicide addition) explained most of the evolutionary changes in growth, but the populations adapted more when these dominant drivers occurred alongside other drivers (Brennan et al., 2017). Since incorporating multiple drivers is not always feasible in a single study, a suggestion has been to test populations with different drivers sequentially (Boyd et al., 2018). This is a step in the right direction, although this approach would not address the potential interactions between responses to the different drivers noted above.

One very promising approach that could overcome the limitations in the use of both simplified populations and simplified environments in experimental evolution studies is to utilize natural populations in *in situ* experiments. Scheinin et al. (2015) utilized marine diatoms (*Skeletonem marinoi*) from a natural plankton community in an *in situ* $p\mathrm{CO_2}$-enriched mesocosm to study experimental evolution in the field. Such a design allows the experimental driver (here: high $p\mathrm{CO_2}$) to interact with other natural drivers and biota in the ecosystem. After ~100 generations of selection under 400 and 1100 ppm $p\mathrm{CO_2}$, *S. marinoi* were isolated from the mesocosm to establish monoclonal cultures for fitness assessment in the laboratory. Under elevated $p\mathrm{CO_2}$ conditions in the laboratory, the growth rate of the field-evolved *S. marinoi* was found to be 1.3 times faster than those evolved under ambient conditions. The $p\mathrm{CO_2}$-evolved *S. marinoi* also had similar growth rates to the control populations under ambient conditions in the laboratory. Scheinin et al. (2015) acknowledged that they could not determine whether the responses observed were driven directly or indirectly by elevated $p\mathrm{CO_2}$, because the control and $p\mathrm{CO_2}$ elevated mesocosms also differed in their phytoplankton communities (Brussaard et al., 2013) and abiotic environments (Schulz et al., 2013).

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

The nature of the data collected

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

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

We have seen that major differences in experimental evolution outcomes can occur under the same thermal conditions between microalgal phyla and between species of the same phylum (Chakravarti & van Oppen, 2018; Huertas et al., 2011), which could in part be related to their modes of reproduction. Although not well characterized, the extent of sexual reproduction in marine microalgae is limited and may be non-existent in some groups (Box 1). However, even occasional recombination will create novel combinations of genotypes (Lobkovsky et al., 2016). Some algae are known to switch from mitosis to meiosis under conditions causing physiological stress (Box 1). Meiosis genes have been identified in the draft genome sequence of *S. goreaui* (Liu et al., 2018) and some of these genes show increased expression under thermal stress (Levin et al., 2016).

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

Conclusions and implications for management

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

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Data Availability Statement

There is no data available.

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Figures and figure legends

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

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

Figure 2. Considerations in terms of population selection, experimental environment and data collection recommended for future experimental evolution studies on marine microalgae. The 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 pCO_2. 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 goreaui	Stable	27, 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	27, 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: C. goreaui	Stable	27, 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: Thalassiosira pseudonana	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: Chaetoceros simplex	Stable	25, 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	380, 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)	Crawfurd et al., 2011
Coccolithophore: Emiliania huxleyi	Stable	15, 26.3°C, 400, 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	20°C and 400 ppm, 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	400, 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: Gephyrocapsa oceanica	Stable	390, 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: E. huxleyi	Stable	260, 1150 ppm	Same	~150. 98 days	Growth $-_{ve}$ Cellular particulate inorganic carbon: total particulate nitrogen - Particulate inorganic carbon: particulate organic carbon /	N/A (due to no fitness again)	Müller et al., 2010
Coccolithus braarudii		260, 930 ppm		~65, 66 days			

Green alga: Micromonas polaris	Stable	2, 6, 13°C[¶]	Same	~170-260, time unknown	Growth $+_{\text{ve}}$	Not observed in the traits measured	Benner et al. 2020
Green alga: Ostreococcus tauri	Stable and fluctuating	430, 430-630, 1000, 700-1300 ppm	Same and 2000 ppm	~100, 100 days ~400, 400 days	Stable conditions[&]: Lipid content $+_{\text{ve}}$ Oxygen evolution - oxygen consumption - R:P ratio - C:N ratio -	Not observed in the traits measured	Schaum et al. 2016
Green alga: Chlamydomonas	Stable	430, 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	380, 750 ppm	Same	~850, 4.5 years	Growth $+_{\text{ve}}$ Nitrogen fixation $+_{\text{ve}}$ (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.

