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# Estimating the Genetic Capability of Different Phytoplankton Organisms to Adapt to Climate Warming

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## Abstract

Current predictions of temperature increase in sea surface water estimate that extensive regions of the ocean will be warmer than at any time in the past million years as a consequence of the present trend of release the $CO_2$ excess into the atmosphere. Studying the capacity of phytoplankton to adapt to warming has become a relevant issue because phytoplankton represents the basis of the aquatic food web supporting about half of the global primary production. Considering the complexity of the phytoplankton community in both taxonomic level and habitat preferences, different responses to increased temperature are expected.We experimentally estimate the potential of different phytoplanktonic populations of 15 species, belonging to different taxonomic groups (Cyanoprokaryota, Dinophyta, Chlorophyta, Haptophyta, Heterokontophyta) and habitat preferences(e.g. coastal waters, open ocean, coral symbiotic), to genetically adapt in an evolutionary sense to marine warming.Since genetic variance in fitness estimates capability for adaptation of a population (Fisher's Fundamental Theorem of Natural Selection) we measured the heritability of fitness (i.e. proportion of variance in fitness that has genetic basis) under increasing temperatures, using an experimental quantitative genetic procedure suitable to phytoplankton populations. Our results reveal that there are interspecific differences in phytoplankton capability for adaptation under a gradual warming process and provides experimental evidences for assessing how phytoplanktonic organisms might evolve under climate warming in the near future.

**Keywords:** Climate change; Genetic adaptation; Global warming; Heritability; Phytoplankton

## Introduction

Climate warming is occurring at an unprecedented rate as a result of human activities. For example, the global mean sea surface temperature increased around 0.678°C during the last century [1]. If the present trend of release of excess $CO_2$ to the atmosphere continues, by the end of the 21st century the sea surface might experience a temperature increase between 1.18°C (under a low $CO_2$ emission scenario) and 6.48°C (under a high $CO_2$ emission scenario) [2]. Consequently, some regions of the ocean will be warmer than at any time in the past million years [3].

Climate warming is causing a variety of emergent challenges for the Earth. Several million populations and many thousand species go extinct annually [4], and nowadays, the massive loss of diversity is the distinctive feature of the Earth [5]. Global warming is expected to have a huge impact on the composition and structure of aquatic ecosystems [6]. Modifications in circulation and stratification patterns are expected, which will have significant results on biota and biogeochemical cycles [7,8]. In this regard, it is troubling that the quantity of phytoplankton on Earth has diminished significantly since 1950 linked to anthropogenic activities [9], and that phytoplankton productivity is decreasing as a consequence of global climate change [7].

However, the effect of global warming on phytoplankton is hardly known, despite the importance of phytoplanktonic organisms. They are the main primary producers in marine and freshwater environments, thereby generating around half of the Earth's atmospheric oxygen through photosynthesis [10,11]. Phytoplankton plays a key role in the biogeochemical cycles of C, P and N [12,13] and fixes around 100 million tons of carbon dioxide per day by mean of the 'biological pump'[14].

Consequently, more investigation on the capacity of adaptation of the different phytoplankton species to temperature increase has interest in order to acquire a further understanding of the future repercussions of climate warming and to make sound predictions about the future.

Phytoplankton includes numerous phyla of prokaryotic and eukaryotic microorganisms, which have different environmental demands, habitat preference and lifestyles[15]. However, from a broad perspective, phytoplankton hasmainlytworesponsesto climate warming: (i) species may disperse to more hospitable habitats, or (ii) species may adapt to the new conditions. In particular, the last mechanism is the most important for the drifting life forms of phytoplanktonic organisms, whose spatial distribution is primarily determined by the motion of the water column.

It is possible to obtain a picture of the capability of different phytoplankton species to adapt to temperature increase based on a classic postulate of evolutionary biology. Fisher [16] published the Fundamental Theorem of the Natural Selection, which says "the rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time". Fisher thought he had made a seminal discovery of biology. Nowadays, Fisher's Fundamental Theorem of Natural Selection is one of the most widely cited works in evolutionary biology. However, most of the citations are in a theoretical context [17-20], but a scarce use was made in experimental approaches.

