The need for unrealistic experiments in global change biology

Sinéad Collins^a, Mridul K. Thomas^b

^a University of Edinburgh, Institute of Evolutionary Biology, The King's Buildings, Charlotte Auerbach Road, Edinburgh, EH9 3FL, UK

^b University of Geneva, Department F.-A. Forel for Environmental and Aquatic Sciences and Institute for Environmental Sciences, CH-1211, Geneva, Switzerland

corresponding author email: s.collins@ed.ac.uk

Abstract

Climate change is an existential threat, and our ability to conduct experiments on how organisms will respond to it is limited by logistics and resources, making it vital that experiments be maximally useful. The majority of experiments on phytoplankton responses to warming and CO₂ use only two levels of each driver. However, to project the characters of future populations, we need a mechanistic and generalizable explanation for how phytoplankton respond to concurrent changes in temperature and CO₂. This requires experiments with more driver levels, to produce response surfaces that can aid in the development of predictive models. We recommend prioritizing experiments or programmes that produce such response surfaces on multiple scales for phytoplankton.

Biological research today is dominated by anthropogenic climate change and the existential threats it poses. Biologists are therefore focused on understanding how rapid environmental change affects organisms, populations, communities and ecosystems, on spatial scales ranging from local to global, and temporal scales ranging from seconds to decades. As in other areas of science, this research is divided into fundamental and applied. Applied research has straightforward practical justifications: we need to improve food production, conservation, predict and mitigate the spread of infections, effectively protect ecosystems to preserve key services, or project how continued human activities will impact other species. However, the majority of research on biological responses to climate change falls under the banner of fundamental science, which aims to provide insights, examples, and theoretical frameworks that help move knowledge (including applied science) forward.

Here we consider the fit-to-purpose of experiments designed to investigate organismal responses to environmental change. Our aim is to evaluate fundamental experiments that use two or more simultaneous environmental changes, which are often called ‘multiple stressor’, ‘multi-stressor’ or ‘multiple driver’ experiments in the aquatic literature [1]. We focus on experiments investigating the joint effects of changing CO₂ and temperature levels as a case study. We look at how their design helps us make progress towards projecting how they jointly affect phytoplankton traits underlying ecological or biogeochemical function in aquatic systems. We highlight how fundamental research can improve our understanding of biotic responses to complex environmental change by expanding experimental designs to include unrealistic environmental conditions that no foreseeable future holds.

BOX 1

Trait: A measurable quantity describing a particular organismal or population function/quantity, usually related to organismal fitness, ecological or biogeochemical function (e.g. population growth rate, cell size, nutrient affinities). For a more detailed discussion of phytoplankton trait types see [2]

Response curve/reaction norm: The *shape* of the biological response to a change in some continuous variable (e.g. temperature response curve). See figures 2-4.

Response/interaction surface: The shape of the surface that describes the biological response to joint changes in two or more continuous variables (e.g. growth responses to concurrent changes in CO₂ and temperature). See figures 2-4.

Experiment: We focus here on manipulative studies carried out in controlled environments, usually in laboratories, where traits are measured and trait changes can be unambiguously linked to specific environmental changes.

END BOX 1

What ocean global change questions do we want to answer with laboratory experiments?

The goals of ocean global change biology are clear. Among them are predictions of how ecosystems and biogeochemical cycles are likely to change globally that are good enough to take effective action or at least anticipate large-scale changes [3]. Fundamental research is aimed at understanding how the environment affects (and is affected by) biota, and at projecting future dynamics and distributions of species, communities, ecosystems, ecosystem services, and biogeochemical cycles. The questions typically asked in global change biology can be summarised as: *what does the future bring at X organisational scale and Y geographic scale?* To project change in specific ecosystems, for specific timescales and projections of environmental change, these questions are made concrete. For primary producers in aquatic systems, such questions include: How much and where will primary production change as a result of expected changes in CO₂, temperature, and nutrient levels? How will warming change the biological carbon pump in the open ocean? How will nitrogen input into the oceans change as warming increases the range of N-fixers?

