



# The need for unrealistic experiments in global change biology

Sinéad Collins<sup>1</sup>, Harriet Whittaker<sup>1</sup> and Mridul K Thomas<sup>2</sup>

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 CO<sub>2</sub> use only two levels of each driver. However, to project the characters of future populations, we need a mechanistic and generalisable explanation for how phytoplankton respond to concurrent changes in temperature and CO<sub>2</sub>. This requires experiments with more driver levels, to produce response surfaces that can aid in the development of predictive models. We recommend prioritising experiments or programmes that produce such response surfaces on multiple scales for phytoplankton.

## Addresses

<sup>1</sup> University of Edinburgh, Institute of Evolutionary Biology, The King's Buildings, Charlotte Auerbach Road, Edinburgh EH9 3FL, UK

<sup>2</sup> University of Geneva, Department F.-A. Forel for Environmental and Aquatic Sciences and Institute for Environmental Sciences, CH-1211 Geneva, Switzerland

Corresponding author: Sinéad Collins ([s.collins@ed.ac.uk](mailto:s.collins@ed.ac.uk))

Current Opinion in Microbiology 2022, 68:102151

This review comes from a themed issue on Environmental Microbiology

Edited by Paul Hoskisson and John Bruce

For complete overview of the section, please refer to the article collection, "[Environmental Microbiology](#)"

Available online 4th May 2022

<https://doi.org/10.1016/j.mib.2022.102151>

1369-5274/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

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 CO<sub>2</sub> and temperature levels on marine diatoms and coccolithophores as a case study, though our argument applies to all multiple driver studies where laboratory culturing is easy enough to allow ~25 simultaneous populations to be grown under different combinations of driver levels (25 populations corresponds to a 5 × 5 factorial experimental design with two drivers, and five points is the minimum number needed to fit most nonlinear response curves of known shape, such as temperature and nutrient response curves). We stress that such experiments are doable; temperature × nutrient response surfaces have been generated for the diatom *Thalassiosira pseudonana* [2] and a handful of freshwater phytoplankton [3]. 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; see 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 [5]. 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

## 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, and nutrient affinities). For a more detailed discussion of phytoplankton trait types see Ref. [4].

Response curve/reaction norm/performance curve: 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  $\text{CO}_2$  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.

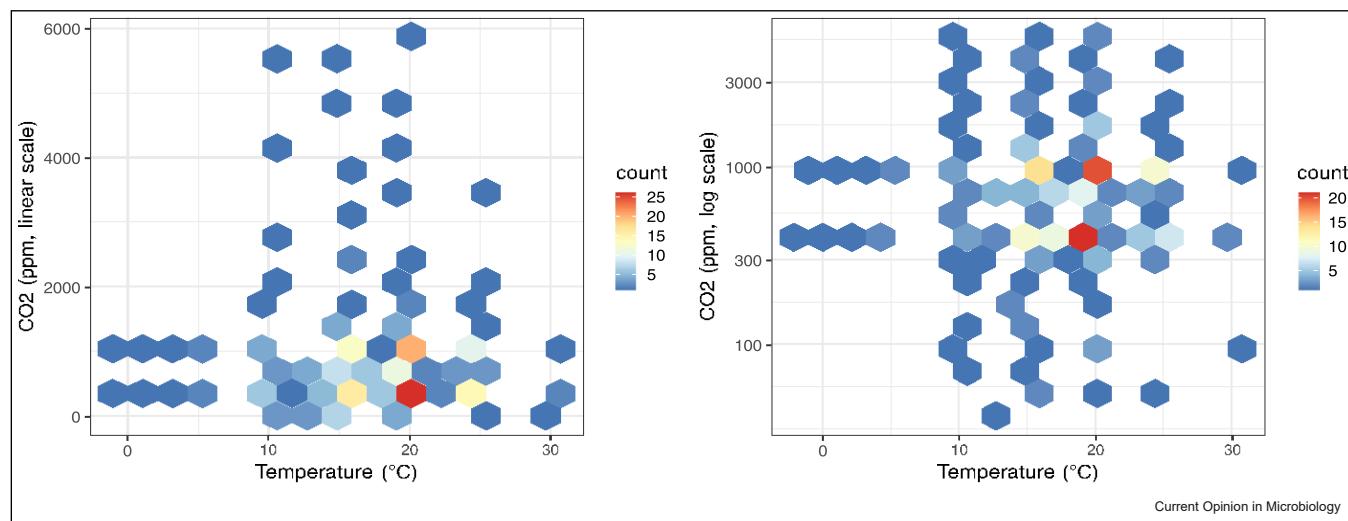
change biology can be summarised as: *what does the future bring at X organisational scale and Y geographic scale?* To project change in specific ecosystems, for specific timescales and projections of environmental change, these questions are made concrete. For primary producers in aquatic systems, such questions include: How much and where will primary production change as a result of expected changes in  $\text{CO}_2$ , 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 [6], 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 [7]. 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  $\text{CO}_2$  levels