However, the Fundamental Theorem of the Natural Selection could be an effective tool for understanding the capacity of phytoplankton to adapt to climate warming.

Phytoplanktonic species are particularly suitable to study genetic variability in fitness. From natural populations of each species, numerous strains (genotypes) can be isolated and maintained in large clonal cultures under asexual growth. The growth rate of many genetically identical individuals can be precisely measured using numerous replicates of clonal culturesgrowing under the same environmental conditions [21,22]. The growth rates of clonal cultures of different strains growing under the same environment can be used to calculate fitness of different genotypes, because the absolute values of growth rate estimates the Malthusian parameter of fitness (reviewed in Crow and Kimura [23] andBrand [21]).

The problem of separating genetic and environmental effects upon the observed phenotypic variability in fitness in phytoplankton can be avoided based on two assumptions: (i) that the variability that could be observed among different strains of a same species grown under the same environmental conditions is mainly due to genetic factors; and (ii) that those variations that could be observed among the members of the same strain are mainly due to non-genetic factors, which are categorized as 'residual'. For that reason, if we measure the fitness of all the different strains under the same environmental conditions, then variation observed between different strains growing in a constant environment are predominantly due to genetic factors, whereas variation observed between replicates of the same strain are due to non-genetic factors [24-27]. Consequently, in populations of clonal organisms the adaptive potential can be estimated by the clonal (or broad-sense) heritability in fitness ($H^2$= VG/VP), i.e. the proportion of phenotypic variation in fitness that is due to genetic factors (reviewed in Falconer and MacKay [24] and Rico et al.[25]).

Here, we measured $H^2$ of fitness in populations of various common phytoplankton species belonging to distinct ecological niches (e.g. planktonic and benthic coastal species, open ocean species) under increasing temperatures to estimate the capacity of adaptation to a warming process. This study provides evidence on the differential capabilities of phytoplanktonic organisms to evolve under temperature increase in the near future.

## Material and Methods

### Species, strains and culture conditions

A total of 215 phytoplankton strains isolated from14 populations of 12 phytoplankton species with different habitat preference (i.e. species from coastal marine waters, species from Open Ocean) were used for estimating genetic variability in fitness. In our study we call "population" to a set of clones of the same species, which were isolated from a geographical area and in a relatively short period of timeto be considered from the same population. For example, strains of populations PmC were isolated from water samples collected in La Ría de la Coruña (between 43°23'N and the coastal line located between 8°25'W and 8°21.5'W) during the first 10 days of July. All the coastal species were isolated from four locations, (Ría de Vigo, Ría de Arosa, Ría de la Coruña and Ría de Vivero, ancient coastal valleys submerged by rising sea level) at the NW of Spain. In these areas, temperature varies strongly throughout the annual cycle (e.g. due to strong vertical stratification, upwelling, among other factors). Data on habitat preference, taxonomic group, species, populations, isolation site, number of isolated strains, and temperature of isolation (°C) are summarized in (Table 1).