Connecting these large-scale questions to the ecophysiological processes that underpin them partly boils down to understanding the environmental dependence of key organismal traits and processes: population growth rates, cell compositions, resource uptake rates, edibility, cell sizes and more. Understanding how the environmental dependence of these traits will shape future ecosystem processes requires integrating and modelling ecophysiology, community ecology, and evolution. The traits that need to be understood in phytoplankton are largely known and measurable in the laboratory [4], and there is sufficient understanding of the physiology underlying these traits to conduct hypothesis-driven experiments on mechanisms underlying known or putative tradeoffs between traits in many cases [5]. At this point, the major empirical knowledge gap is robustly connecting variation in trait values to variation in the environment. This is a tractable problem that can be addressed with experiments.

Dynamic models can simulate the growth of species (or higher taxonomic units) alone or in mixed communities, with and without predators, at temperatures or CO₂ levels that were not measured (extrapolating requires caution, however), in constant or variable environments, and many can account for eco-evolutionary processes [6-9]. These involve important assumptions, but represent vital steps towards the larger goal of making accurate predictions of future populations and ecosystem change. To model how populations and communities will change when both temperature and CO₂ change, these dynamic models need parameterised equations that capture the temperature- CO₂ *response surface*. At present, we do not have the data to tell us the shape of this surface, so we lack an equation to describe it (see Figs. 2-4 for one possibility, based on [10]). However, this can be solved with experiments that we

have the methods to do, but rarely carry out. To produce response surfaces, it can be unhelpful to replicate only realistic environmental conditions. Equations for temperature- CO₂ response surfaces would be constrained better if experiments included high temperature-low CO₂ treatments, and vice versa.

How are we studying driver interactions at present?

We compiled data on temperature x CO₂ interaction experiments involving two key phytoplankton taxa (diatoms and coccolithophores). We included data from studies that manipulated both CO₂ and temperature, with at minimum a 2 x 2 design, and which measured population growth rate. We found 16 studies that met these criteria (see [11,12] for a representative example). Nearly all the data from these studies have been gathered at just 2 levels of CO₂ (~400ppm and ~1000ppm), at or near 20°C (Fig. 1). Despite omitting simple cases where drivers were not independently manipulated, the median experiment is still a 2 x 2 study. Overall, the published data do a poor job of sampling the interaction surface for temperature and CO₂. This is concerning because while we can (and do) produce explanations and models based on basic physiology and conserved tradeoff phenomena that are consistent with the variation in outcomes in the published data [13-15], our current understanding of physiology cannot build interaction surfaces from first principles.

Fig. 1. What temperature and CO₂ values are we measuring? The colour of each hexagon indicates the number of measurements in our compiled dataset at (narrowly binned) combinations of temperature and CO₂ values. Note that

different studies have different numbers of measurements. Even allowing for the fact that very high concentrations are of less interest for forecasting future responses, there are large gaps in the data we collect. Tropical and polar temperatures are underrepresented, as well as CO₂ levels between 400 and 1000 ppm. Data are from single-genotype laboratory experiments with diatoms and coccolithophores where at least 2 levels of CO₂ and 2 levels of temperature were used, and growth rates were reported [11,16-31]. See Supplementary information for details of search and data.

Key limitations of the current approach to driver interaction experiments

Temperature response curves exist for >100 species representing all major phytoplankton functional groups [32]. CO₂ response curves at different temperatures are scarce, so expectations of temperature- CO₂ response surfaces cannot be generated. Our estimates of organismal responses to the joint effects of rising CO₂ and warming are therefore sensitive to small errors in both predictions of future environments (Figure 3) and assumptions about the shape of the response surface (Fig. 4). Despite the knowledge that warming and changes to CO₂ are key drivers in marine systems, and decades of experiments with temperature and CO₂, we have yet to build robust hypotheses of how even model phytoplankton should respond to the joint action of these drivers.

Our compilation shows that the literature is dominated by ANOVA experimental designs, which are appropriate for discrete variables (e.g. species A vs species B). These designs treat continuous variables as discrete (e.g. temperature treated as 'present' vs 'future'). In contrast, regression designs appropriate for continuous variables involve using many experimental levels of the variable and modelling a continuous response. Response surface designs are multi-dimensional versions of regression designs (Fig. 2).

Fig. 2. Experimental design for continuous variables like temperature and CO₂. Present experiments treat both temperature and CO₂ as discrete and measure them at 2 levels (left panels). This limits our understanding, inferences and ability to build on experiments with modelling efforts because in reality, responses to both are continuous and nonlinear. The response to temperature is left-skewed and unimodal (top right). The response to CO₂ is right-skewed and unimodal (not shown). Jointly, they form a complex response surface that we do not understand well at present for lack of data. Bottom right shows a hypothetical surface based on a temperature x CO₂ model described in the supplementary information. Growth rates below -0.25 are suppressed (dark grey) to highlight variation in positive growth rates.

