Title

New niches for larger phytoplankton in a warmer, more resource-limited ocean

Authors

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Abstract

Warming and nutrient limitation are major stressors that affect primary production in the ocean, with cascading impacts on the food web. Yet, we lack a mechanistic understanding of how phytoplankton manage the combined stress of heat-damage and nutrient-limitation and the implications of these responses for phytoplankton biogeography. By combining theory, proteome allocation modeling, and climate projections, we identified two potential competing strategies for multi-stressor growth: (1) increase growth temperature optima through higher nutrient uptake efficiency and smaller cells, or (2) invest in heat-mitigation mechanisms achieving higher thermal tolerance at the cost of growth and larger cells. By simulating the optimal metabolic strategies of different phytoplankton functional types across a latitudinal gradient, we found that *Prochlorococcus* are more vulnerable in warmer, 'heat-stressed', tropical regions due to greater heat sensitivity and lower storage capacity, indicating a potential ecological niche for larger phytoplankton with lower sensitivity to oxidative stress, such as *Synechococcus* and picoeukaryotes. Our findings advocate for the inclusion of phytoplankton heat-stress responses in global models to more accurately predict their ecological niches as the climate warms.

1 Introduction

Future climate scenarios project an average increase of 1.5°C by 2040 in the global ocean (IPCC, 2023) with vast regions of the ocean warming to above 30°C by the end of the century (Kwiatkowski et al., 2020). Ocean warming is expected to promote stronger stratification, potentially decreasing the influx of nutrients from below the mixed layer (Bopp et al., 2001). Changing ocean conditions have already resulted in decreased primary production (Boyce et al., 2010) and shifts in species distributions (Thomas et al., 2012) and size spectra (Atkinson et al., 2024). As phytoplankton drive ocean carbon cycling and fuel the ocean food web (Worden et al., 2015), understanding how these microbes respond to changes in temperature and nutrient availability is critical for predicting ecosystem function as the climate changes (Raven and Geider, 1988; Boyd et al., 2018; Van de Waal and Litchman, 2020).

A key driver of microbial response to resource availability and temperature is cell size, more specifically surface area-to-volume ratio (Litchman et al., 2009; Finkel et al., 2010; Marañón, 2015; Sommer et al., 2017). Under low nutrient concentrations, uptake is limited by molecular diffusion. Thus, a phytoplankter cell can increase nutrient uptake by increasing the number of transporters in the outer membrane (Aksnes and Egge, 1991). As there is a limit to the number of transporters a membrane can support, cells maximize the nutrient flux per unit of cellular volume by decreasing cell size and thereby increasing their surface area-to-volume ratio (Finkel et al., 2010; Sommer et al., 2017; Van de Waal and Litchman, 2020) (Fig. 1). While warming temperatures often result in smaller cell sizes (Atkinson et al., 2003; Daufresne et al., 2009), experimental (Schaum et al., 2018; Liang et al., 2019; Barton et al., 2023; Labban et al., 2023) and field (Hattich et al., 2024) studies have shown that phytoplankton increase their cell volume at critically high temperatures. In a previous study, we suggested that these larger cell sizes are a result of increased investment in energy metabolism and heat-mitigation, both of which require a lower surface area-to-volume ratio, i.e., larger cell sizes, to accommodate carbon storage and antioxidant machinery (Leles and Levine, 2023) (Fig. 1). This sets up a challenge for phytoplankton cells facing both nutrient and heat stress: invest in uptake which requires more transporters and higher surface area-to-volume ratios (smaller sizes), or invest in heat-stress mitigation strategies which require lower surface area-to-volume ratios (larger sizes) (Fig. 1).

The global ocean has well known biogeographical distributions of phytoplankton (Longhurst, 2010) with *Prochlorococcus* dominating the oligotrophic gyres and larger eukaryotic phytoplankton thriving in more nutrient-rich temperate and coastal regions (Anderson et al., 2024). Current models predict that, as the climate warms, the oligotrophic gyres will expand poleward resulting in an expanded ecological niche for *Prochlorococcus* (Dutkiewicz et al., 2013;

Flombaum et al., 2013). However, these predictions are based on a simplified representation of phytoplankton physiology that does not account for cellular strategies to mitigate heat stress and store carbon, both of which can promote increases in cell volume (Yang et al., 2014; Baroni et al., 2019; Liang et al., 2019; Labban et al., 2023; Marañón et al., 2024; Hattich et al., 2024; Miettinen et al., 2024). In addition, these models assume fixed thermal traits when in reality phytoplankton can shift their thermal optimum for growth (T_{opt}) through evolutionary adaptation and acclimation to changing environmental conditions (Hattich et al., 2024). Empirical studies suggest that the T_{opt} of phytoplankton often shifts left under nutrient limitation resulting in additional growth reductions at high temperatures (Thomas et al., 2017; Bestion et al., 2018). However, some species are able to avoid this "metabolic meltdown" (Huey and Kingsolver, 2019) by increasing their T_{opt} under nutrient stress (Aranguren-Gassis and Litchman, 2020). It remains unclear how physiological responses, such as shifts in T_{opt} and cell size, interact to shape phytoplankton biogeography under climate change.

Here, we investigate underlying mechanisms behind phytoplankton responses to co-occuring heat-stress and nutrient limitation using a cellular allocation model. We demonstrate that T_{opt} can shift to either warmer or cooler temperatures under nitrogen limitation and that this shift is modulated by the heat-mitigation capacity of the cell. We then expand our theoretical predictions by calibrating the model to data for relevant phytoplankton functional types and simulate optimal growth strategies across a latitudinal transect under current and future projections of temperature and nitrate concentrations in the ocean. While the niche of cyanobacteria expands towards higher latitudes, *Prochlorococcus* are more vulnerable in the warmer tropical seas due to their low heat-mitigation capacity and small cell sizes. We thus identify a critical new niche in warm tropical seas where phytoplankton with larger cell sizes, such as *Synechococcus* or even small eukaryotes, might thrive due to their larger flexibility in cell size and thermal tolerance. Our results suggest that representing the interaction between heat-stress and nutrient limitation in climate models is paramount for predicting species extinction and succession.