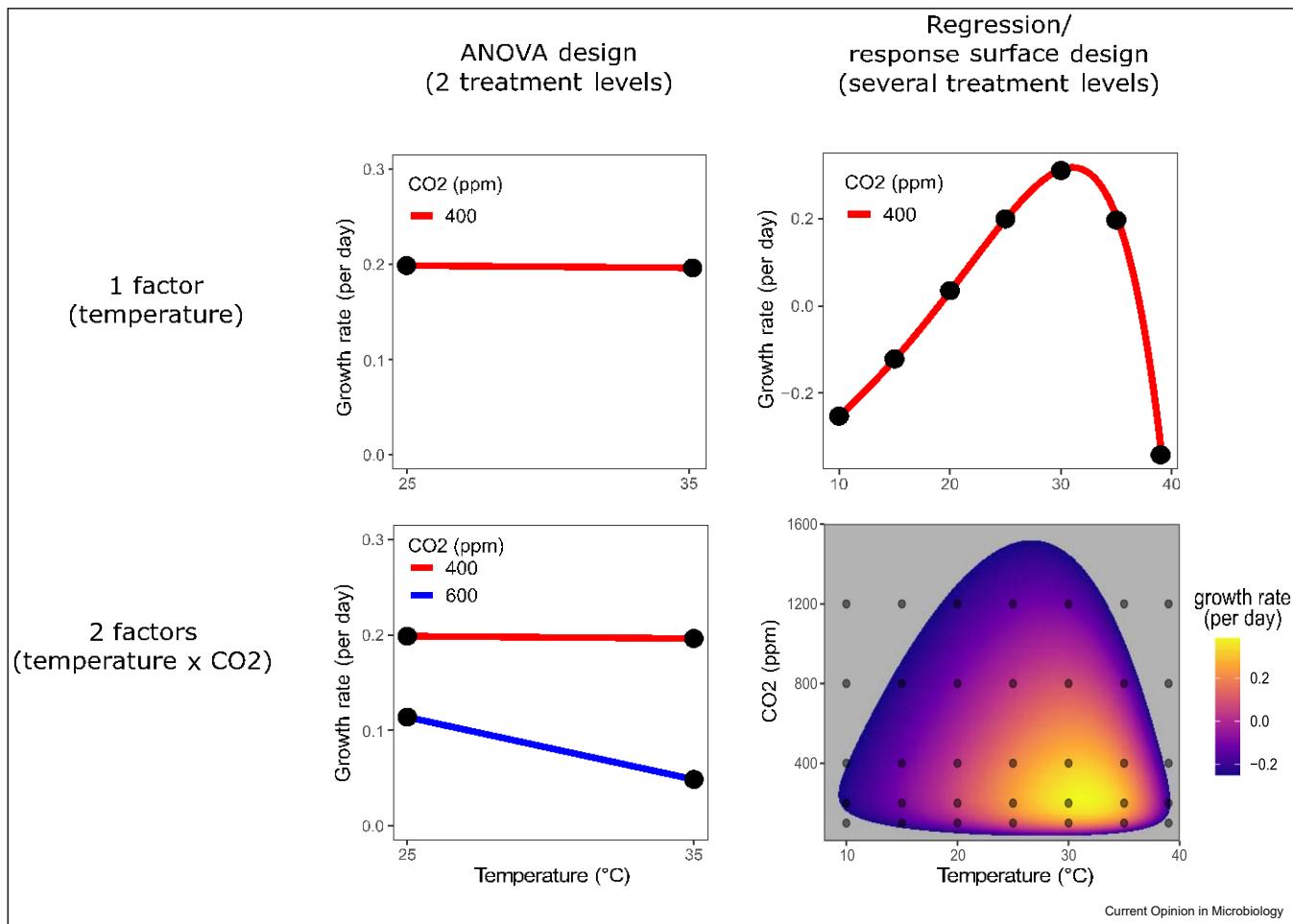
Figure 1



Current Opinion in Microbiology

What temperature and  $\text{CO}_2$  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  $\text{CO}_2$  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 under-represented, as well as  $\text{CO}_2$  levels between 400 and 1000 ppm. These intermediate  $\text{CO}_2$  levels represent levels that will drive phytoplankton responses as we progress towards year 2100; responses at  $\text{CO}_2$  levels between 400 and 1000 ppm are vital for projections. Data are from single-genotype laboratory experiments with diatoms and coccolithophores where at least 2 levels of  $\text{CO}_2$  and 2 levels of temperature were used, and growth rates were reported [13,18–35]. See Supplementary information for details of search and data.