When would 2 x 2 ANOVA experiments achieve the goal of understanding and predicting how projected environmental change affects organisms? If we had precise and accurate forecasts of temperature and CO₂ levels, it could be argued that predicting changes in a species' performance only requires comparing performance under present and perfectly-predicted future conditions. However, we do not know precisely what CO₂ levels or even average global temperatures will be decades from now, with uncertainties running to several degrees [33]. Simulations from a realistic response surface (Fig. 3) show that modest uncertainty in either dimension can lead to substantially different outcomes. This uncertainty is amplified by variation through time and across space, and in other environmental dimensions. Organismal responses also vary based on evolutionary differences and ecological interactions. This variation in outcomes does not mean experiments measuring biological responses to the environment are fruitless. Uncertainty in biological responses can

be accounted for if we measure and model responses appropriately. An ANOVA-based approach cannot account for most of this uncertainty. Indeed, it is difficult from the current literature to partition how much of the observed variation in responses to combinations of ocean acidification and warming in phytoplankton [13,34] is attributable to biological variation in responses, and how much is due to uncertainty introduced by small differences in the way experiments sample interaction surfaces.

Fig. 3. Uncertainty in both temperature and future CO₂ is hard to account for in ANOVA designs. Growth rates in present day conditions (represented by black point) may increase, decrease or stay substantially the same based on small differences in future conditions (represented by coloured points). Coloured curves in the right panel represent temperature curves at different CO₂ concentrations i.e. horizontal slices through the response surface in the left panel. Conclusions drawn from existing 2 x 2 experiments are therefore heavily dependent on the reliability of CO₂ projections - which themselves depend on the phytoplankton response - and climate sensitivity (compare blue points, which represent different amounts of warming based on the same amount of CO₂). We can escape this problem of circularity only by understanding the response surface better.

The ANOVA approach is not rescued by binning responses into qualitative levels. The common additive/synergistic/antagonistic interaction framework and its extensions or variations [35,36] is subject to the same weaknesses described above, and additional ones too. Differences in the precise driver levels chosen, and the choice of the control/reference level can lead to any of the three possible outcomes from the same response surface (Fig. 4). The additive/ synergistic/ antagonistic framework is often applied to higher levels of organisation such as ecosystem responses and it is possible that some of our objections do not apply there; this merits more investigation than it has received.

Fig. 4. Response surface experiments can lead to very different inferences than ANOVA experiments. In this example, the same response surface (left panel) is reduced first to 2 response curves (middle panel) and finally to 2 x 2 ANOVA experiments (right panels). The specific ANOVA experimental levels chosen (vertical black lines in middle panel) and the choice of reference level alters the nature of the inferred interaction. Note that the synergistic and antagonistic interactions shown are from the same set of 4 growth rates. In the synergistic case, the low CO₂, low temperature point is chosen as control, while in the antagonistic case, the high CO₂, high temperature point is chosen as control. Additive/synergistic/antagonistic interactions are context-dependent, whereas capturing the response surface largely eliminates the need for this misleading taxonomy.

ANOVA-based approaches also make it challenging to draw conclusions about responses at higher taxonomic levels without additional experiments targeting those levels. In contrast, response surfaces allow scaling across levels more easily. The 'Eppley Curve' is a classic oceanography community pattern that arises from species temperature response curves [37,38]. It defines an upper boundary on maximum growth rate that increases exponentially with temperature and can be constructed as the 'envelope' of a series of temperature response curves. This connection between an important community-level pattern (the Eppley curve) and population-level response curves allows for easy switching between scales of interest - a clear advantage for modelling and prediction. A multi-dimensional version of the Eppley curve that varies with CO₂ concentration (an Eppley surface) should be possible with sufficient data on variation in temperature- CO₂ response surfaces.

