Phycologia

ISSN: 0031-8884 (Print) 2330-2968 (Online) Journal homepage: http://www.tandfonline.com/loi/uphy20

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To cite this article: Ellen S. Cameron, Michael T. Arts & Lesley G. Campbell (2018): Mutation in algae – the increasing role of anthropogenic environmental stress, Phycologia

To link to this article: https://doi.org/10.1080/00318884.2018.1517530

PHYCOLOGIA
https://doi.org/10.1080/00318884.2018.1517530

Mutation in algae – the increasing role of anthropogenic environmental stress

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ABSTRACT

Algae are globally important primary producers and when faced with anthropogenic pollutant stress (APS; e.g. heavy metals, herbicides) or naturally occurring stress (NOS; e.g. naturally acidic conditions, high sulphide concentrations), physiological processes may be disrupted. This might lead to abnormal growth and, potentially, individual mortality, or, in extreme cases, extirpation. However, algal populations could persist, in the face of such stressors, if they were able to evolve rapidly based on genetic variation generated through induced mutation and/or recombination. We searched the literature for studies which assessed rates of recombination and mutation under a diversity of environmental conditions. Unfortunately, we did not encounter studies which provided estimates of recombination rates in algae, thus identifying a major gap in the literature. Our meta-analysis of published mutation rates raised the intriguing hypothesis that algae may have higher mutation rates when exposed to APS vs NOS. We conclude that more studies examining algae from diverse habitats are needed to bolster our understanding of the mechanisms behind this increased discrepency in DNA replication, and that elevated mutation rates may contribute to evolutionary rescue in algal populations experiencing declines in water quality on both local and global scales.

ARTICLE HISTORY
Received 7 February 2018
Accepted 13 August 2018
Published online 10 December 2018

KEYWORDS
Algae; Evolutionary potential; Evolutionary rescue; Global change; Induced mutation; Recombination

Algae are a diverse group of organisms occurring in a variety of environments ranging from oceans to moist soils (Gamal 2010). Algae contribute roughly half of global primary productivity and are therefore critical to the survival of life on earth (Chapman 2013; Guiry 2012). As primary producers, algae provide energy and essential fatty acids (e.g. the omega-3, long-chain polyunsaturated fatty acids eicosapentaenoic acid [EPA, 20:5n-3] and docosahexaenoic acid [DHA, 22:6n-3]) to primary and higher order consumers in aquatic systems (invertebrates and fish; Colombo *et al.* 2017; Hixson *et al.* 2015) and to terrestrial organisms through food chain transfers from aquatic to terrestrial systems (Gladyshev *et al.* 2009). Because algae play such fundamental roles in aquatic ecosystems, global primary productivity, human health and alternative energy production, it is important to understand their tolerance of, and responses to, environmental variation, especially in the face of increasing anthropogenic stress. Algal species with unspecialised habitat requirements may tolerate more climatic and ecosystem change than species with specialised requirements. However, vulnerability may be exacerbated when the habitats of algae with narrow range tolerances are particularly sensitive to climate change impacts (e.g. shallow aquatic ecosystems like mangroves or small freshwater lakes).

Human population growth, accompanied by urbanisation and increased agricultural and industrial activities, has altered the physicochemical characteristics of aquatic environments (Feuchtmayr *et al.* 2009; Harley *et al.* 2006; Ma *et al.* 2015; Mayer-Pinto & Ignacio 2015; Priyadarshani *et al.* 2015) with potentially serious consequences for many algal populations (Benedetti-Cecchi *et al.* 2001; Hare *et al.* 2007). For example, anthropogenic pollutant stressors (APS), including pesticides, pharmaceuticals, and municipal wastewater effluents, may be discharged directly into water bodies or enter water bodies through runoff and/or leaching processes and may expose aquatic biota to new chemical stresses either singly or in combination (Carneiro *et al.* 2015; Gracia-Vásquez *et al.* 2014; Kushwaha 2013). Many of these contaminants are resistant to degradation, forcing organisms such as algae to respond physiologically or evolutionarily to their toxic effects (Almeida *et al.* 2014; McKnight *et al.* 2015). In contrast, we categorised naturally occurring stressors (NOS) to include natural variation in water acidity, sulphide concentration, or thermal gradients.