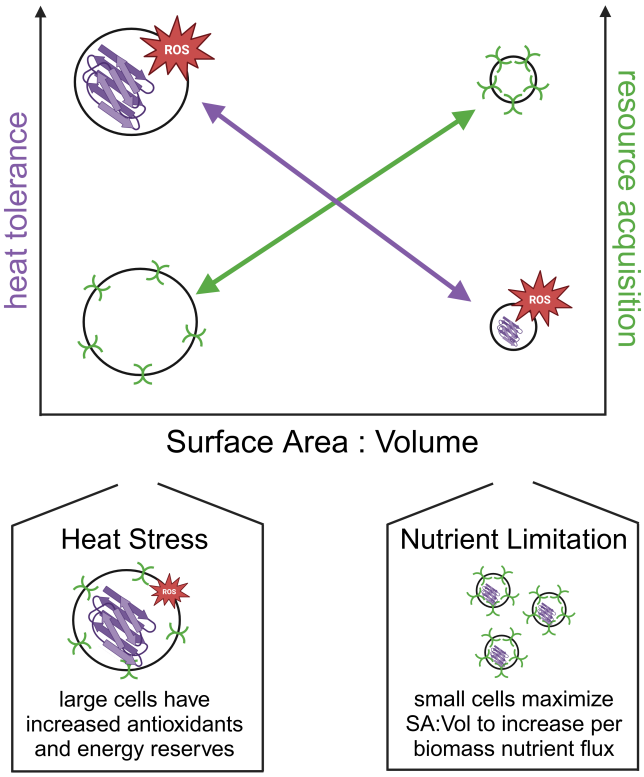

Figure 1: **Predicted trade-off for phytoplankton under multi-stressor growth.** Under nutrient limitation, phytoplankton need to invest more in transporters increasing their surface area-to-volume ratio, which requires smaller cell sizes. Under heat-stress, the cell must invest in heat-mitigation strategies decreasing their surface area-to-volume ratio to accommodate antioxidants and other proteomic investments which requires larger cell sizes. A trade-off between decreasing cell size to optimize nutrient uptake and increasing cell size to optimize heat-mitigation emerges under the multiple stressor scenario.

2 Results

2.1 The proteome allocation model

We used a phytoplankton proteome allocation model to understand the molecular mechanisms behind changes in T_{opt} and cell size under multi-stressor growth (Fig. 1). Here, we expand the model of Leles and Levine (2023) to simulate a range of phytoplankton phenotypes under the multiple stressor condition of heat-stress and resource limitation. Given that nitrogen is the most often limiting nutrient within oligotrophic gyres (Moore et al., 2013), we investigated phytoplankton responses to nitrogen limitation. However, the model dynamics of resource limitation are

broadly applicable to other limiting nutrients (e.g., phosphorus). We then use the model to simulate phytoplankton growth along a latitudinal gradient under current and future ocean conditions (see Methods).

Our proteome model consists of a system of ordinary differential equations defined as a constrained optimization problem (see Supplemental Methods for complete list of model equations). The model predicts cellular traits and physiological states by maximizing the steady-state growth rate of a phytoplankton cell under specific environmental conditions (i.e., irradiance, external inorganic nitrogen and carbon concentrations, and temperature). Specifically, the model simulates cellular resource allocation by optimizing the relative proteome investment across metabolic functions (e.g., nutrient uptake, biosynthesis, respiration), intracellular macromolecular concentrations (e.g., proteins, carbon storage), and the surface area-to-volume ratio of the phytoplankton cell (Table S1). The model resolves carbon and nitrogen metabolisms, including transport of nitrogen into the cell and synthesis of all critical cellular components. Parameter values are given in the Supplemental Methods (Tables S2 and S3).

Temperature accelerates all enzymatic reactions in the model (Eq. 3, Methods) except photochemical reactions, which we assumed to be temperature independent (Raven and Geider, 1988; Leles and Levine, 2023). Nitrogen transporters have a lower thermal dependency than other reactions in the model based on previous empirical (Kristiansen, 1983; Berges et al., 2002; Baker et al., 2016; Tomczyk et al., 2022) and modeling (Leles and Levine, 2023) studies. All other processes are assumed to have the same thermal dependency (defined by the parameter E_a). Warming also results in protein damage in the model, thus mechanistically representing the decay in growth rates as temperatures increase beyond the optimum. To minimize heat-damage, the model cell can invest in repair pathways and in heat-mitigation strategies, such as producing antioxidant enzymes and/or storing fatty acids (Hemme et al., 2014; Jarc and Petan, 2019; McCain and Bertrand, 2022). Heat-mitigation capacity is controlled by the parameter α, with higher values enabling the cell to build more antioxidant machinery which, in turn, allows the cell to be more efficient at mitigating oxidative stress (Eq. 4, Methods). While high α values provide protection against oxidative stress, this strategy comes with a trade-off of requiring intracellular space and proteomic investment that divests resources away from nutrient acquisition and/or growth.

We solve the proteome model over a range of temperatures and nitrogen concentrations, allowing thermal growth curves to emerge as a function of molecular constraints. Key to our analysis is that we are able to evaluate the underlying physiological and molecular mechanisms driving changes in thermal traits (e.g., T_{opt}) and cell size. The optimal surface area-to-volume ratio of the cell is solved by the model as a function of surface area required for transporters in the cell membrane and cellular volume required for intracellular machinery, such as photosystems,

nucleus, and antioxidants. Therefore, our model offers a platform to test, mechanistically, the cell size trade-off that phytoplankton face under the combined effects of heat-stress and nitrogen limitation (Fig. 1).

2.2 Understanding growth trade-offs under multi-stressor conditions

To understand the fundamental trade-offs associated with multi-stressor growth, we simulated phytoplankton cells with varying thermal dependencies (E_a) and heat-damage mitigation ability (α) and analyzed the emergent thermal growth responses (see Sensitivity Analyses, Methods). For this analysis, the cells were otherwise equal (i.e., all other parameters were equal). The model captured the expected trade-offs where warm-adapted cells (higher E_a) have faster growth at higher temperatures but slower growth at lower temperatures (Fig. 2A) and cells with higher heat-mitigation capacity (higher α) survive at higher temperatures at the cost of lower maximum growth rates (Fig. 2B).