Conclusions

To have a robust understanding of how phytoplankton (and other organisms) respond to multiple simultaneous drivers, we need to move beyond ANOVA-based experiments that categorise interactions as additive, synergistic, antagonistic (or variations thereof), and towards experiments that produce interaction/response surfaces. Regression designs are logistically more challenging and often require better experimental facilities than ANOVA-based ones. The nonlinearity of the temperature response curve is not captured well with fewer than 5 temperature levels; more is better. Based on data thus far, the same appears to be true for CO₂ response curves [10]. ‘Collapsed designs’ can be useful in reducing the size of experiments relative to fully-factorial designs (see [39]) and the more information we have about the shape of the surface, the better equipped we will be to intelligently sample it and parameterise it for a species. However, collapsed designs do not replace fully factorial experiments, which are often doable for microbes, given adequate resourcing and prioritization. For example, a 6 x 6 experiment can involve just 36 experimental units (replication is not necessary for regression designs though it can be helpful if specific levels are particularly important for parameter estimation). Depending on the traits under investigation, this is a challenging but doable experiment. Indeed, both Sett (2014) [19] and Zhang (2020) [25] conducted experiments with enough total experimental units to have calculated rough response surfaces had more levels of temperature been used; both used more CO₂ levels than necessary.

There is substantial variation in responses to changes in temperature and CO₂ both within and between functional groups (and within and between species) [10,32,34,40,41]. While studying response surfaces for model organisms from each functional group will produce general insights, estimates of how much taxonomic variation exists for response surfaces are also needed. Replicate experiments are necessary in order to estimate the extent of biological variation in the biological responses that form interaction surfaces; repeating experiments, either within single studies that encompass multiple genotypes or species (e.g. [40]), or as coordinated networks of experiments (e.g.[42]) is both useful and necessary; funding and publication success must begin to reflect this. Finally, phytoplankton have the capacity to evolve between now and nearly any future date we make projections for, and evolutionary responses can differ from and even reverse plastic ones [43-45]. There is also good evidence that adaptation to warming is affected by other drivers [46]. We need generalizable insights into how response surfaces evolve under different rates and patterns of multidriver environmental change.

We suggest prioritizing the measurement of response surfaces at multiple timescales to capture plastic/acclimatory as well as eco-evolutionary responses for

phytoplankton that can be grown in the laboratory. This calls for allocating resources to larger or coordinated experiments. We recognize this is challenging given funding cycles and the need to publish frequently, both of which favour smaller, shorter, stand-alone experiments. However, producing data that reveals response surfaces is what basic science is meant to do: lead to usable insights about real-world phenomena and inspire theoretical frameworks that help us understand and predict the world better. We think that this is a better investment of limited resources than the continued production of smaller or isolated experiments focused almost entirely on realistic environments that, from our current position, have made limited headway towards a usable understanding of phytoplankton responses to multidriver environmental change.

Acknowledgments

We thank Allanah Paul and Lennart Bach for discussions of their model. The work presented in this article results, in part, from funding provided by national committees of the Scientific Committee on Oceanic Research (SCOR) and from a grant to SCOR from the U.S. National Science Foundation (OCE-1840868) to the Changing Oceans Biological Systems project, and in part from funding to SC from the Gordon and Betty Moore Foundation (MMI 7397).

Declaration of interest: none.

1. Orr JA, Vinebrooke RD, Jackson MC, Kroeker KJ, Kordas RL, Mantyka-Pringle C, Van den Brink PJ, De Laender F, Stoks R, Holmstrup M: **Towards a unified study of multiple stressors: divisions and common goals across research disciplines.** *Proceedings of the Royal Society B* 2020, **287**:20200421.
2. Weithoff G, Beisner BE: **Measures and Approaches in Trait-Based Phytoplankton Community Ecology – From Freshwater to Marine Ecosystems.** *Frontiers in Marine Science* 2019, **6**:40.
3. Duarte CM: **Global change and the future ocean: A grand challenge for marine sciences.** *Frontiers in Marine Science* 2014, **1**:1-16.
4. Litchman E, Klausmeier CA: **Trait-Based Community Ecology of Phytoplankton.** *Annual Review of Ecology, Evolution, and Systematics* 2008, **39**:615-639.
5. van de Waal DB, Litchman E: **Multiple global change stressor effects on phytoplankton nutrient acquisition in a future ocean.** In *Philosophical Transactions of the Royal Society B: Biological Sciences*. Edited by; 2020:1-8. vol 375.]
6. Cotto O, Wessely J, Georges D, Klöner G, Schmid M, Dullinger S, Thuiller W, Guillaume F: **A dynamic eco-evolutionary model predicts slow response of alpine plants to climate warming.** In *Nature Communications*. Edited by; 2017. vol 8.]