Figure 2

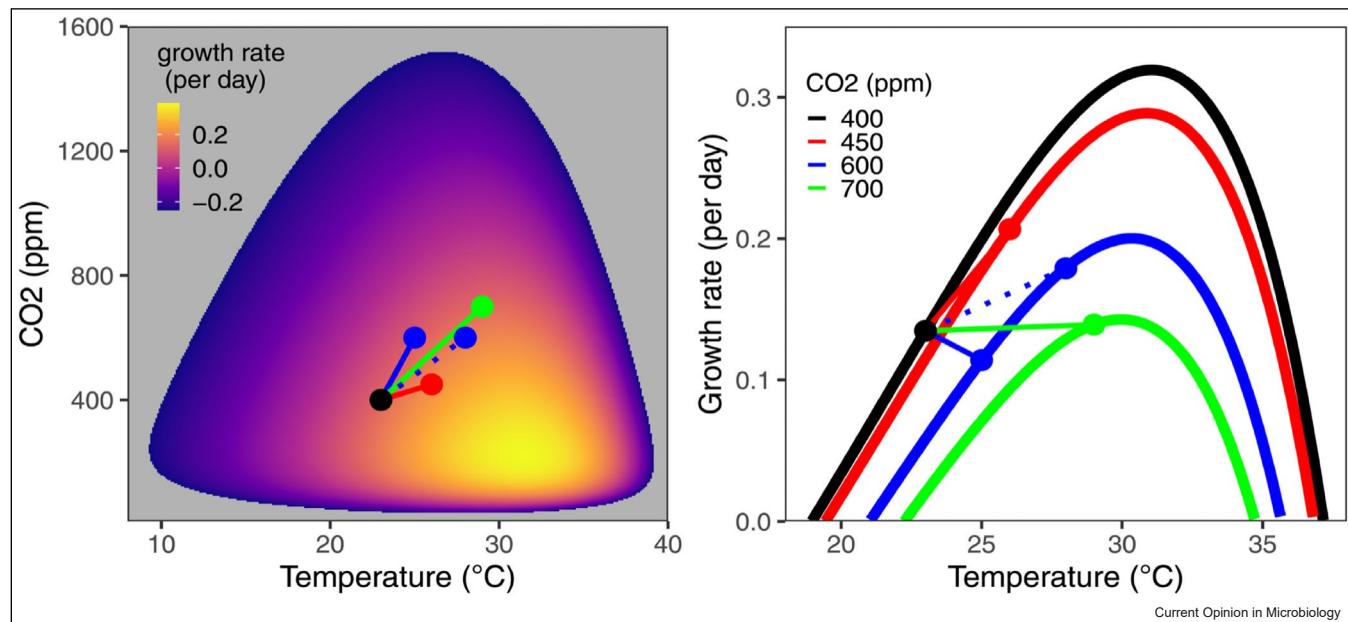


Experimental design for continuous variables like temperature and CO<sub>2</sub>. Present experiments treat both temperature and CO<sub>2</sub> as discrete and measure them at 2 levels (left panels). This limits our understanding, inferences and ability to build on experiments with modelling efforts because in reality, responses to both are continuous and nonlinear. The response to temperature is left-skewed and unimodal (top right). The response to CO<sub>2</sub> 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 CO<sub>2</sub> model described in the [supplementary information](#). Growth rates below -0.25 are suppressed (grey) to highlight variation in positive growth rates.