The model predicts both increases and decreases in T_{opt} under nitrogen limitation (Figs. 2C and D), consistent with experimental results (Thomas et al., 2017; Bestion et al., 2018; Aranguren-Gassis and Litchman, 2020). The model suggests that warm-adapted cells (higher E_a) are more likely to increase their T_{opt} under nitrogen limitation (Fig. 2D), allowing cells to maximize nitrogen uptake rates at higher temperatures (Fig. 2E). Higher heat-mitigation capacity allows the cell to survive at higher maximum temperatures (Fig. 2F) but it also results in a decrease in T_{opt} and lower maximum growth rates under nutrient stress (Figs. 2D and G). Thus, two competing strategies for coping with multi-stressor conditions emerge from the model results: 'nutrient-efficient' or 'thermal-tolerant' (Fig. 2C). The 'nutrient-efficient' strategy couples higher E_a values with lower α values. Higher E_a values allow cells to invest primarily in growth (ribosomes). In turn, lower α values results in less investment in heat-mitigation strategies which allows for smaller cell sizes and more efficient nutrient uptake. This comes with the trade-off that cells are more susceptible to heat-damage and so have lower maximum growth temperatures. Alternatively, the 'thermal-tolerant' strategy cells invest heavily in heat mitigation (higher α). This results in increased proteomic investment in heat mitigation proteins which diverts resources away from growth (ribosomes) and results in lower maximum growth rates (Fig. 2G). This strategy also requires more antioxidant machinery that results in larger cells. However, this investment allows for a higher maximum critical thermal limit (Fig. 2F). Because the 'thermal-tolerant' strategy requires larger cell sizes, cells cannot be both thermally tolerant and nutrient efficient.

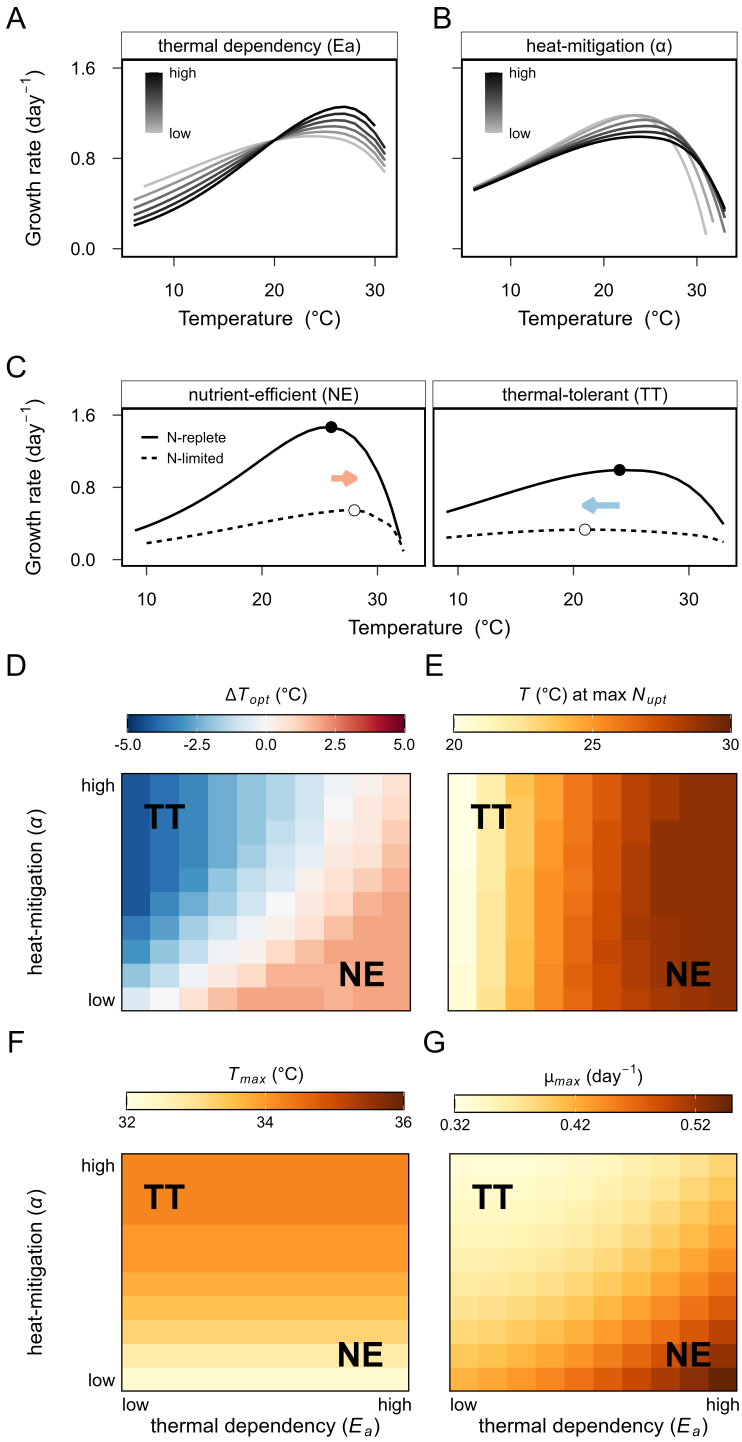

Figure 2: **Growth trade-offs as a function of thermal dependency (Ea) and heat-mitigation capacity (α).** Thermal growth curves under nitrogen-replete conditions are given for (A) cells with variable E_a and (B) cells with variable α. (C) Thermal growth curves indicate shifts in T_{opt} (open circles) from N-replete to N-limited conditions as a result of different metabolic strategies. (D) The change in T_{opt} from N-replete to N-limited conditions (ΔT_{opt}) showing increases (positive and red values) and decreases (negative and blue values) in T_{opt}. (E) Temperature (T) where nitrogen uptake rates (N_{upt}) are maximized under N-limited. (F) Maximal growth temperatures (T_{max}) under N-limited. (G) Maximal growth rates (μ_{max}) under N-limited. The two competing strategies are highlighted: nutrient-efficient (NE) and thermal-tolerant (TT).