7. Beckmann A, Schaum CE, Hense I: **Phytoplankton adaptation in ecosystem models.** *Journal of Theoretical Biology* 2019, **468**:60-71.

* Phytoplankton adaptation to different temperature regimes is modelled in two different ways (individual based models and trait diffusion) that explicitly consider the process of mutation and intraspecific competition. This is a great example of a model that relates directly to and is informed by experiments, and is a necessary step before scaling up to global ecosystem models where processes cannot be explicitly represented.

8. Laufkötter C, Vogt M, Gruber N, Aita-Noguchi M, Aumont O, Bopp L, Buitenhuis E, Doney S, Dunne J, Hashioka T, et al.: **Drivers and uncertainties of future global marine primary production in marine ecosystem models.** *Biogeosciences* 2015, **12**:6955-6984.
9. Dutkiewicz S, Morris JJ, Follows MJ, Scott J, Levitan O, Dyhrman ST, Berman-Frank I: **Impact of ocean acidification on the structure of future phytoplankton communities.** *Nature Climate Change* 2015:1002-1006.
10. Paul AJ, Bach LT: **Universal response pattern of phytoplankton growth rates to increasing CO₂.** *New Phytol* 2020, **228**:1710-1716.

** Using published data from diatoms and coccolithophores, the authors propose that phytoplankton growth rates follow a universal optimum curve response when grown over a large range of H⁺ (corresponding to a large range in CO₂), and that this constitutes a “universal reaction norm” to CO₂. While further data is needed to test the hypothesis, this is the first time a single overarching explanation has been proposed for CO₂ reaction norms in phytoplankton.

11. Tong S, Hutchins DA, Gao K: **Physiological and biochemical responses of *Emiliania huxleyi* to ocean acidification and warming are modulated by UV radiation.** *Biogeosciences* 2019, **16**:561-572.

* Key traits for a model calcifying coccolithophore, *E.huxleyi*, are measured at 2 levels of CO₂ and 3 temperatures, with and without UV. This is a current example of a 2x3 CO₂ x temperature study that incorporates a third driver, and finds evidence that UV, which is usually neglected in laboratory experiments, but is present in natural marine environments where phytoplankton grow, has the potential to affect the shape of the temperature x CO₂ interaction surface.

12. Zhong J, Guo Y, Liang Z, Huang Q, Lu H, Pan J, Li P, Jin P, Xia J: **Adaptation of a marine diatom to ocean acidification and warming reveals constraints and trade-offs.** *The Science of the total environment* 2021, **771**:145167.

* Uses experimental evolution with a marine diatom with a 2x2 design for temperature and CO₂, and finds that, at these driver levels, warming is the main driver of trait change, but some traits are also affected by CO₂ levels. This study considers mainly photosynthesis-related traits. This demonstrates that interaction effects between warming and CO₂ depend on the traits under consideration, as well as on the precise levels of each driver used.

13. Gao K, Beardall J, Häder D-P, Hall-Spencer JM, Gao G, Hutchins DA: **Effects of ocean acidification on marine photosynthetic organisms under the concurrent influences of warming, UV radiation, and deoxygenation.** *Frontiers in Marine Science* 2019, **6**:322.

14. van de Waal DB, Litchman E: **Multiple global change stressor effects on phytoplankton nutrient acquisition in a future ocean.** *Philosophical Transactions of the Royal Society B: Biological Sciences* 2020, **375**:1-8.

** A paper that combines theoretical insights with data to offer new, testable hypotheses about how interactions will affect phytoplankton of different sizes. Such hypotheses can form the basis of new models of temperature x CO₂ (and other) interactions, and also point towards important and neglected trade-offs.

15. Serra-Pompei C, Hagstrom GI, Visser AW, Andersen KH: **Resource limitation determines temperature response of unicellular plankton communities.** *Limnology and Oceanography* 2019:1-14.

* A model of phytoplankton growth that incorporates temperature-dependencies at the level of cellular processes, enabling the incorporation of interactions with CO₂, nutrients, and other factors at a sub-cellular scale. This is likely to be a productive path towards developing better models of temperature-CO₂ (and other) interactions.