Several mechanisms, including recombination and stress-induced mutagenesis, may accelerate evolution and increase survival of populations in new and stressful environments (Galhardo *et al.* 2007). Enhanced mutation rates increase standing genetic variation and positively influence the rate at which a clonal or sexually reproducing population can evolve in response to selection (Collins & de Meaux 2009; Goho & Bell 2000). Genetic diversity within sexually reproducing algal populations can also increase via genetic recombination during meiosis (Barton & Charlesworth 1998; Grimsley *et al.* 2010; Lagator *et al.* 2014). Because recombination and sex in algal populations tend to occur under particular environmental conditions, one might also

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Supplemental data for this article can be accessed on the publisher's website.

expect recombination rates to respond to environmental variation (Togashi & Cox 2001, 2008). Thus, increased genetic diversity leads to persistence and growth in local populations, even in the face of extreme environmental change (Lagator *et al.* 2014; Smith *et al.* 2012).

Although adaptation of plants in response to NOS may often be accomplished through polygenic inheritance (i.e. the accumulation of minor mutations at several loci; Lande 1983), the development of resistance to toxic substances of anthropogenic origin (i.e. in response to APS) is more frequently a result of major gene evolution (i.e. few mutations in one or few loci of major effect; Kawecki & Ebert 2004; McGregor *et al.* 2007). However, to our knowledge, this kind of analysis has not been extended to include algae. In summary, APS may influence biological evolution in fundamentally unique ways relative to NOS.

Environmental conditions that exceed the physiological tolerance thresholds of algae could result in various levels of ecological impairment for that ecosystem (Harley *et al.* 2006; Hays *et al.* 2005; Occhipinti-Ambrogi 2007). However, the health and persistence of algal populations may be increased if they possess or produce enough genetic variation. This may result from enhanced mutation rates, caused by responses to abiotic challenges, a process known as 'evolutionary rescue' (Bell & Collins 2008; Bell & Gonzalez 2009; Collins & Bell 2004). The probability that a given population will be rescued by evolution depends upon several critical variables: population size, degree of maladaptation (i.e. difference between the average phenotype and optimal phenotype within the population) to the environment or stressor, and the availability of genetic variation in traits that promote survival in the face of environmental variation (Gomulkiewicz & Holt 1995; Holt & Gomulkiewicz 2004). The degree of maladaptation exhibited by populations varies with environments through time (Gomulkiewicz & Holt 1995). Because of their relatively small volumes, shallow freshwater lakes face more rapid changes in water conditions in short timescales; whereas, large marine systems likely do not experience the same level of environmental variability as in these sorts of freshwater systems (Andersen *et al.* 2013; Moisan *et al.* 2002). Organisms can alter their physiology and developmental trajectories through phenotypic plasticity (i.e. nonevolutionary developmental changes), to acclimate to short-term environmental variation (i.e. phenotypic changes within an individual's lifetime (Miner *et al.* 2005; Shaum & Collins 2014). However, plastic responses are often energetically costly (Dewitt & Scheiner 2004; Windig *et al.* 2004). We hypothesised, therefore, that due to the shorter timescales of environmental variability in freshwater compared with marine habitats, freshwater species may respond with phenotypic plasticity over evolved changes in phenotype over generations (i.e. adaptation).

Because phytoplanktonic algal populations are often composed of many ramets (i.e. largely reproducing clonally), population size and the degree of maladaptation are expected to be key drivers promoting evolutionary rescue. We expect that APS, because of its relative novelty (from an evolutionary and ecological perspective) and because organisms tend to adapt to the effects of APS with shifts in genes of major effect, is generally more stressful than NOS. Therefore, we expect that APS will impose selection on parents to generate genetically diverse offspring via elevated mutation or recombination rates in algal populations (Almeida *et al.* 2014; Jarvis & Bielmyer-Fraser 2015; Mayer-Pinto & Ignacio 2015).