2.3 Predicting phytoplankton optimal growth strategies in a changing ocean

We investigate the implications of different growth strategies in a changing ocean using four contrasting phytoplankton functional types: *Prochlorococcus*, *Synechococcus*, warm-adapted diatom, and cold-adapted diatom. *Prochlorococcus* are small and are optimized for high nutrient use efficiency (Edwards et al., 2012). In contrast, Synechococcus have slightly larger cell sizes and are less sensitive to oxidative stress than *Prochlorococcus* (Mella-Flores et al., 2012). The two diatoms have substantially larger cell sizes and carry large numbers of genes for mitigating temperature and oxidative stress (Drábková et al., 2007; Bernroitner et al., 2009; Rosenwasser et al., 2014). Diatoms were selected as a representative bloom forming eukaryotic phytoplankton due to their abundance throughout the oceans, the critical role they play in marine ecosystems, and the availability of data to validate the model. The dichotomy between cyanobacteria and diatoms in our model also mirrors the way phytoplankton diversity is typically simplified in biogeochemical models (see Discussion) (Danabasoglu et al., 2020).

We parameterized the proteome model to represent these four strategies based on differences in nutrient uptake efficiency, thermal traits, and heat-mitigation capacity (Table S3, Methods). We validated our model phenotypes against thermal growth data from 167 species obtained from Anderson et al. (2021). The maximum growth rate and slope of the thermal curves for our model phenotypes (Fig. S1) show good agreement with observed values (Fig. 3A). In addition, the emergent cell sizes in the model were consistent with the expected differences between these two groups (Fig. 3B). We also qualitatively validated modeled shifts in proteome investment under heat or nutrient stress using proteomic datasets, based on the up- or down-regulation of specific pathways (Tables S4 and S5, see Methods). Unfortunately, no proteomic datasets were available to validate predictions under combined heat and nutrient stress.

Under heat stress, all phenotypes divested resources away from nitrogen transporters to invest in heat-mitigation strategies and glycolysis to support increased respiration demands (Fig. S3). This trade-off is supported by observed proteomic shifts in the marine diatom *Chaetoceros simplex* (Aranguren-Gassis et al., 2024), where populations down-regulated the investment in nitrogen transporters when grown at temperatures that induced heat-stress, while up-regulating the investment in chaperones, ROS scavenging, heat shock proteins, and glycolysis (Table S4). Under nitrogen stress, all modeled phenotypes maximized the specific affinity for nitrogen uptake by decreasing cell size (Fig. S3). Cells also divested resources away from photosystems, rubisco, ribosomes, and respiration under nitrogen limitation, while investing more in transporters (Fig. S3). These metabolic shifts are overall consistent with empirical observations for both diatoms and cyanobacteria (summarized in Table S5).

Under multi-stressor growth, the 'nutrient-efficient' strategy allowed cyanobacteria to achieve the fastest maximum growth rates (Fig. S1) and shift T_{opt} towards warmer temperatures due to their higher nitrogen use efficiency (Figs. 3B and C). However, at critically high temperatures, the 'thermal-tolerant' strategy allowed the warm-adapted diatom to achieve higher carbon and nitrogen use efficiency (Fig. 3C), resulting in better survival at higher maximum temperatures (Fig. S1).