16. Feng Y, Roleda MY, Armstrong E, Summerfield TC, Law CS, Hurd CL, Boyd PW: **Effects of multiple drivers of ocean global change on the physiology and functional gene expression of the coccolithophore *Emiliana huxleyi*.** *Global Change Biology* 2020, **26**:5630-5645.
17. Feng Y, Warner ME, Zhang Y, Sun J, Fu F-X, Rose JM, Hutchins DA: **Interactive effects of increased pCO₂, temperature and irradiance on the marine coccolithophore *Emiliana huxleyi* (Prymnesiophyceae).** *European Journal of Phycology* 2008, **43**:87-98.
18. De Bodt C, Van Oostende N, Harlay J, Sabbe K, Chou L: **Individual and interacting effects of pCO₂ and temperature on *Emiliana huxleyi* calcification: study of the calcite production, the coccolith morphology and the coccosphere size.** *Biogeosciences Discussions* 2009, **6**.
19. Sett S, Bach LT, Schulz KG, Koch-Klavsen S, Lebrato M, Riebesell U: **Temperature modulates coccolithophorid sensitivity of growth, photosynthesis and calcification to increasing seawater pCO₂.** *PLoS One* 2014, **9**:e88308.
20. Milner S, Langer G, Grelaud M, Ziveri P: **Ocean warming modulates the effects of acidification on *Emiliana huxleyi* calcification and sinking.** *Limnology and Oceanography* 2016, **61**:1322-1336.
21. Li X, Røevros N, Dehairs F, Chou L: **Biological responses of the marine diatom *Chaetoceros socialis* to changing environmental conditions: A laboratory experiment.** *PloS one* 2017, **12**:e0188615.
22. Passow U, Laws EA: **Ocean acidification as one of multiple stressors: growth response of *Thalassiosira weissflogii* (diatom) under temperature and light stress.** *Marine Ecology Progress Series* 2015, **541**:75-90.
23. Torstensson A, Chierici M, Wulff A: **The influence of increased temperature and carbon dioxide levels on the benthic/sea ice diatom *Navicula directa*.** *Polar Biology* 2012, **35**:205-214.
24. Torstensson A, Hedblom M, Andersson J, Andersson M, Wulff A: **Synergism between elevated pCO₂ and temperature on the Antarctic sea ice diatom *Nitzschia lecontei*.** *Biogeosciences* 2013, **10**:6391-6401.
25. Zhang Y, Collins S, Gao K: **Reduced growth with increased quotas of particulate organic and inorganic carbon in the coccolithophore *Emiliana huxleyi* under future ocean climate change conditions.** *Biogeosciences* 2020, **17**:6357-6375.

26. Li F, Beardall J, Gao K: **Diatom performance in a future ocean: interactions between nitrogen limitation, temperature, and CO₂-induced seawater acidification.** *ICES Journal of Marine Science* 2018, **75**:1451-1464.
27. Qu P, Fu F, Hutchins DA: **Responses of the large centric diatom *Coscinodiscus* sp. to interactions between warming, elevated CO₂, and nitrate availability.** *Limnology and Oceanography* 2018, **63**:1407-1424.
28. Laws EA, McClellan SA, Passow U: **Interactive Effects of CO₂, Temperature, Irradiance, and Nutrient Limitation on the Growth and Physiology of the Marine Diatom *Thalassiosira pseudonana* (Coscinodiscophyceae).** *Journal of Phycology* 2020, **56**:1614-1624.
29. Hyun B, Choi K-H, Jang P-G, Jang M-C, Lee W-J, Moon C-H, Shin K: **Effects of increased CO₂ and temperature on the growth of four diatom species (*Chaetoceros debilis*, *Chaetoceros didymus*, *Skeletonema costatum* and *Thalassiosira nordenskiöldii*) in laboratory experiments.** *Journal of Environmental Science International* 2014, **23**:1003-1012.
30. Seebah S, Fairfield C, Ullrich MS, Passow U: **Aggregation and sedimentation of *Thalassiosira weissflogii* (diatom) in a warmer and more acidified future ocean.** *PLoS One* 2014, **9**:e112379.
31. Wolf KK, Romanelli E, Rost B, John U, Collins S, Weigand H, Hoppe CJ: **Company matters: The presence of other genotypes alters traits and intraspecific selection in an Arctic diatom under climate change.** *Global change biology* 2019, **25**:2869-2884.
32. Thomas MK, Kremer CT, Litchman E: **Environment and evolutionary history determine the global biogeography of phytoplankton temperature traits.** *Global Ecology and Biogeography* 2016, **25**:75-86.
33. Masson-Delmotte V, Zhai P, Pirani A, Connors S, Péan C, Berger S, Caud N, Chen Y, Goldfarb L, Gomis M: **Global Warming of 1.5 C—Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.** Edited by: Cambridge University Press: Cambridge, UK; 2021.
34. Seifert M, Rost B, Trimborn S, Hauck J: **Meta-analysis of multiple driver effects on marine phytoplankton highlights modulating role of pCO₂.** *Global Change Biology* 2020, **26**:6787-6804.