| Habitat preference<br>Taxonomic group<br>Species | Population | Isolation site | No. of isolated strains | Temperature of(°C)* |
|---|---|---|---|---|
| **Coastalwaters** | | | | |
| **Dinophyta:** | | | | |
| *Prorocentrum micans Ehrenberg* | PmC | (Ría de la Coruña) | 17 | 17 |
| *Prorocentrum micans Ehrenberg* | PmV | (Ría de Vigo) | 13 | 18 |
| *Prorocentrum triestimun Schiller* | PtC | (Ría de la Coruña) | 18 | 17 |
| *Alexandrium minutum Halim* | AmG | (Ría de Arosa) | 22 | 17 |
| *Schrippsiella trochoidea Stein* | StC | (Ría de la Coruña) | 15 | 16 |
| **Chlorophyta** | | | | |
| *Tetraselmis suecica(Kylin) Butch* | TsC | (Ría de la Coruña) | 20 | 13 |
| **Bacillariophyta** | | | | |
| *Chaetoceros curvisetum Cleve* | CcC | (Ría de la Coruña) | 13 | 13 |
| *Skeletonema costatum Cleve* | SkV | (Ría de Vigo) | 21 | 14 |
| Skeletonema costalum *Cleve* | SkL | (Ría de Vivero) | 21 | 15 |
| *Nitzschiaclosterium Ehrenberg* | NcC | (Ría de la Coruña) | 14 | 18 |
| **Heterokontophyta** | | | | |
| *Olisthodiscus luteos Carter* | OlC | (Ría de la Coruña) | 9 | 14 |
| **Open Ocean** | | | | |
| **Cyanophyta** | | | | |
| *Prochlorococcus marinus Chisholm* | PmN | (North Atlantic) | 14 | 15 |
| **Haptophyta** | | | | |
| *Prymnesium polylepis Manton* | PpN | (North Atlantic) | 6 | 14 |
| *Isochrysis galbana Parke* | IgN | (Nord Atlantic) | 12 | 14 |

**Table 1:** Species, populations and strains used to estimate H2 of fitness

Each strain was obtained by isolating a single cell using a micromanipulator (Zeiss Eppendorf), this unique cell was asexually grown under axenic conditions in ventilated cell-culture flasks covered with a filter cap (Greiner, Bio-One Inc., Longwood, NJ, USA) containing 20 ml of f/2 medium (Sigma Aldrich) until obtaining a clonal culture (strain). Strains were placed initially at its isolation temperature under a continuous photon flux density of 60-80 µmol m-2 s-1 over the waveband 400–700 nm provided by cool white fluorescent tubes. The strains were maintained in mid-log exponential growth by serial transfers of a cell inoculum to fresh medium. Under these conditions, all the cultures grew asexually.

### Estimation of fitness values

Malthusian parameter of fitness (r) was estimated as the acclimated maximal growth rates in exponentially growing cultures, according to Crow and Kimura [23] as:

$$r = Log_e (N_t/N_0) / t$$

Where = growth rate, $N_t$and $N_0$ are the cell number at time t = 5 and 10 days, respectively. For this purpose, the values of $N_t$ and $N_0$ were determined at 5 and 10 days after the transference of cells to fresh medium. Cell numbers in experiments and controls were counted (in triplicate) using a particle counter (Beckman CA, USA). In addition, two independent observers also made cell counts by Haemocytometer (Double Neubauer ruling, Fortuna W.G. Co., Germany) to check theaccuracy of the particle counter.Only cultures under balanced growth (mid-log exponential phase) were usedto ensure that the global expression of quantitative traits is independent of the moment when samples are collected from a culture [28]. Mean Malthusian parameter of fitness (ř) of each population was calculated as the mean of the Malthusian fitness of the different strains constituting the population.

The relative fitness (w) (i.e. Darwinian fitness) of each genotype in each population was estimated as the ratio:

$$r_i/r_{max}$$

Where $r_i$ is the Malthusian parameter of the strain i and $r_{max}$ is the maximum Malthusian parameter of the population. Consequently, the maximum value of fitness will be 1. We work with Darwinian fitness to compare different populations and species.

### Calculation of heritability

The observed phenotypic variation (Vp) in fitness is due to variability of genetic factors (Vg) and variability of non-genetic residual factors (Vr). The relative importance of genetic factors on the observed variability of fitness, or broad-sense heritability ($H^2$), was estimated as the ratio between genetic variance (Vg) and phenotypic variance (Vp), this latter considered the sum of genetic Vg and the residual variance (Vr) (in our model, Vr accounts for differences due to non-genetic effects, which are mainly environmental factors). Consequently, a high value of $H^2$ indicates preponderance of genetic factors and low values of $H^2$ indicates preponderance of environmental factors. The ratio Vg/Vp was estimated using analyses of variance (ANOVA) involving among and within clone variation. Ten replicates of each clone were used in each environmental condition. Variation within clones estimates Vr, while that variation among clones is due to Vg + Vr. Consequently, we calculated the genetic variation among clones by subtracting the residual variance from the total phenotypic variance (Vg = Vp-Vr) and estimated heritability as $H^2$= Vg/Vp [24]. More details are given in Rico et al. [25] and López-Rodas et al.[27].