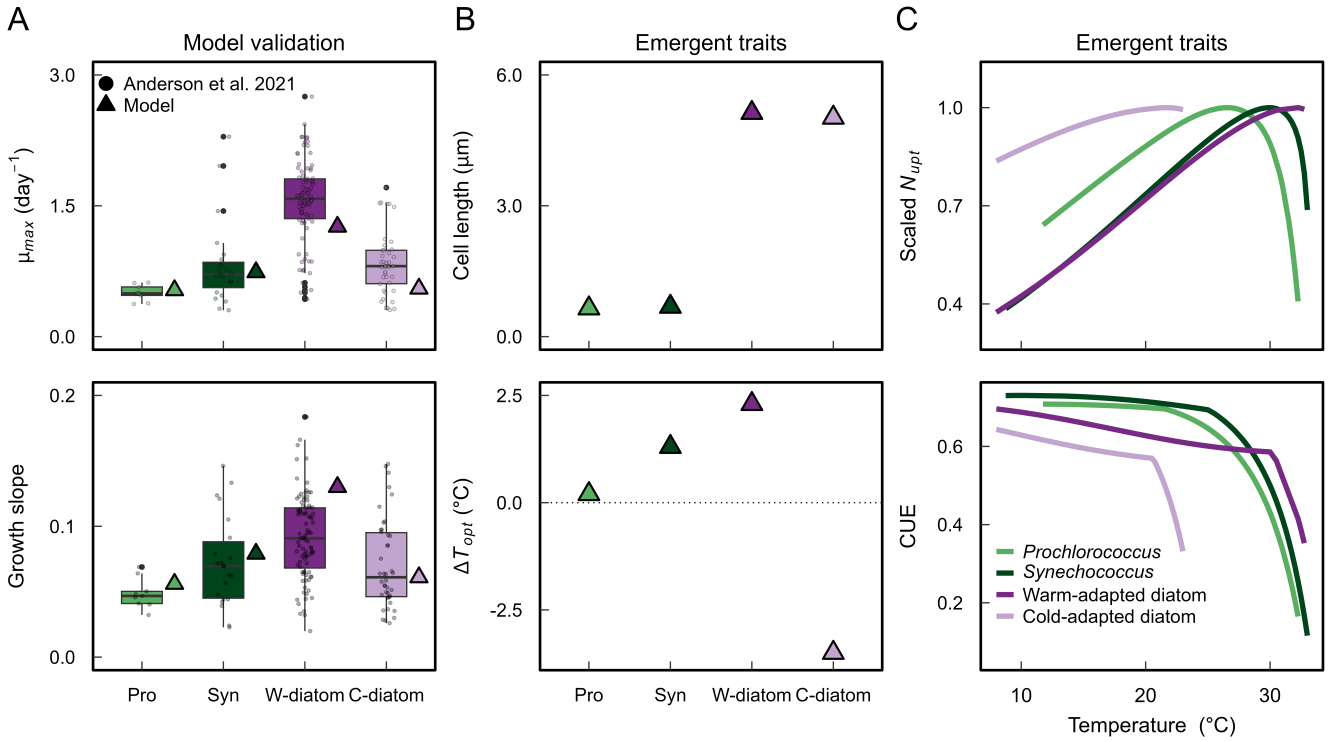

Figure 3: **Emergent traits and trade-offs for different phytoplankton functional types.** (A) Model validation against empirical thermal curve data from Anderson et al. (2021) for the cyanobacteria *Prochlorococcus* (n = 9) and *Synechococcus* (n = 23) as well as cold-adapted (n = 42) and warm-adapted (n = 93) diatoms under nitrogen replete conditions. Maximum growth rates (μ_{max}) and the slope at which growth rates increase as a function of temperature are shown. (B) Emergent model cell length at T_{opt} under nitrogen limitation (see Fig. S2 for N-replete conditions) and the shift in T_{opt} from N-limited to N-replete conditions. (C) Model nitrogen uptake rates (N_{upt}) scaled by maximum observed rate and Carbon Use Efficiencies (CUE) under nitrogen limitation. Carbon use efficiency is defined as 1 - respiration/photosynthesis. See Figs. S1 and S3 for full thermal curves and proteome investments in both nutrient regimes.

We then use these four phenotypes to investigate optimal growth strategies across a latitudinal transect in the Atlantic Ocean under present day (1980-2000) and future (2080-2100) conditions (see Methods; Fig. 4). For

this analysis, we use temperature and nitrate concentrations for these two time periods from the CESM-WACCM model (Fig. 4B). The proteome model captured present-day biogeographical conditions. We observed the expected distributions of cold-adapted diatoms at the poles and warm-adapted diatoms in the temperate ocean due to their different thermal dependencies (Figs. 4C and D). The cyanobacteria showed the highest growth potential in the oligotrophic gyres as expected (Fig. S4), especially in the ultra-oligotrophic South Atlantic, due to their high specific nutrient affinity (Figs. 4C and D). The warm-adapted diatom dominated again near the Equator due to increases in nitrate concentrations, except in the latitudinal range 0-7°N where nitrate concentrations were minimal and temperatures were maximal (Figs. 4C and D).

Under the future climate scenario, the model predicts a drastic increase globally in surface waters experiencing temperatures above 30°C from only 2.7% under present day to 34.5% by the end of the century (Figs. 4A and S5). Temperatures along the transect also warmed significantly with average increase of 2.8 °C (maximum of 4.76 °C) and nitrate concentrations were on average 33% lower (maximum of 86%). The poleward expansion of the oligotrophic gyres, where decreases in nitrate concentrations were greater than the increases in temperature, resulted in the poleward expansion of cyanobacteria due to their higher nutrient affinity, i.e. 'nutrient-efficient' strategy (Fig. 4D). The cold-adapted diatom phenotype still achieved the highest growth rates in the poles, however, the potential ecological niche for this group was narrower in the future projection due to the higher temperatures (Fig. 4D). The proteome model results also suggest a new niche where the projected increases in temperature will have a larger impact in governing phenotype shift than the projected change in nutrients ('heat-stressed regions', Fig. 4D). This niche occurred in regions of the ocean that are severely nutrient-limited under present-day conditions and were predicted to experience significant warming (e.g., 12°S to 22°N). We hypothesize that the 'thermal-tolerant' strategy rather then the 'nutrient-efficient' strategy will have a competitive advantage in these regions, resulting in increases in cell size corresponding to the need to combat oxidative damage and meet dark respiration demands (Fig. 4E). Thus, the *Synechococcus* and the warm-adapted diatom functional types are predicted to fair better in these 'heat-stressed' regions than *Prochlorococcus*. While the specific latitudinal range over which these changes would occur depend on the CESM model projections for changes in temperature and nitrate concentrations, our hypothesized shift in ecological niches provide insight into how the phytoplankton community will respond to multi-stressor change.

The underlying metabolic shifts in response to future multi-stressor change varied across oceanographic regions and by phenotype (Figs. S6 and S7). At the poles, all phenotypes invested more in transporters (Fig. S6) and decreased their cell size (white filled symbols in Fig. 4E) to maximize nitrogen use efficiency under future more

nitrogen limited conditions. This result is consistent with empirical observations that nitrogen limitation plays a more important role than temperature in determining phytoplankton cell size (Sommer et al., 2017). In the tropics, all phenotypes divested away from transporters and invested more in photosystems due to critical high temperatures, which increased respiration demands and oxidative stress, resulting in lower growth rates relative to current conditions (Figs. S6 and S7). This strategy required larger cell sizes relative to present conditions (black filled symbols in Fig. 4E). While *Prochlorococcus* ('nutrient-efficient' strategy) increased cell size to accommodate intracellular energy storage compounds to manage increasingly high rates of heat-damage and respiration, *Synechococcus* and the warm-adapted diatom ('thermal-tolerant' strategy) increased cell size to both store carbon for dark respiration and accommodate intracellular machinery for combating oxidative damage, thus better managing levels of heat-stress. This difference explains why the growth potential of *Prochlorococcus* was lower in the tropics. Overall, *Prochlorococcus* must devote a larger fraction of the proteome to respiration relative to *Synechococcus* and diatoms due to their lower heat-mitigation capacity (Fig. S7). As a result, our model suggests that cyanobacteria, in particular the streamlined ultra-oligotrophic 'nutrient-efficient' *Prochlorococcus*, will have lower metabolic flexibility to manage a warmer climate.