that were not measured (extrapolating requires caution, however), in constant or variable environments, and many can account for eco-evolutionary processes [8–11]. These involve important assumptions but represent vital steps towards the larger goal of making accurate predictions of future population and ecosystem change. Currently, projections of ocean primary production by earth systems models disagree in both sign and magnitude. Variation in model predictions is highest in the tropics [10], where nutrients are lowest and organisms are near the edge of their thermal tolerances. All of the models reviewed parameterised phytoplankton growth as a multiplicative function of maximum growth rate, temperature limitation, nutrient limitation, and light limitation. These purely multiplicative interactions *may* be appropriate for community/ecosystem

responses, but the scant empirical evidence on population-level interaction surfaces indicates that this strong assumption warrants some scrutiny. For example, Thomas et al. [2] measured a population-level interaction surface, and found that the optimum growth temperature (T<sub>opt</sub>) and maximum growth temperature (T<sub>max</sub>) decreased at low nutrient concentrations for the model diatom *Thalassiosira pseudonana*. Implementing this interaction into a mechanistic species distribution model predicted shifts in growth rates and ranges relative to the same model that used a multiplicative interaction. Community-level response surfaces may not exhibit a T<sub>opt</sub> or T<sub>max</sub> as a result of species turnover — but this remains an unverified assumption, and one that is less likely to be met in regions with extreme environmental conditions or species-poor communities.

Figure 3



Uncertainty in both temperature and future CO<sub>2</sub> is hard to account for in ANOVA designs. Growth rates in present day conditions (represented by black point) may increase (red point, blue point with dotted line), decrease (blue point with solid line) or stay essentially the same (green point) based on small differences in future conditions. Coloured curves in the right panel represent temperature curves at different CO<sub>2</sub> concentrations i.e. horizontal slices through the response surface in the left panel. Conclusions drawn from existing 2 × 2 experiments are therefore heavily dependent on the reliability of CO<sub>2</sub> 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 CO<sub>2</sub>). We can escape this problem of circularity only by understanding the response surface better.

To model how populations and communities will change when both temperature and CO<sub>2</sub> change, these dynamic models need parameterised equations that capture the temperature–CO<sub>2</sub> response surface. At present, we do not have the data to tell us the shape of the temperature–CO<sub>2</sub> surface, so we lack an equation to describe it (see Figures 2–4) for one possibility, based on [12]. 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–CO<sub>2</sub> response surfaces would be constrained better if experiments included high temperature–low CO<sub>2</sub> treatments, and vice versa. Though we focus on temperature and CO<sub>2</sub> here, the same approach is needed to understand other important driver interactions underlying projections of global marine primary production [10].