In order not to overestimate the variance of non-genetic factors only cultures fully acclimatized to the experimental conditions were used according to Brand[21] and Costas [26]. Cultures were grown at least for 2 months under the previous constant experimental conditions to ensure full environmental acclimation [21,26].

The magnitude of genetic variability in fitness can vary in different environments due to genotype – environment interaction [24,29]. Therefore, we estimate heritability in fitness in each population in 3 different environments: 1) at the nowadays temperature (isolation temperature); 2) at the predicted sea surface temperature augmentation expected to the end of the 21st century under a low $CO_2$ emission scenario (isolation temperature + 2°C increase); and 3) at the predicted sea surface temperature augmentationexpectedto the end of the 21st century under a high $CO_2$emission scenario (isolation temperature + 7°C increase).

### Time course of the experiments

Logically, the vast amount of experimental work (215 strains x 10 replicates x 3 temperatures) prevents to perform the experiment simultaneously. All the experimental work was extended for 4 years. However, all the different clones of the same population of a given species were analyzed simultaneously.

## Results and Discussion

Estimating genetic variance in fitness under increasing temperatures in different populations of diverse phytoplankton species is a slightly used way to explore the capacity for genetic adaptation to the future thermal scenario in phytoplankton key groups. However, it could be a predictive approach with great potential as was demonstrated by Császár et al. [30] using the heritability ($H^2$) in thermal tolerant traits of different coral algal photosymbionts strains to estimate the potential for adaptation of corals to climate warming. Of course, this conceptual approach constitutes a reductionist line to attack reality, which cannot respond to all the complex aspects of the global warming effects on phytoplankton. However, the reductionist approaches were the key to the development of modern science [31]. Besides, the Neo-Darwinist concept of evolution is based on natural selection acting on genetic variability maintained within the natural populations [32,33].

An important finding of our study is that all the natural populations of diverse phytoplankton species seem to have a high potential for adaptation to a light thermal-stress. In fact, most of populations maintain considerable genetic variation in fitness at the temperature of isolation, as well as under temperatures expected in a scenario of low $CO_2$ emission (Table 2). All strains survived under a slight increase in temperature, and each population maintains a high mean fitness (Table 3).This result is particularly relevant, becauseas there are no data to delimit the borders of the population of phytoplankton organisms, we obtain each experimental population from small areas to ensure no mixing of different populations, which could overestimate the genetic variability.

In contrast, genetic variability in fitness progressively decreases in the phytoplankton populations under the temperatures expected under a high CO2 scenario (Table 2), indicating that, as a general rule, warming exerts a strong selective pressure on phytoplankton. In

several of the populations analyzed the number of strains (genotypes) that survive under high temperatures drops significantly (Table 3). (e.g. in the *P. triestinum* population, nine strains died at the temperature expected under high $CO_2$ scenario). This loss of genetic variability reaches critical values in the population of *N. closterium* , in which only survives one strain at high temperature (Table 3). Of particular concern is that the Malthusian parameters of fitness show a significant reduction as temperature increase (Table 3). In fact, the analyzed phytoplankton populations reached maximum values of mean Malthusian parameter under nowadays temperatures or under the slight increase of temperatures expected in a scenario of low $CO_2$ emission (Table 3). However, mean Malthusian parameter of fitness significantly decrease under the temperatures expected in a high $CO_2$ scenario (Table 3).