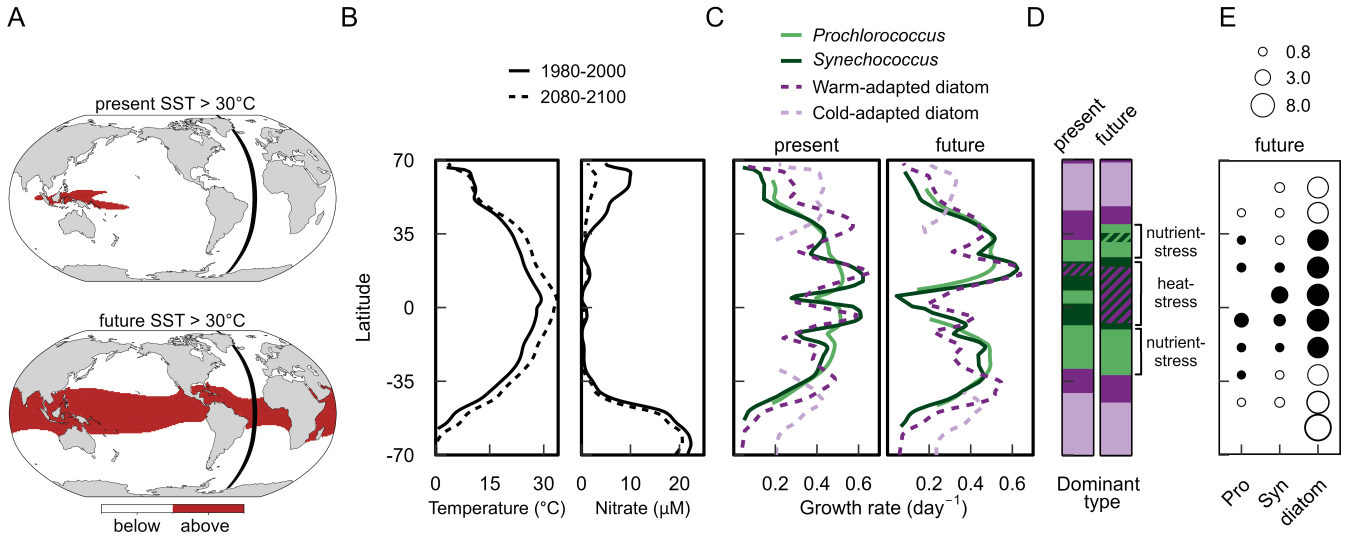

Figure 4: **Phytoplankton optimal growth strategies across a latitudinal gradient.** Future projections from the CESM-WACCM model indicate a 34.5% global increase in surface waters experiencing temperatures above 30°C (A). Simulations were run using current (1980-2000) and future (2080-2100) temperature and nitrate concentrations along a latitudinal transect in the Atlantic Ocean (B). Also shown are the optimal growth rates (C), dominant functional type by latitude (D), and optimal cell diameter (μm) under the future climate scenario (E), where white fill and black fill shows decreases and increases in cell size from present to future, respectively. See Fig. S6 for modeled proteome investments.

3 Discussion

By combining ecological theory, proteome allocation modeling, and future climate projections, we were able to identify proteomic and cell size constraints underlying phytoplankton response to multi-stressor change. Specifically, our approach allows us to differentiate between 'hard constraints' imposed by physics (e.g., space and energy limitations) and 'flexible constraints' due to biology (e.g., enzyme kinetics) (Leles and Levine, 2023). Unlike 'hard constraints', 'flexible constraints' can be mitigated through adaptation. We identified two different strategies that allow phytoplankton to minimize the negative impacts of climate warming under nitrogen limitation: 'nutrient-efficient' and 'thermal-tolerant'. Due to hard constraints on cells, these two strategies are mutually exclusive - a cell cannot be both maximally nutrient efficient and have a high thermal tolerance. The cyanobacteria *Prochlorococcus* is a key example of the nutrient-efficient strategy. This strategy has allowed this group to thrive in present day oligotrophic gyres and we predict that their niche will expand polewards under future conditions, consistent with previous studies (Dutkiewicz et al., 2013; Flombaum et al., 2013). However, we demonstrate that evolving to become maximally

nutrient-efficient, as Prochlorococcus has done, comes at a cost of thermal-tolerance. We therefore predict that future warming will result in the expansion of a 'heat-stressed' niche in the tropics where 'thermal-tolerant' phytoplankton will gain an advantage over 'nutrient-efficient' phytoplankton, due to their higher cell size flexibility and ability to manage heat-damage.

Our results highlight that cell size constraints are an important driver of phenotype. All phenotypes were predicted to decrease cell size in the polar and temperate oceans in response to nutrient stress and increase cell size in the tropics due to temperature stress (Fig. 4E). This increase in cell volume for our model diatoms is consistent with previous work which has shown that diatoms and other eukaryotes increase cell size when exposed to temperatures above their growth maxima (Schaum et al., 2018; Liang et al., 2019; Barton et al., 2023; Hattich et al., 2024) and when deprived of nutrients, due to an accumulation of carbohydrates (Baroni et al., 2019; Miettinen et al., 2024) and/or lipids (Yang et al., 2014). Similarly, our model predicted that, in order to survive in the tropics, the cyanobacteria functional types would need to significantly increase their cell diameter (up to 4-fold for *Prochlorococcus* and 3-fold for *Synechococcus*) to store carbon and support high respiration costs. This is consistent with recent laboratory (Labban et al., 2023; Barton et al., 2023) and field manipulative experiments by Marañón et al. (2024), which found that cyanobacteria increased their cell diameter under warming conditions up to 2-fold in the Atlantic Ocean, and that increases in cell volume were associated with slow or negative growth rates.