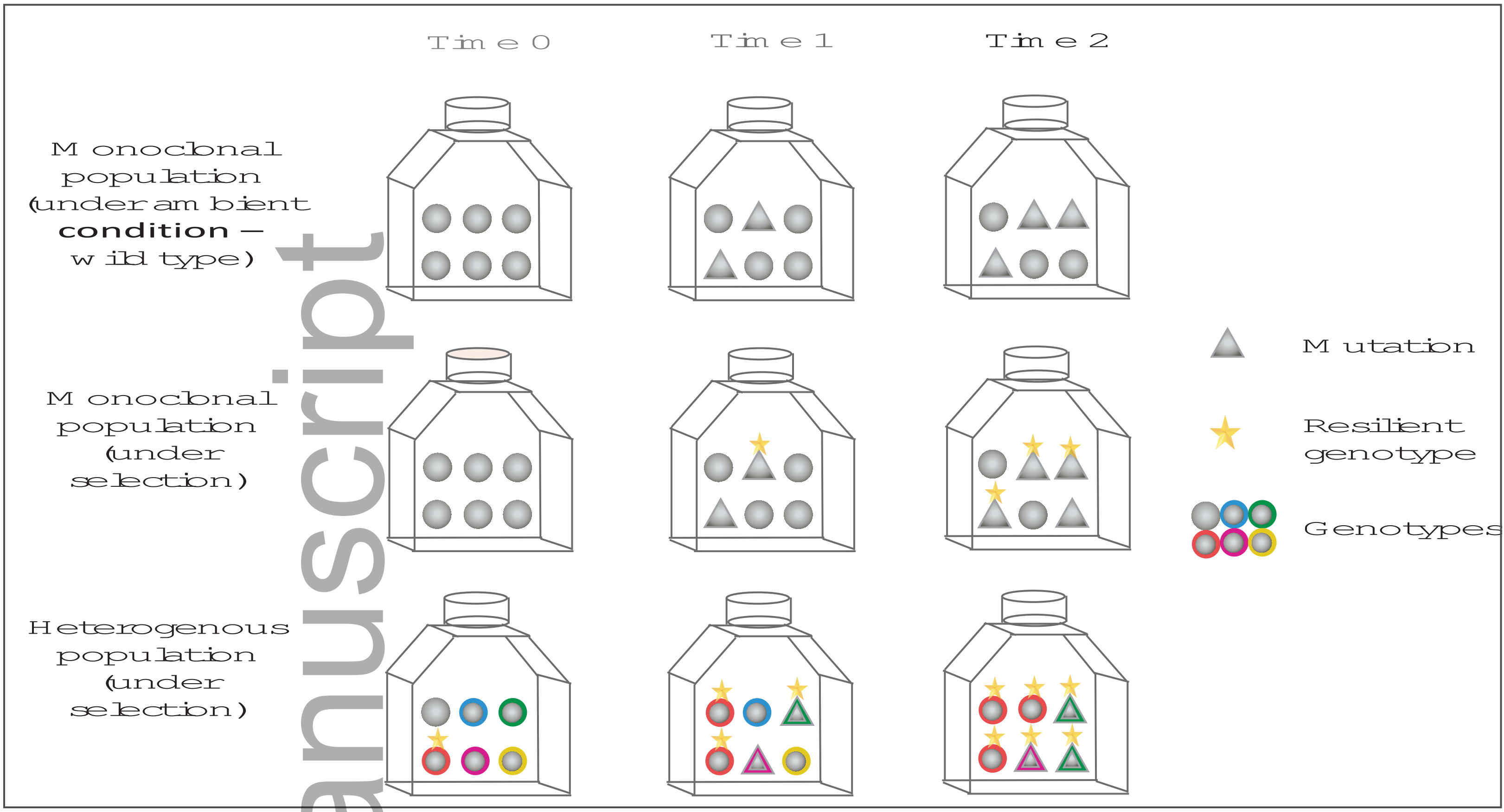
[&] The phenotypic effects listed are based on the comparison between wild-type population and those evolved under stable high pCO_2 conditions. Population evolved under fluctuation high pCO_2 conditions respired more, were smaller in size, and had lower C:N ratio and chlorophyll a content than those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

[¶] All cultures were under 110 µmol photos m^{-2} s^{-1} of light, except the 13°C treatment had an addition of high light treatment of 220µmol photos m^{-2} 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 12-23°C. Refer to Table 1 in Huertas et al. (2011) for details of each strain.