| Habitat preference Taxonomic group Species | Population | $H^2$ at nowadays temperature | $H^2$ at low temperature increase | $H^2$ at high increase temperature |
|---|---|---|---|---|
| **Coastal waters** | | | | |
| **Dinophyta:** | | | | |
| *P.micans* | PmC | 0.91 | 0.82 | 0.82 |
| *P.micans* | PmV | 0.87 | 0.86 | 0.84 |
| *P.triestimun* | PtC | 0.69 | 0.69 | 0.21 |
| *A.minutum* | AmG | 0.93 | 0.86 | 0.83 |
| *S.trochoidea* | StC | 0.79 | 0.59 | 0.23 |
| *Chlorophyta:* | | | | |
| *T.suecica* | TsC | 0.95 | 0.91 | 0.86 |
| *Bacillariophyta* | | | | |
| *C.curvisetum* | CcC | 0.90 | 0.74 | 0.53 |
| *S.costatum* | SkV | 0.81 | 0.64 | 0.51 |
| *S.costatum* | SkL | 0.72 | 0.62 | 0.52 |
| *N.closterium* | NcC | 0.52 | 0.44 | 0.00 |
| *Heterokontophyta* | | | | |
| *O.luteus* | OlC | 0.71 | 0.61 | 0.41 |
| *Open Ocean* | | | | |
| *Cyanophyta:* | | | | |
| *P.marinus* | PmN | 0.46 | 0.27 | 0.11 |
| *Haptophyta* | | 0.90 | 0.87 | 0.51 |
| *P.polylepis* | PpN | 0.90 | 0.87 | 0.51 |
| *I. galbana* | IgN | 0.93 | 0.92 | 0.92 |

**Table 2:** Broad sense heritability ($H^2$ = Vg/Vp) of relative fitness atnowadays temperatures (isolation temperature), at low temperature increase (as the expected under alow $CO_2$emission scenario, i.e. isolation temperature + 2°C), and at high temperature increase (as the expected under a high $CO_2$ emission scenario, i.e. isolation temperature + 7°C)

Our data also show that a wide variety of interspecific responses to global warming are expected to occur based on the different potentialities of phytoplankton to adapt to temperature increase. The populations of some species show great potential to adapt to global warming, with values of heritability of fitness greater than 0.5 even at the high temperatures expected under a high $CO_2$ emission scenario (Table 2). This occurs in the populations of coastal species of diatoms (*C. curvisetus*, *S. costatum*), dinoflagellates (*P.micans*, *A. minutum*), and chlorophytes (*T. suecica*) as well as in the populations of two oceanic haptophytes (*P. polilepis*, *I. galbana*). In fact, one of them (*I. galbana*) is the one with greater genetic variability in fitness. In contrast populations of other species have a low adaptive potential such as the coastal dinoflagellates (*S. trochoidea*, *P. triestinum*), the coastal diatom (*Nitzxchia closterium*) and the oceanic cyanoprokaryota (*P. marina*).

| Habitat preference Taxonomic group Species | Population | Nowadays temperature | | Low increase temperature | | High increase temperature | |
|---|---|---|---|---|---|---|---|
| | | no. | ř | no. | ř | no. | ř |
| **Coastal waters** | | | | | | | |
| **Dinophyta:** | | | | | | | |
| *P.micans* | PmC | 17 | 0.33 | 17 | 0.35 | 17 | 0.30 |
| *P.micans* | PmV | 13 | 0.30 | 13 | 0.33 | 13 | 0.29 |
| *P.triestimun* | PtC | 18 | 0.46 | 18 | 0.31 | 9 | 0.20 |
| *A.minutum* | AmG | 22 | 0.40 | 22 | 0.42 | 22 | 0.37 |
| *S.trochoidea* | StC | 15 | 0.31 | 15 | 0.27 | 9 | 0.21 |
| **Chlorophyta:** | | | | | | | |
| *T.suecica* | TsC | 20 | 0.82 | 20 | 0.85 | 20 | 0.78 |
| Heterokontophyta | | | | | | | |
| *C.curvisetum* | CcC | 13 | 0.61 | 13 | 0.63 | 12 | 0.57 |
| *S. costatum* | SkV | 21 | 0.51 | 21 | 0.45 | 21 | 0.37 |
| *S. costatum* | SkL | 21 | 0.51 | 21 | 0.47 | 20 | 0.33 |
| *N.closterium* | NcC | 14 | 0.67 | 14 | 0.68 | 1 | 0.35 |
| Open Ocean | | | | | | | |
| *Cyanophyta* | | | | | | | |
| *P.marinus* | PmN | 14 | 0.32 | 13 | 0.32 | 9 | 0.17 |
| Haptophyta | | | | | | | |
| *P.polylepis* | PpN | 6 | 0.38 | 6 | 0.40 | 6 | 0.33 |
| *I.galbanaParke* | IgN | 12 | 0.69 | 12 | 0.71 | 12 | 0.66 |