#### How are we studying driver interactions at present?

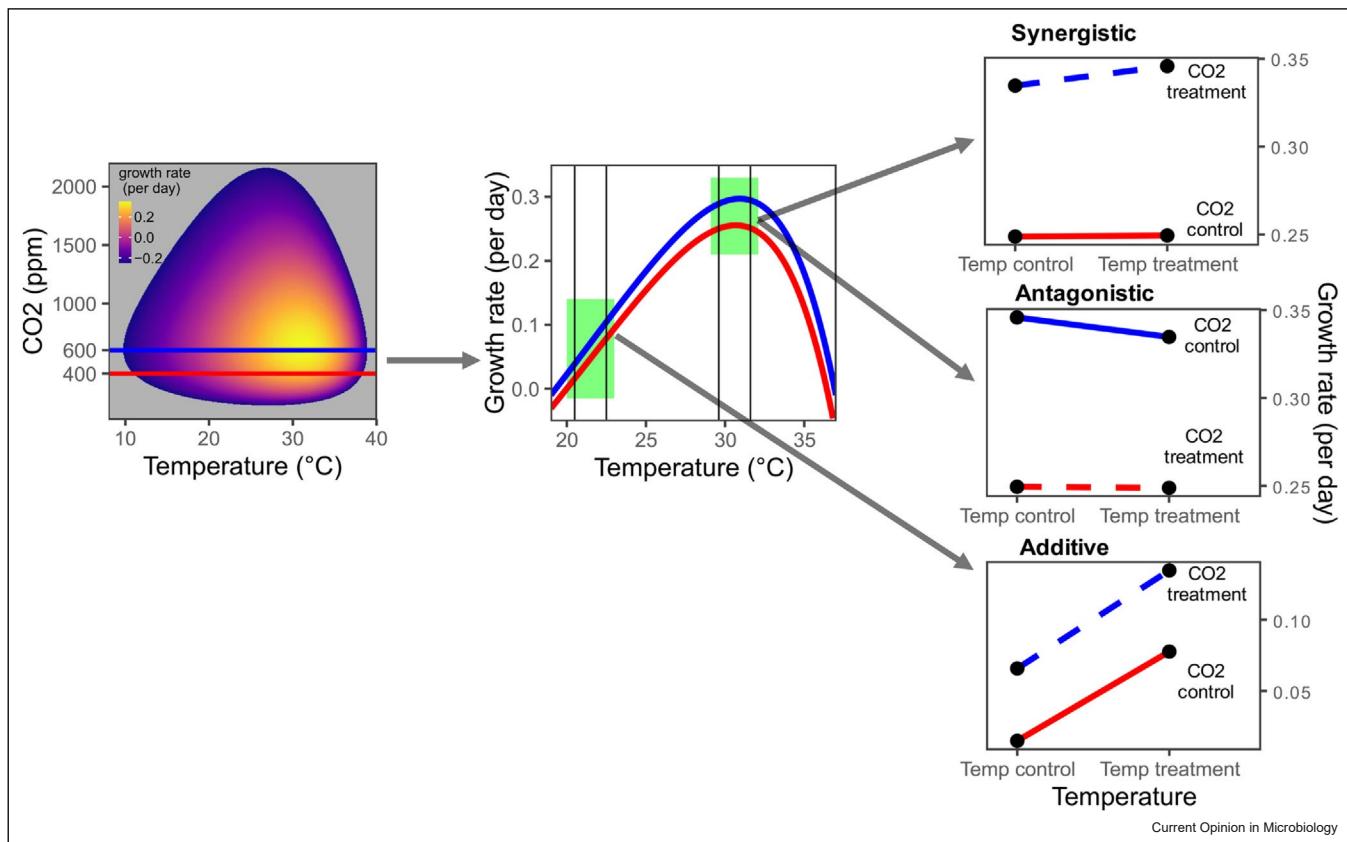
We compiled data on temperature × CO<sub>2</sub> interaction experiments involving two key marine phytoplankton taxa (diatoms and coccolithophores). We included data from studies that manipulated both CO<sub>2</sub> and temperature, with at minimum a 2 × 2 factorial experimental design, and which measured population growth rate. We found 16

studies that met these criteria (see Refs. [13,14] for a representative example). Nearly all the data from these studies have been gathered at just 2 levels of CO<sub>2</sub> (~400 ppm and ~1000 ppm), at or near 20°C (Figure 1; these two levels of CO<sub>2</sub> contain hexagons in red or orange). Despite omitting simple cases where drivers were not independently manipulated, the median experiment is still a 2 × 2 study. Overall, the published data do a poor job of sampling the interaction surface for temperature and CO<sub>2</sub>. 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 [15–17], our current understanding of physiology cannot build interaction surfaces from first principles.

#### Key limitations of the current approach to driver interaction experiments

Temperature response curves exist for > 100 species representing all major phytoplankton functional groups [36]. CO<sub>2</sub> response curves at different temperatures are scarce, so expectations of temperature–CO<sub>2</sub> response surfaces cannot be generated. Our estimates of organismal responses to the joint effects of rising CO<sub>2</sub> and warming are therefore sensitive to small errors in both predictions of future environments (Figure 3) and assumptions about the

Figure 4



Response surface experiments can lead to very different inferences than an ANOVA experiments. In this example, the same response surface (left panel) is reduced first to 2 response curves (middle panel) and finally to  $2 \times 2$  ANOVA experiments (right panels). The specific ANOVA experimental levels chosen (vertical black lines in middle panel) and the choice of reference level alter 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 CO<sub>2</sub> and low temperature are chosen as control conditions, while in the antagonistic case, the high CO<sub>2</sub> and high temperature are chosen as controls. Additive/synergistic/antagonistic interactions (or other descriptions of how interactions depart from additivity) are context dependent, whereas capturing the response surface largely eliminates the need for this misleading nomenclature. Note that the parameters for this surface differ from Figures 2 and 3.

shape of the response surface (Figure 4). Despite the knowledge that warming and changes to CO<sub>2</sub> are key drivers in marine systems, and decades of experiments with temperature and CO<sub>2</sub>, 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 versus species B). These designs treat continuous variables as discrete (e.g. temperature treated as 'present' versus '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 multidimensional versions of regression designs (Figure 2).