We used a meta-analytical approach to evaluate the question: do algae growing in the presence of APS show evidence of elevated mutation rates over and above the mutation rate that might occur in algae exposed to NOS? To assess the potential for algal populations to respond to changing environmental conditions associated with either NOS or APS, we surveyed primary peer-reviewed literature (See Supplemental Appendix 1 for a full list of references and detailed selection criteria) to discern whether algae modify their mutation rate (μ; see Supplemental Appendix 2 for definition), recombination rate [likelihood of recombination occurring during meiosis (θ or r)] or linkage disequilibrium [affiliation between alleles at different loci (r_d or LD); Barton & Charlesworth 1998; Stumpf & McVean 2003] based on habitat type (freshwater vs marine) or exposure to APS vs NOS. We found no studies that reported on algal recombination rate or linkage disequilibrium (a gap in the literature discussed in greater detail below). Therefore, our analysis focused on the response of mutation rate to environmental variation (habitat type and stressor type). We identified studies that used algal stress scenarios to measure mutation. We selected studies that clearly identified algal species, source of stress, and genetic variation generated during the experiment (i.e. mutation rate, μ). Phytoplanktonic taxa were commonly used in evolutionary response studies that we collected, with no representation of benthic macroalgae in our collected dataset. Reported mutation rate estimates in algae were assessed using a modified Luria-Delbrück model (Luria & Delbrück 1943), so we also collected mutation frequency (ρ) and cellular growth rate (β) estimates from these studies (see Supplemental Appendix 2 for definitions). The original Luria–Delbrück's combined experimental and statistical procedure to study resistant variants in bacterial populations was modified for use with a wide group of organisms, from algae to human cells (Cole *et al.* 1976; Costas *et al.* 2001; Crane *et al.* 1996; Jones *et al.* 1994). Note that, although other methods have been used to estimate mutation rate (e.g. Lea & Coulson 1949; Zheng 2002), we did not uncover publications that used these methods for algae.

The mutation rates of 12 algal taxa were reported in the literature sources that met our search criteria (Supplemental Appendix 1), including *Chlamydomonas reinhardtii* (Chlorophyta), *Dictyosphaerium chlorelloides* (Chlorophyta), *Dunaliella tertiolecta* (Chlorophyta), *Emiliania huxleyi* (Haptophyta), *Microcystis aeruginosa* (Cyanobacteria), *Navicula ramossisima* (Heterokontophyta), *Prochloron* sp. (Cyanobacteria), *Pseudabaena planctonica* (Cyanobacteria), *Scenedesmus intermedius* (Chlorophyta), *Scenedesmus* sp. (Chlorophyta), *Spirogyra insignis* (Chlorophyta) and *Tetraselmis seucica* (Chlorophyta). In studies where habitat type could not be identified from the source publication, data points were used only for stressor-type analysis (APS and NOS) and information from that publication was excluded from the habitat-based analysis. The surficial habitat classification into freshwater and marine habitats was identified using the habitat type listed on AlgaeBase (Guiry & Guiry 2018). However, it is important to acknowledge that the categorisation

into freshwater and marine habitats does not account for natural variation and stressors that may be found in individual systems. Though the authors cited here indicated that the particular strains of their study species were either freshwater or marine, we recognise that this distinction is often more complex because, for example, some members of the genera *Dunaliella, Navicula* and *Tetraselmis* may be quite adaptable and thus capable of inhabiting waters of varying salinity. As more data become available, habitat designations of specific strains and species will be better defined, allowing for more precise descriptions of salinity tolerances.

Of the taxa examined, only *M. aeruginosa* and *D. chlorelloides* were reported to be exposed to both APS and NOS; the remaining taxa were exposed either to only APS or to only NOS. Thus, for most of the algal species examined, variation in mutation rates could be due to phylogenetic differences in algal response to stress rather than specifically in response to the differential effect of the two stressor classes (APS or NOS) on algal mutation rates. To account for the effect of phylogeny, species were classified into appropriate phyla. We calculated the arithmetic mean values of mutation rate, mutation frequency and cellular growth rate using species-level data points for each phylum, and data were re-analysed using average values to account for the effect of phylogeny.