**Table 3:** Number of strains surviving (no.) and mean Malthusian parameter (ř) at nowadays temperatures (isolation temperature), at low temperature increase (as the expected under a low CO2 emission scenario, i.e. isolation temperature + 2°C), and at high temperature

increase (as the expected under a high $CO_2$ emission scenario, i.e. isolation temperature + 7°C)

A similar result is obtained by comparing values of mean fitness. The different species of Dinophyta from coastal waters shown statistically significant differences in mean fitness ($p< 0.001$; Kruskal-Wallis H-test) at nowadays temperatures, as well as at low increase of temperature ($p< 0.01$; Kruskal-Wallis H-test) and under a high increase of temperature ($p< 0.01$; Kruskal-Wallis H-test). Similar results were observed within populations of coastal Bacillariophyta (($p< 0.01$; Kruskal-Wallis test in all the temperatures tested). Populations of Open Ocean Haptophyta also follow a similar pattern ($p< 0.001$; Mann-Whitney U-test under the 3 temperatures).These differences indicate that within each of the different taxonomic groups as well as within the different habitat preferences groups we can find species that will be able to adapt to higher temperatures while others do not. It could predict that a strong environmental change could extinguish populations and species within any taxonomic group or habitat preference, but should not remove any of the taxonomic groups nor extinguish the entire phytoplankton of some habitats.

In a previous work, Huertas et al. [34] estimated the maximum capacity of genetic adaptation to global warming of 12 species of marine and freshwater phytoplankton, using a ratchet procedure that maximizes the appearance of new mutants conferring warming-resistance. They found interspecific different of capacity for genetic adaptation to thermal stress. Four of the species used were the same as those used in this work, and although the procedure used by Huertas et al.[34] to estimate the capacity to warming adaptation is very different to that those used in this work, the results coincide completely. The ratchet procedure indicates that *T. suecica* and *I. galbana* have high adaptability, *P. triestinum* shows a medium capacity, while *N. closteriun* has a low capability to warming adaptation, just as show our estimation based on heritability of fitness. The agreement between the results using as different procedures to estimate capability of adaptation provides reliability to these predictions. Such differences in capability for adaptation to warming will undoubtedly cause shifts in the composition of the phytoplankton community, as well as replacement of impaired individuals by others that are more resistant; or low-latitude marine species could even colonize higher latitudes as the global sea surface temperature becomes warmer. Although an absolute scenario cannot be envisaged at this point, it is certain that genetics will ultimately determine which species will survive to the environmental forcing.

Climate warming is causing a variety of emergent challenges for the Earth including the massive loss of diversity, which can be considered the distinctive feature of the future biosphere [5]. Millions of populations and many thousand species go extinct annually [4]. Although numerous studies deal on the effects of global warming on diverse aspects of physiology, ecology and distribution of marine phytoplankton, the effect of global warming on phytoplankton is only limitedly known, despite that the phytoplankton is responsible for about half of the global net primary production. One reason could be that the studies that seek to make predictions based on capability to genetic adaptation of phytoplanktonic organisms are scarce. Global warming not seems to be a low environmental stress that soon will revert to the initial conditions. By contrast global warming appears as a powerful change that may not revert soon to the initial conditions.Recent work are accumulating experimental evidence that phytoplankton is able to survive under a low environmental stress by means of physiological acclimatization by modifying gene expression[35,36], but when the environmental stress increases, only genetic variability allow adaptation [37-41]. Thus, the classical neo-Darwinist assumption that genetic variance in fitness estimates capability for adaptation of a population should also be applied to natural populations of phytoplankton. Further studies estimating the heritability of adaptive traits in phytoplankton populations could help us to understand the future of the oceans.