When would  $2 \times 2$  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 CO<sub>2</sub> 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 CO<sub>2</sub> levels or even average global temperatures will be decades from now, with uncertainties running to several degrees [37]. Simulations from a realistic response surface (Figure 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 [15,38] 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.

The ANOVA approach is not rescued by binning responses into qualitative levels. The common additive/synergistic/antagonistic interaction framework and its extensions or variations [39,40] are 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 (Figure 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.

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 [41,42]. 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 CO<sub>2</sub> concentration (an Eppley surface) should be possible with sufficient data on variation in temperature–CO<sub>2</sub> 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 logically 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 five temperature levels; more is better. Based on data thus far, the same appears to be true for CO<sub>2</sub> response curves [12]. 'Collapsed designs' can be useful in reducing the size of experiments relative to fully-factorial designs (see Ref. [43]) 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 prioritisation. For example, a 6 × 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) [21] and Zhang (2020) [27] conducted experiments with enough total experimental units to have calculated rough response surfaces had more levels of temperature been used; both used more CO<sub>2</sub> levels than necessary.

There is substantial variation in responses to changes in temperature and CO<sub>2</sub> both within and between functional groups (and within and between species) [12,36,38,44,45]. 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. [44]), or as coordinated networks of experiments (e.g. [46]) 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 trait change can differ in both magnitude and direction from plastic trait changes [47–49]. There is also good evidence that adaptation to warming is affected by other drivers [50]. We need generalisable insights into how response surfaces evolve under different rates and patterns of multidriver environmental change.

We suggest prioritising 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 recognise 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.

## Author contributions

SC designed the study. HW conducted the initial literature survey. MKT analysed data and models. SC and MK jointly wrote the manuscript.

## Conflict of interest statement

None.

## Acknowledgements

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 US 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).

## Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.mib.2022.102151](https://doi.org/10.1016/j.mib.2022.102151).