* A synopsis of the current data on phytoplankton responses to temperature and CO₂, with conclusions based mainly on 2x2 experimental designs. This considers data to date on such interactions nearly exhaustively. This is a summary of the current state of the art in terms of experiments and interpretation.

35. Piggott JJ, Townsend CR, Matthaei CD: **Reconceptualizing synergism and antagonism among multiple stressors.** *Ecology and Evolution* 2015, **5**:1538-1547.
36. Galic N, Sullivan LL, Grimm V, Forbes VE: **When things don't add up: quantifying impacts of multiple stressors from individual metabolism to ecosystem processing.** *Ecology Letters* 2018, **21**:568-577.
37. Eppley RW: **Temperature and phytoplankton growth in the sea.** *Fishery Bulletin* 1972, **70**:1063-1085.
38. Bissinger JE, Montagnes DJS, Sharples J, Atkinson D: **Predicting marine phytoplankton maximum growth rates from temperature: Improving on the Eppley curve using quantile regression.** *Limnology and Oceanography* 2008, **53**:487-493.
39. Boyd PW, Collins S, Dupont S, Fabricius K, Gattuso J-P, Havenhand J, Hutchins DA, Riebesell U, Rintoul MS, Vichi M, et al.: **Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change—A review.** *Global Change Biology* 2018, **24**:2239-2261.

40. Anderson SI, Ryneerson TA: **Variability approaching the thermal limits can drive diatom community dynamics.** *Limnology and Oceanography* 2020, **65**:1961-1973.

* Thermal reaction norms were constructed for 24 strains of the diatom *Skeletonema* from a single location, and found that most of the biological variation was at the extremes of thermal tolerance. This variation has the potential to affect species succession as well as elemental cycling. This highlights the value of including environmental extremes in experimental designs, as well as the value of repeating experiments to estimate biological variation.

41. Schaum E, Rost B, Millar AJ, Collins S: **Variation in plastic responses of a globally distributed picoplankton species to ocean acidification.** *Nature Climate Change* 2013, **3**:298-302.
42. Boyd PW, Ryneerson TA, Armstrong EA, Fu F, Hayashi K, Hu Z, Hutchins DA, Kudela RM, Litchman E, Mulholland MR, et al.: **PLOS ONE: Marine Phytoplankton Temperature versus Growth Responses from Polar to Tropical Waters – Outcome of a Scientific Community-Wide Study.** *Multiple values selected* 2013, **8**:e63091.
43. Barton S, Jenkins J, Buckling A, Schaum CE, Smirnoff N, Raven JA, Yvon-Durocher G: **Evolutionary temperature compensation of carbon fixation in marine phytoplankton.** *Ecology letters* 2020, **23**:722-733.

* Uses experimental evolution to show that adaptation to temperature directly alters carbon metabolism, which is a strong indicator from basic physiology that evolving under changing temp + changing CO₂ together will be different from dealing with either alone.

44. Schaum CE, Rost B, Collins S: **Environmental stability affects phenotypic evolution in a globally distributed marine picoplankton.** *The ISME Journal* 2015, **10**:75-84.
45. Lindberg RT, Collins S: **Quality–quantity trade-offs drive functional trait evolution in a model microalgal ‘climate change winner’.** *Ecology letters* 2020, **23**:780-790.
46. Aranguren-Gassis M, Kremer CT, Klausmeier CA, Litchman E: **Nitrogen limitation inhibits marine diatom adaptation to high temperatures.** *Ecology letters* 2019, **22**:1860-1869.

* Uses experimental evolution to demonstrate that a marine diatom (*Chaetoceros*) adapts to extreme temperatures under nitrogen replete, but not nitrogen starved, conditions, using 2 levels of nitrogen. This highlights that interaction surfaces themselves can evolve, but does not characterise how they do.