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## References

1. Trenberth KE, Jones PD, Ambenje P, Bojariu R, Easterling D et al. (2007) Observations: surface and atmospheric climate change In Climate change 2007: the physical science basis Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge UK: Cambridge University Press and New York NY, USA.
2. Meehl GA, Cove C, Delworth T, Latif M, McAvaney B, et al. (2007) The WCRP CMIP3 Multimodel Dataset: A New Era in Climate Change Research. Bull AmerMeteorolSoc 88: 1383-1394.
3. Belkin I (2009) Rapid warming of large marine ecosystems. ProgOceanogr 81: 207–213.
4. Woodruff DS (2001) Declines of biomes and biotas and the future of evolution. ProcNatlAcadSci USA 98: 5471–5476.
5. Myers N and Knoll AH (2001) The biotic crisis and the future of evolution. ProcNatlAcadSci USA 98: 5389–5392.
6. Wrona FJ, Prowse TD, Reist JD, Hobbie JE, Lévesque LMJ, et al. (2006) Climate Change Effects on Aquatic Biota Ecosystem Structure and Function. AMBIO: A Journal of the Human Environment 35: 359-369.
7. BehrenfeldMJ, O'Malley RT, Siegel DA, McClain CR, Sarmiento JL, et al. (2006) Climate-driven trends in contemporary ocean productivity. Nature 444: 752–755.
8. Jöhnk KD, Huiman J, Sharples J, Sommeijer B, VisserPM and Stroom JM (2008) Summer heat waves promote blooms of harmful cyanobacteria Global Change. Biol 14: 495-512.
9. Boyce D, Lewis M and Worm B (2010) Global phytoplankton decline over the past century. Nature 466: 591-596.
10. Kirk JTO (1994) Light and photosynthesis in aquatic ecosystems (2nd edn) Cambridge University Press. New York NY, USA.
11. Falkowski PG and Raven JA (1997) Princeton University Press, 484pp.
12. Falkowski PG, Barber RT and Smetacek V (1998) Biogeochemical controls and feedbacks on ocean primary production. Science 281: 200–206.
13. Behrenfeld MJ, Randerson JT, McClain CR, Feldman GC, Los SO, et al., (2001) Biospheric primary production during an ENSO transition. Science 291: 2594–2597.
14. Schiermeier Q (2010) Ocean greenery under warming stress. Nature.
15. Margulis L and Schwartz VK (1982) Five kingdoms: An illustrated guide to the phyla of life on Earth.WHFreeman.New York NY, USA.
16. Fisher RA (1930) The Genetical Theory of Natural Selection Clarendon Press Oxford.
17. Li CC (1967) Fundamental Theorem of Natural Selection. Nature 214: 505 – 506.
18. Edwards AWF (1994) The fundamental theorem of natural selection Biological. Reviews 69: 443–474.
19. Franka SA and Slatkinb M (1992) Fisher's fundamental theorem of natural selection. Trends in Ecology and Evolution 7(3): 92–95.