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

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. *Proc R Soc B* 2020, 287:20200421.
2. Thomas MK, Aranguren-Gassis M, Kremer CT, Gould MR, Anderson K, Klausmeier CA, Litchman E: Temperature-nutrient interactions exacerbate sensitivity to warming in phytoplankton. *Glob Chang Biol* 2017, 23:3269-3280.
3. Bestion E, Schaum C-E, Yvon-Durocher G: Nutrient limitation constrains thermal tolerance in freshwater phytoplankton. *Limnol Oceanogr Lett* 2018, 3:436-443.
4. Weithoff G, Beisner BE: Measures and approaches in trait-based phytoplankton community ecology — from freshwater to marine ecosystems. *Front Mar Sci* 2019, 6:40.
5. Duarte CM: Global change and the future ocean: a grand challenge for marine sciences. *Front Mar Sci* 2014, 1:1-16.
6. Litchman E, Klausmeier CA: Trait-based community ecology of phytoplankton. *Annu Rev Ecol Evol Syst* 2008, 39:615-639.
7. van de Waal DB, Litchman E: Multiple global change stressor effects on phytoplankton nutrient acquisition in a future ocean. *Philos Trans R Soc B: Biol Sci* 2020, 375:1-8.
8. Cotto O, Wessely J, Georges D, Klonner G, Schmid M, Dullinger S, Thüller W, Guillaume F: A dynamic eco-evolutionary model predicts slow response of alpine plants to climate warming. *Nat Commun* 2017, 8:p1-9.
9. Beckmann A, Schaum CE, Hense I: Phytoplankton adaptation in ecosystem models. *J Theor Biol* 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.
10. 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.
11. 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. *Nat Clim Chang* 2015, 5:1002-1006.
12. Paul AJ, Bach LT: Universal response pattern of phytoplankton growth rates to increasing CO<sub>2</sub>. *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<sup>+</sup> (corresponding to a large range in CO<sub>2</sub>) and that this constitutes a 'universal reaction norm' to CO<sub>2</sub>. While further data are needed to test the hypothesis, this is the first time a single overarching explanation has been proposed for CO<sub>2</sub> reaction norms in phytoplankton.
13. 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 two levels of CO<sub>2</sub> and three temperatures, with and without UV. This is a current example of a 2 × 3 CO<sub>2</sub> × 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 × CO<sub>2</sub> interaction surface.
14. 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. *Sci Total Environ* 2021, 771:145167.
- Uses experimental evolution with a marine diatom with a 2 × 2 design for temperature and CO<sub>2</sub>, and finds that, at these driver levels, warming is the main driver of trait change, but some traits are also affected by CO<sub>2</sub> levels. This study considers mainly photosynthesis-related traits. This demonstrates that interaction effects between warming and CO<sub>2</sub> depend on the traits under consideration, as well as on the precise levels of each driver used.
15. 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. *Front Mar Sci* 2019, 6:322.
16. van de Waal DB, Litchman E: Multiple global change stressor effects on phytoplankton nutrient acquisition in a future ocean. *Philos Trans R Soc B: Biol Sci* 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 × CO<sub>2</sub> (and other) interactions, and also point towards important and neglected trade-offs.
17. Serra-Pompeu C, Hagstrom GI, Visser AW, Andersen KH: Resource limitation determines temperature response of unicellular plankton communities. *Limnol Oceanogr* (4) 2019, 64:1-14.
- A model of phytoplankton growth that incorporates temperature dependencies at the level of cellular processes, enabling the incorporation of interactions with CO<sub>2</sub>, nutrients, and other factors at a subcellular scale. This is likely to be a productive path towards developing better models of temperature–CO<sub>2</sub> (and other) interactions.
18. 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 *Emiliania huxleyi*. *Glob Chang Biol* 2020, 26:5630-5645.
19. Feng Y, Warner ME, Zhang Y, Sun J, Fu F-X, Rose JM, Hutchins DA: Interactive effects of increased pCO<sub>2</sub>, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae). *Eur J Phycol* 2008, 43:87-98.
20. De Bodt C, Van Oostende N, Harlay J, Sabbe K, Chou L: Individual and interacting effects of pCO<sub>2</sub> and temperature on *Emiliania huxleyi* calcification: study of the calcite production, the coccolith morphology and the coccospHERE size. *Biogeosci Discuss* 2009, 6:p1401-1412.

21. 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 pCO<sub>2</sub>. *PLoS One* 2014, 9:e88308.

22. Milner S, Langer G, Grelaud M, Ziveri P: Ocean warming modulates the effects of acidification on *Emiliania huxleyi* calcification and sinking. *Limnol Oceanogr* 2016, 61:1322-1336.

23. Li X, Roevros 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.

24. Passow U, Laws EA: Ocean acidification as one of multiple stressors: growth response of *Thalassiosira weissflogii* (diatom) under temperature and light stress. *Mar Ecol Prog Ser* 2015, 541:75-90.

25. Torstensson A, Chierici M, Wulff A: The influence of increased temperature and carbon dioxide levels on the benthic/sea ice diatom *Navicula directa*. *Polar Biol* 2012, 35:205-214.

26. Torstensson A, Hedblom M, Andersson J, Andersson M, Wulff A: Synergism between elevated pCO<sub>2</sub> and temperature on the Antarctic sea ice diatom *Nitzschia lecoitei*. *Biogeosciences* 2013, 10:6391-6401.

27. Zhang Y, Collins S, Gao K: Reduced growth with increased quotas of particulate organic and inorganic carbon in the coccolithophore *Emiliania huxleyi* under future ocean climate change conditions. *Biogeosciences* 2020, 17:6357-6375.

28. Li F, Beardall J, Gao K: Diatom performance in a future ocean: interactions between nitrogen limitation, temperature, and CO<sub>2</sub>-induced seawater acidification. *ICES J Mar Sci* 2018, 75:1451-1464.

29. Qu P, Fu F, Hutchins DA: Responses of the large centric diatom *Coscinodiscus* sp. to interactions between warming, elevated CO<sub>2</sub>, and nitrate availability. *Limnol Oceanogr* 2018, 63:1407-1424.