We expected that, in aquatic environments, APS would be more stressful than NOS, and that this would result in elevated algal mutation rates in APS- relative to NOS-conditions. Further, we analysed the effect that natural habitat type had on evolutionary potential, to determine whether freshwater populations or marine populations had predictable variation in mutation rate.

Table 1. Stressors imposed on algal populations were categorised as chemical or natural stressors for the purpose of quantitative review.

Stress factor category	Stress factors
Natural	Warm water
	Naturally acidic conditions
	Fumaroles
	Seltzer hot springs
Chemical	Uranium mining
	Simazine
	Chromium VI
	Sulphur
	Formaldehyde
	High metals
	Dimethyl urea
	Osmium
	Copper sulphate
	Glyphosate
	Petroleum
	Diesel
	Osmium
	Lindane
	Diquat
	Chloramphenicol
	Acid wastes
	2,4,6-trinitrotoluene
	3-(3,4-dichlorophenyl)-1,1-dimethylurea
	Tributylin
	Erythromycin

Table 2. Organism classification into water habitat and summary of which category of stressor to which each species was exposed.

Species name	Habitat type	Stressor exposed to
Chlamydomonas reinhardtii P.A.Dangeard	Freshwater	Chemical
Dictyosphaerium chlorelloides (Nauman) Komárek & Perman	Freshwater	Chemical Natural
Dunaliella tertiolecta Butcher	Marine	Chemical
Emiliania huxleyi (Lohman) W.W.Hay & H.P.Mohler	Marine	Chemical
Microcystis aeruginosa (Kützing) Kützing	Freshwater	Chemical Natural
Navicula ramossisima (C.Agardh) Cleve	Marine	Chemical
Scendesmus intermedius Chodat	Freshwater	Chemical
Spirogyra insignis (Hassall) Kützing	Freshwater	Chemical
Tetraselmis seucica (Kylin) Butcher	Marine	Chemical

To that end, we compared mean rank mutation rate and mutation frequency in APS- vs NOS-waters (see Table 1 for categorisation of stressors) and marine vs freshwater species (Table 2) using a Wilcoxon rank sum test. Because μ, ρ, and β are interdependent in the Luria-Delbrück model, we did not analyse the data for cellular growth rate (β).

We found 26 publications, the majority of which were from three laboratories that dealt with this topic. Therefore, the results of our analysis should be useful to foster further inquiry into this research topic and to generate intriguing hypotheses rather than provide a definitive/substantive database from which to draw more sweeping conclusions. We found that marine algae appear to be understudied with respect to their mutation rate in the face of environmental stress (i.e. we did not find any studies that explored the response of mutation rate of marine algae in the face of NOS). Further, because mutation-rate estimates exist for so few algal species, our analysis likely underestimates the diversity of genetic responses that algae may have to environmental variation (Felsenstein 1988).

Here we describe some results of a quantitative analysis of the available literature with the intention of generating predictions and identifying key research directions, rather than identifying definitive patterns. Mutation rates in algal populations varied from 2.35×10^{-7} to 2.76×10^{-5} mutants per cell division; whereas, mutation frequency varied from 1.00×10^{-5} to 3.50×10^{-3} mutants per total number of cells cultured. Mutation rates and mutation frequency were higher for algae from marine compared to freshwater environments ($Z_{72,12} = 4.35$, $P < 0.0001$ and $Z_{72,12} = 4.14$, $P < 0.0001$, respectively) and under conditions of APS relative to NOS ($Z_{56,31} = -5.18$, $P < 0.0001$ and $Z_{56,31} = -5.03$, $P < 0.0001$, respectively; Fig. 1). A subsequent analysis was performed excluding two phyla with small sample sizes, Haptophyta and Heterokontophyta. Consistent with the initial analysis, mutation rates and mutation frequency were still higher for marine environments compared to freshwater environments ($Z_{72,10} = 5.27$, $P < 0.0001$ and $Z_{72,10} = 5.27$, $P < 0.0001$, respectively) and for algae living under conditions of APS. Therefore, we