20. Okasha S (2008) Fisher's Fundamental Theorem of Natural Selection—A Philosophical Analysis. Br J PhilosSci 59(3): 319-351.
21. Brand LE (1981) Genetic variability in reproduction rates in marine phytoplankton populations. Evolution 35: 1117-1127.
22. Bañares-España E, López-Rodas V, Costas E, Salgado C and Flores-Moya A (2007) Genetic variability associated with photosynthetic pigment concentration and photochemical and nonphotochemical quenching in strains of the cyanobacterium *Microcystis aeruginosa*. FEMS microbial Ecol 60 (3): 449-455.
23. Crow JF and Kimura M (1970) An introduction to population genetics theory. Harper and Row, New York NY, USA. pp 59.
24. Falconer DS and Mackay TFC (1996) Introduction to Quantitative Genetics (4th edn) Longmans Green Harlow, Essex, UK.
25. Rico M, Altamirano M, López-Rodas V and Costas E (2006) Analysis of polygenic traits of *Microcystis aeruginosa* (Cyanobacteria) strains by Restricted Maximum Likelihood (REML) procedures: 1 Size and shape of colonies and cells. Phycologia 45: 237-242.
26. Costas E (1990) Genetic variability in growth rates in marine dinoflagellates. Genetica 83: 99-102.
27. López-Rodas V, Costas E, Bañares E, García-Villada L, Altamirano M, et al. (2006) Analysis of polygenic traits of *Microcystis aeruginosa* (Cyanobacteria) strains by Restricted Maximum Likelihood (REML) procedures: 2 Microcystin net production photosynthesis and respiration. Phycologia 45: 243-248.
28. Cooper S (1991) Bacterial growth and division Biochemistry and regulation of prokaryotic and eukaryotic division cycles. Harcourt Brace Jovanovich. London, Academic Press.
29. Mather K and Jinks JL (1971) Biometrical Genetics London Chapman Hall. London, UK.
30. Császár NBM, Ralph PJ, Frankham R, Berkelmans R and van Oppen MJH (2010) Estimating the Potential for Adaptation of Corals to Climate Warming. Plos one 5: e9751.
31. Bunge (1967) Scientific Research Strategy and Philosophy. Berlin, New York, Springer-Verlag.
32. Lewontin RC (1975) The problem of genetic diversity. Harvey Lecture Series 70: 1-20.
33. Gould SJ (2002) The structure of evolutionary theory, Belknap press Harvard.
34. Huertas IE, Rouco M, López-Rodas V and Costas E (2011) Warming will affect phytoplankton differently: evidence through a mechanistic approach. Proc Roy Soc B 278: 3534–3543.
35. López-Rodas V, Flores-Moya A, Maneiro E, Perdigones N, Marva F, et al. (2007) Resistance to glyphosate in the cyanobacterium *Microcystis aeruginosa* as result of pre-selective mutations. Evolutionary Ecology 21: 535-547.
36. Costas E, Flores-Moya A and López-Rodas V (2008) Rapid adaptation of algae to extreme environments (geothermal waters) by single mutation allows "Noah's Arks" for photosynthesizers during the Neoproterozoic "Snowball Earth"?. New Phytologist 189: 922-932.
37. Romero J, López-Rodas V and Costas E (2012) Estimating the capability of microalgae to physiological acclimatization and genetic adaptation to petroleum and diesel oil contamination. Aquat Toxicol 124: 227- 237.
38. González R, García-Balboa C, Rouco M, Lopez-Rodas V and Costas E (2013) Adaptation of microalgae to lindane: A new approach for bioremediation. Aquat Toxicol 109: 25-32.
39. Marvá F, López-Rodas V, Rouco M, Navarro M, Toro FJ, et al. (2010) Adaptation of green microalgae to the herbicides simazine and diquat as result of pre-selective mutations. Aquat Toxicol 96(2): 130-134.
40. Marvá F, García-Balboa C, Baselga-Cervera B and Costas E (2013) Rapid adaptation of some phytoplankton species to osmium as a result of spontaneous mutations. Ecotoxicology.
41. García-Balboa C, Baselga-Cervera B, García-Sanchez A, Mariano-Igual J, et al. (2013) Rapid adaptation of microalgae to extremely polluted waterbodies from uranium mining: an explanation of how the mesophilic organisms can rapidly colonize extremely toxic environments. Aquat Toxic 144-145: 166-123.