30. Laws EA, McClellan SA, Passow U: Interactive effects of CO<sub>2</sub>, temperature, irradiance, and nutrient limitation on the growth and physiology of the marine diatom *Thalassiosira pseudonana* (Coscinodiscophyceae). *J Phycol* 2020, 56:1614-1624.

31. Hyun B, Choi K-H, Jang P-G, Jang M-C, Lee W-J, Moon C-H, Shin K: Effects of increased CO<sub>2</sub> and temperature on the growth of four diatom species (*Chaetoceros debilis*, *Chaetoceros didymus*, *Skeletonema costatum* and *Thalassiosira nordenskioeldii*) in laboratory experiments. *J Environ Sci Int* 2014, 23:1003-1012.

32. 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.

33. 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. *Glob Chang Biol* 2019, 25:2869-2884.

34. Fiorini S, Middelburg JJ, Gattuso J-P: Effects of elevated CO<sub>2</sub> partial pressure and temperature on the coccolithophore *Syracospaera pulchra*. *Aquat Microb Ecol* 2011, 64:221-232.

35. Gao G, Fu Q, Beardall J, Wu M, Xu J: Combination of ocean acidification and warming enhances the competitive advantage of *Skeletonema costatum* over a green tide alga, *Ulva linza*. *Harmful Algae* 2019, 85:101698.

36. Thomas MK, Kremer CT, Litchman E: Environment and evolutionary history determine the global biogeography of phytoplankton temperature traits. *Glob Ecol Biogeogr* 2016, 25:75-86.

37. 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. Cambridge University Press; 2021.

38. Seifert M, Rost B, Trimborn S, Hauck J: Meta-analysis of multiple driver effects on marine phytoplankton highlights modulating role of pCO<sub>2</sub>. *Glob Chang Biol* 2020, 26:6787-6804.

A synopsis of the current data on phytoplankton responses to temperature and CO<sub>2</sub>, with conclusions based mainly on 2 × 2 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.

39. Piggott JJ, Townsend CR, Matthaei CD: Reconceptualizing synergism and antagonism among multiple stressors. *Ecol Evol* 2015, 5:1538-1547.

40. 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. *Ecol Lett* 2018, 21:568-577.

41. Eppley RW: Temperature and phytoplankton growth in the sea. *Fish Bull* 1972, 70:1063-1085.

42. Bissinger JE, Montagnes DJS, Sharples J, Atkinson D: Predicting marine phytoplankton maximum growth rates from temperature: improving on the Eppley curve using quantile regression. *Limnol Oceanogr* 2008, 53:487-493.

43. 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. *Glob Chang Biol* 2018, 24:2239-2261.

44. Anderson SI, Ryneerson TA: Variability approaching the thermal limits can drive diatom community dynamics. *Limnol Oceanogr* 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.

45. Schaum E, Rost B, Millar AJ, Collins S: Variation in plastic responses of a globally distributed picoplankton species to ocean acidification. *Nat Clim Chang* 2013, 3:298-302.

46. Boyd PW, Ryneerson TA, Armstrong EA, Fu F, Hayashi K, Hu Z, Hutchins DA, Kudela RM, Litchman E, Mulholland MR, et al.: Marine phytoplankton temperature versus growth responses from polar to tropical waters — outcome of a Scientific Community-Wide Study. *PLOS One* 2013, 8:e63091.

47. Barton S, Jenkins J, Buckling A, Schaum CE, Smirnoff N, Raven JA, Yvon-Durocher G: Evolutionary temperature compensation of carbon fixation in marine phytoplankton. *Ecol Lett* 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 CO<sub>2</sub> together will be different from dealing with either alone.

48. Schaum CE, Rost B, Collins S: Environmental stability affects phenotypic evolution in a globally distributed marine picoplankton. *ISME J* 2015, 10:75-84.

49. Lindberg RT, Collins S: Quality-quantity trade-offs drive functional trait evolution in a model microalgal 'climate change winner'. *Ecol Lett* 2020, 23:780-790.

50. Aranguren-Gassis M, Kremer CT, Klausmeier CA, Litchman E: Nitrogen limitation inhibits marine diatom adaptation to high temperatures. *Ecol Lett* 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 two levels of nitrogen. This highlights that interaction surfaces themselves can evolve, but does not characterise how they do.