Global climate models predict a drastic increase in ultra-warm oligotrophic waters (Kwiatkowski et al., 2020), from 2 to 34% by the end of the century (Figs. 4A and S5). These projections are based on the average temperature and do not show additional effects of temporal variability such as heatwaves, which will further exacerbate the situation. The two closest analogies in the present day ocean for these future conditions are the South Pacific Gyre and the Red Sea. Red Sea surface temperatures can reach record values of 33°C in the surface (Chaidez et al., 2017) and nutrient concentrations can be as low as 0.1 μM (Al-Otaibi et al., 2020). Field observations show that the larger, more thermally tolerant cyanobacteria *Synechococcus* can dominate over *Prochlorococcus* in the surface waters of the Red Sea (Al-Otaibi et al., 2020; Coello-Camba and Agustí, 2021) and that Prochlorococcus is absent in the warmest (> 30°C) waters of the southern Red Sea (Kheireddine et al., 2017). While not conclusive, this does suggest that the Red Sea strains of *Prochlorococcus* have not been able to evolve to be competitive in the high temperature (> 30°C) niche (Labban et al., 2023). In contrast, Synechococcus was shown to be able to easily evolve to growth at elevated temperatures (> 30°C) by increasing cell size and chlorophyll per cell (Barton et al., 2023). Consistent with the observations from the Red Sea, while *Prochlorococcus* is abundant and the key contributor to carbon fixation around

the rims of the South Pacific Subtropical Gyre, carbon fixation within the gyre is dominated by small photosynthetic eukaryotes (Duerschlag et al., 2022). Thus, both our work and experimental and field evidence suggest that elevated temperatures in the equatorial and tropical regions may favor small eukaryotes and larger cyanobacteria relative to *Prochlorococcus*.

We hypothesize that the ability to manage oxidative stress will play a key role in the success of different phenotypes in the future oceans that experience high levels of warming. *Synechococcus* are less sensitive to oxidative stress than *Prochlorococcus* (Mella-Flores et al., 2012). Overall, eukaryotes are better at sensing the environment and controlling oxidative stress relative to prokaryotes (Drábková et al., 2007), which is probably related to the lack of a large number of antioxidant genes in the latter, especially for *Prochlorococcus* (Bernroitner et al., 2009). Previous work suggests that *Prochlorococcus* may have lost many antioxidant genes potentially because they are costly and, instead, rely on other microbes to degrade reactive oxygen species for them (Morris et al., 2012; Weenink et al., 2021). Our model did not account for the potential benefit of these "helpers". Yet, relying on other microbes to perform this important function poses a risk to the adaptive potential of *Prochlorococcus*, as the "helpers" will also rapidly evolve under changing ocean conditions with no guarantee that the current interactions will persist (Hennon et al., 2018; Lu et al., 2024). Finally, diatoms and other eukaryotes have been found to associate symbiotically with nitrogen-fixing cyanobacteria, representing yet another strategy that enables larger eukaryotic cells to thrive under nitrogen depleted conditions (Schvarcz et al., 2022; Coale et al., 2024). Exploring how these microbial interactions will shift under multi-stressor changes is an important avenue for future work.

A key challenge faced broadly by numerical models is how to represent the vast diversity of marine microbes. For this study, we used diatoms as our example eukaryotic phytoplankton type to facilitate validation against experimental datasets and to be consistent with the simplified set of phytoplankton functional groups typically used in global biogeochemical models (Kwiatkowski et al., 2020). However, as we do not incorporate key diatom specific metabolisms such as silicate frustule synthesis into the proteome model, these findings are broadly applicable across bloom forming eukaryotic phytoplankton (e.g., haptophytes). A key area for future research, both modeling and laboratory, is to understand the different strategies for coping with high temperature growth and oxidative stress in eukaryotic phytoplankton. Similarly, we focus on the upper water column (upper 20 m) where temperature and nutrient stress are greatest. However, there are sharp gradients within the euphotic zone and key differences between high and low light adapted *Prochlorococcus*. An additional area for future work would be to explore how the environment at the deep-chlorophyll maximum is predicted to shift and the impact on the relative competitive advantage of different

strategies.

Here we highlight the key importance that heat stress can play in determining biogeographical distributions of phytoplankton. Moreover, we hypothesize that due to inherent space and energy trade-offs, there are two alternative strategies for dealing with multi-stressor growth: 'nutrient-efficient' and 'thermal-tolerant'. Our work advocates for the inclusion of heat stress strategies, carbon storage, and changes in cell size into Earth System Models used for future climate predictions. This would allow for future studies to investigate the interactive effects of cellular-level responses to multi-stressor growth with populations dynamics and ecological interactions. Specifically, the creation of a new niche for larger phytoplankton in the equatorial and tropical ocean under warming conditions has the potential to reshape microbial interactions, food web structure, and nutrient cycling. Eukaryotic phytoplankton typically release more diverse and abundant organic compounds than cyanobacteria, fueling heterotrophic bacterial growth and enhancing nutrient remineralization (Amin et al., 2012). These interactions can support larger zooplankton previously constrained by the small size of cyanobacterial prey, enhancing trophic transfer efficiency to higher predators (Ward and Follows, 2016). Moreover, larger phytoplankton have higher cellular carbon content, produce sticky exopolymers that promote particle aggregation (Amin et al., 2012), and are consumed by zooplankton that generate denser fecal pellets. Together, these traits could increase the potential for vertical carbon export to deeper waters, altering the stoichiometry and depth of nutrient recycling. Integrating the insights from cellular scale models into biogeochemical models provides a powerful tool for synthesizing experimental and field data on cellular responses and scaling-up to understand large-scale climate impacts.

4 Methods

The model

We use a proteome allocation model modified from Leles and Levine (2023) to predict the optimal metabolic strategy and cell size of a phytoplankton cell under the multi-stressor growth condition of heat and nutrient stress. We further integrate the model to empirical data from 167 species previously compiled by Anderson et al. (2021) to model the optimal strategies of different phytoplankton functional types across a latitudinal transect under current and future ocean conditions. Our coarse-grained model is defined as a constrained optimization problem that maximizes the steady-state growth of the cell. Here, we give the general equations governing the model behavior and focus on the

processes relevant for the present work. For the full model equations and list of optimized variables (Table S1) and parameter values (Tables S2 and S3), we refer the reader to the Supplemental Methods and the code available on GitHub (`https://github.com/LevineLab/ProteomePhyto/tree/main`).