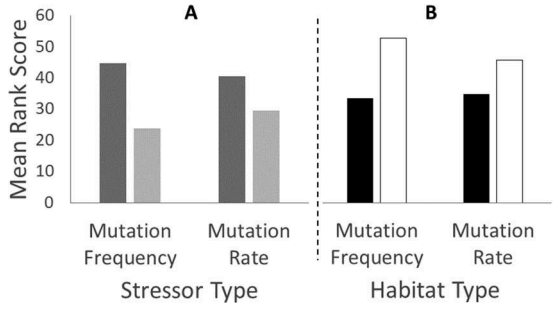

Fig. 1. Algal cellular growth rate, mutation rate and mutation frequency in relation to (a) type of habitat and (b) type of stressor.

conclude, based on current data, that mutation rate and mutation frequency in algae appear to be elevated under exposure to APS relative to NOS, suggesting that APS mutation rates in algae may be environmentally sensitive.

The ability of algae to generate mutations that increase survival and growth in the face of persistent chemical contaminants indicates that there is a potential for algal populations to evolve more rapidly in response to increasing discharge of contaminants into global water sources. This may indicate that NOS does not impose conditions that induce as severe a genetic response as APS, or it may indicate other responses to these conditions (e.g. pre-existing natural variation in mutation rate, high mortality coupled in low-mutation-rate genotypes). A diversity of factors may cause the presence of APS to drive enhanced mutation rates and mutation frequencies in algae, including that APS contaminants are toxicants (whereas NOS may or may not be toxicants) and some have at least mildly mutagenic properties; thus, APS may be expected to cause more DNA replication errors (e.g. Johnson 1998; Tabrez & Ahmad 2011); APS may include harsher stressors, due to their complex structures, (Schwarzenbach *et al.* 2006) than NOS and thus cause more mutations (Tabrez & Ahmad 2011); the additional challenge to algae, because of the xenobiotic nature of many modern contaminants, may enhance mutation (Chen & White 2004; Ohe *et al.* 2004). Alternatively, APS could foster selection for particular genotypes that are more prone to mutation than genotypes selected under NOS conditions (e.g. nitrogen). Moreover, because aquatic ecosystems naturally experience variation in NOS, e.g. temperature and pH fluctuations (Andersen *et al.* 2013; Moisan *et al.* 2002), aquatic organisms may already have nonevolutionary coping mechanisms for experimental treatments that impose extreme levels of these environmental conditions. We encourage future research to discern among these hypotheses. Marine populations of algae exhibited enhanced mutation rates relative to freshwater populations, which was surprising. We postulate that these differences could be driven by the natural environmental variation, such as temperature and pH fluctuations, which occur regularly in freshwater ecosystems (Andersen *et al.* 2013; Moisan *et al.* 2002). This, in turn, results in freshwater organisms opting for quick phenotypic responses to changes in environmental conditions, relative to marine environments.

Our data analysis suggests that, along with the influence of environmental variation on mutation rate, algal phyla varied in their mutation rate from 0 to 0.00001100 mutations per cell (Supplemental Appendix 1: Baos *et al.* 2002; Carrera-Martínez *et al.* 2010; Carrera-Martínez *et al.* 2011; Costas *et al.* 2001, 2007, 2008, 2013; del Mar Fernández-Arjona *et al.* 2013; Flores-Moya *et al.* 2005; García-Balboa *et al.* 2013; García-Villada *et al.* 2002, 2004; González *et al.* 2012; López-Rodas *et al.* 2001, 2007, 2009, 2011; López-Rodas, Marvá, Costas & Flores-Moya 2008; López-Rodas, Marvá, Rouco *et al.* 2008; López-Rodas, Perdigones *et al.* 2008; Marvá *et al.* 2010, 2014; Romero-Lopez *et al.* 2012; Sánchez-Fortün *et al.* 2009; Wurtz *et al.* 1979) when grown under a diversity of environmental conditions (APS or NOS), as described in our results above. This suggests potential for phylogenetically unique responses to environmental stress (Fig. 1). For example, data collected for members of the phylum Chlorophyta, which was well represented in our small dataset, exhibited the most plastic response to environmental variation (i.e. their mutation rate varied the most between environments), relative to Cyanobacteria, Haptophyta or Heterokontophyta. The difference in evolutionary response between phyla identifies interesting taxonomic variation that should be explored in future research. For instance, mutation rates in Chlorophyta were found to be higher than mutation rates in Cyanobacteria, suggesting that members of Chlorophyta may generate genetic variation as an evolutionary response to environmental stressors. Alternatively, members of Cyanobacteria that were included in this study may not be able to evolve in response to environmental stressors and instead may rely more on rapid, plastic responses. However, in gradient stress studies, when stress levels were too extreme, both Cyanobacteria and Chlorophyta experienced population-wide mortality (see Supplemental Appendix 1; Carrera-Martínez *et al.* 2011; Romero-Lopez *et al.* 2012) and, thus, no mutation, suggesting that there will always be a potential threshold for survival and evolutionary response.