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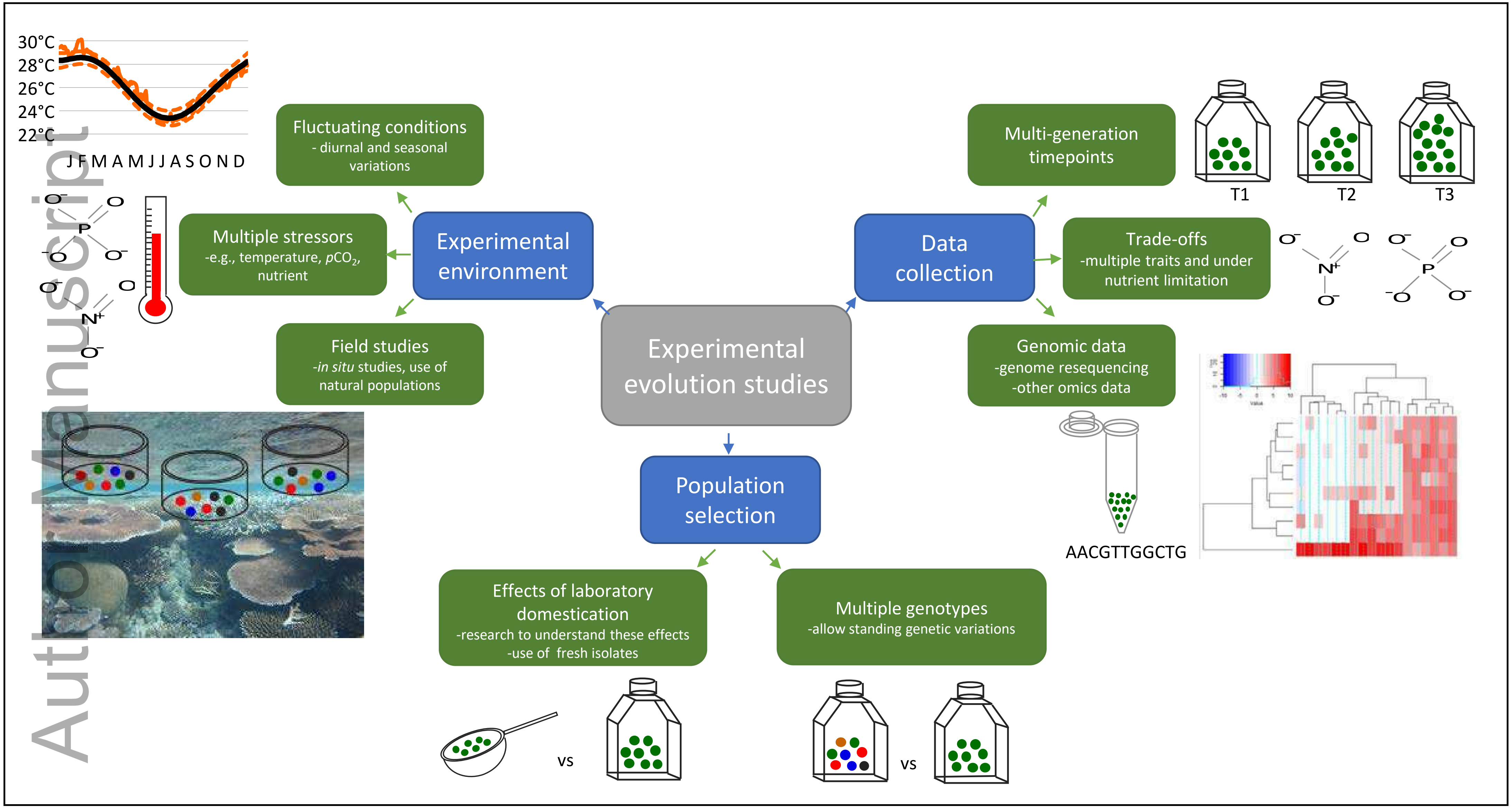
30°C
28°C
26°C
24°C
22°C
J F M A M J J A S O N D
Fluctuating conditions
- diurnal and seasonal variations
Multiple stressors
-e.g., temperature, pCO_2, nutrient
Field studies
-*in situ* studies, use of natural populations
Experimental environment
Experimental evolution studies
Data collection
Multi-generation timepoints
T1
T2
T3
Trade-offs
-multiple traits and under nutrient limitation
Genomic data
-genome resequencing
-other omics data
AACGTTGGCTG
Population selection
Effects of laboratory domestication
-research to understand these effects
-use of fresh isolates
vs
Multiple genotypes
-allow standing genetic variations
vs