Despite the importance of algae in global food webs and the urgency in dealing with global change, research on mutational responses by algae to stressors is still limited. For instance, although research has documented that algae can rapidly adapt to anthropogenic stress (e.g. Bell 2013; Bell & Gonzalez 2009), the source of the genetic variation necessary for this response is relatively underexplored. Moreover, others have hypothesised that, despite documentation of elevated mutation rates in the face of abiotic stress, many species will not be able to generate the necessary adaptive variation to cope with global change (Bradshaw & McNeilly 1991). Thus, persistence of algae in the face of anthropogenic pollution represents an urgent contest among demography, evolutionary history, and contemporary evolutionary processes (Maynard Smith 1989). We suggest that future research should explore which stressful environments lead to the highest mutation rates, and at what mutation rate the opportunity for evolutionary rescue is maximised. Our preliminary analysis suggests that some algal phyla may have greater potential in developing the necessary genetic variation to evolve

when exposed to stress. To further elucidate the phylogenetic differences in evolutionary responses of algal populations, macroalgal species should be researched to determine differences in evolutionary capability between microalgae and macroalgae taxa, especially given that clonal reproduction is far more common in phytoplankton.

Finally, due to the lack of research on this topic, we call for a concerted effort to measure recombination rates in algae. Of the collected papers on the topic of recombination and sexual reproduction in algae, none of the papers include a recombination rate. In some studies, estimates of algal recombination rates were replaced with data collected on unicellular fungi (e.g. recombination in *Saccharomyces paradoxus Bachinskaga* was used as an estimate for *Ostreococcus* in Grimsley *et al.* 2010). Replacing algal mutation rates with that of *S. paradoxus* may be problematic due to their distinct biological differences; these organisms may sexually (vs asexually) reproduce at different rates or maintain different population sizes and densities because one is autotrophic and the other is not (Brand & Guillard 1981; Ruderfer *et al.* 2006). Other reports of recombination and sexual reproduction in algae focus on overall demographic potential of the organism after recombination and sexual reproduction, instead of measuring molecular genetic recombination rates (e.g. Bell 2013; Colegrave *et al.* 2002; Goho & Bell 2000; Kaltz & Bell 2002; Renaut *et al.* 2006). The lack of published recombination rates for algae points to a paucity of fundamental research on the evolutionary mechanisms in these globally important primary producers in response to stress, and makes it impossible to assess the relative importance of mutation and recombination in the generation of genetic diversity within algal populations. The ability to generate new genotypes is fundamental to the process of evolutionary rescue in algal populations. The generation of new diversity will be accelerated when mutation or recombination rates increase under stressful conditions. Because aquatic ecosystems are becoming increasingly stressful environments in the face of global climate change and increased anthropogenic activity, it is critical to understand how elevated mutation rates contribute to algal fitness.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Heather Roshon for useful discussions.

FUNDING

This work was supported by the Natural Sciences and Engineering Research Council (NSERC) Discovery Grants program (#402305-2011 to LGC and #04537-2014 to MTA) and Ryerson University.

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