The model assumes a cell that is growing exponentially given a certain temperature and nitrogen concentration. The macromolecular concentrations (c) can thus be obtained assuming that the sum of the syntheses rates ($v_{synthesis}$) minus the sum of the degradation rates ($v_{degradation}$) are balanced by the cellular growth rate, μ:

$$\frac{dc}{dt} = \sum v_{synthesis} - \sum v_{degradation} - \mu c \quad = \quad 0. \tag{1}$$

Each reaction rate catalyzed by a given protein i is described following a Michaelis-Menten type equation:

$$v_i = k_i p_i \frac{S_i}{S_i + K_i}, \tag{2}$$

where k_i is the maximum turnover rate per protein, S_i is the substrate concentration, K_i is the half-saturation constant, and p_i is the concentration of protein i. k_i is derived from literature values and corrected to a reference temperature of 20 °C. We assume all reaction rates in the cell increase with temperature following the Arrhenius equation, such that:

$$\begin{aligned} k_i &= k_{ref_i} \gamma_{Ta}, \\ \gamma_{Ta} &= \exp\left(\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \end{aligned} \tag{3}$$

where k_{ref_i} is the maximum turnover rate at a reference temperature T_{ref} (in Kelvin) for a given protein i, E_a is the thermal dependency of metabolic rates (eV), R is the Boltzmann's constant (eV K^{-1}) and T is the actual temperature (K). The only exception is the maximum rate of photosynthesis, which we assume to be independent of temperature (Raven and Geider, 1988). All reactions were assumed to have the same E_a, with the exception of nitrogen uptake, for which we halved the thermal dependency relative to other reactions in the model according to previous empirical (Kristiansen, 1983; Berges et al., 2002; Baker et al., 2016) and modeling (Leles and Levine, 2023)

studies.

Warming also results in protein damage v_d which increases with increasing temperature, however, cells can invest in heat-mitigation strategies that reduce the impact of heat damage:

$$v_d = k_{ref_d} \gamma_{Td} \frac{\alpha}{p_{ox}},$$
$$\gamma_{Td} = \exp\left(\frac{E_d}{R}\left(\frac{1}{T_d} - \frac{1}{T}\right)\right) \tag{4}$$

in which k_{ref_d} is the maximum rate of damage at a given temperature, E_d is the thermal dependency of protein damage, α is the heat-mitigation capacity of the cell, and p_{ox} is the pool of antioxidant machinery. p_{ox} is constrained to be lower or equal to α. Thus, higher α enables the cell to build more antioxidant machinery p_{ox}, which increases the efficiency of the cell in managing oxidative stress, but it comes at the cost of lower maximum growth rates (Fig. 2B) due to higher intracellular space and proteomic costs required to build p_{ox} that are ultimately divested away from growth. Our model can represent two different strategies in which a cell can mitigate heat-damage: storing lipids to sequester reactive oxygen species (Jarc and Petan, 2019) or producing antioxidant enzymes to decrease oxidative stress (McCain and Bertrand, 2022). Here, we present the results for the model version in which a cell utilizes lipid storage. The results for antioxidant enzymes are quantitatively the same and are provided in the Supplemental Materials (Fig. S8).

The model optimizes for the surface area-to-volume of the cell. The cell size is a trade-off between 1) surface area required for nitrogen transporters and 2) intracellular space required for intracellular machinery, including photosystems, carbon storage, nucleus, and antioxidants (see Supplementary Methods). The model can be applied to simulate a phytoplankton cell of any shape, but here we assume a spherical cell for simplicity.

Sensitivity analyses

Empirical observations show that phytoplankton species display diverse thermal growth curves (Thomas et al., 2012; Anderson et al., 2021) and sensitivities to oxidative stress (Drábková et al., 2007; Mizrachi et al., 2019). To capture such trait diversity, we varied two parameters in our simulations: the thermal dependency of metabolic reactions (E_a) and the heat-mitigation capacity of the cell (α). We leveraged 167 phytoplankton thermal growth curves

from previous compilations (Thomas et al., 2012; Anderson et al., 2021) to select the range of E_a values to test in our sensitivity analyses. Note that the value for E_a in our model corresponds to the metabolic reaction and not necessarily the E_a for growth rates, which is the commonly described parameter in the literature. In turn, the value of α is poorly constrained in the literature. The range of α values used in our simulations was guided by a previous study, which found that phytoplankton sensitivity to oxidative stress can vary up to 10-fold between species (Drábková et al., 2007). We further assumed that, the higher the maximum concentration of antioxidants α, the lower the energetic cost associated with protein damage e_d; all remaining parameter values were held constant for these analyses (Table S2).

We performed simulations for 100 different cells based on a 10x10 grid combining different E_a and α values. We solved the model across a wide range of temperatures for every 0.25 °C to capture the full thermal growth curves and derive the T_{opt} for each phenotype from our simulations. We repeated this process for two different nutrient regimes to simulate nitrogen-replete (DIN = 20 μM) and nitrogen-deplete (DIN = 0.1 μM) conditions. In total, we simulated 200 thermal growth curves and found optimal solutions for approximately 10,000 combinations of temperature, DIN, E_a and α (see Supplementary Methods).

Model comparison against proteomic data

We validated the heat-stress predictions from our model against data from an experimental evolution study that quantified the proteomic responses of the marine diatom *Chaetoceros simplex* (Aranguren-Gassis et al., 2024). In the experiment, protein differential expression was compared between three different populations: ancestral population acclimated to control temperature (25 °C), ancestral population acclimated to heat-stress (31 °C), and warm-evolved population acclimated to heat-stress (34 °C). Functional annotation of all identified proteins and gene ontology (GO) enrichment analysis were conducted using the Omicsbox software (version 1.4.12), employing the Fisher exact test with a false discovery rate (FDR) of 0.05. The analysis utilized the reduced option to provide more specific GO terms (Table S4). We compared our simulations against the proteomic dataset qualitatively, based on the up-regulation or down-regulation of specific pathways. This expands our previous work by providing empirical evidence that supports the modeled trade-off between nutrient acquisition and heat-mitigation for both ancestral and evolved populations. To validate the model predictions between the two different nutrient regimes, we compared model output to literature values for the proteomic responses of different phytoplankton species between nitrogen-replete and nitrogen-deplete

conditions (Table S5, Supplementary Methods). No proteomic datasets were available to validate predictions under combined heat and nutrient stress.

Phytoplankton functional types and latitudinal simulations

We solved the proteome model across a latitudinal transect in the Atlantic Ocean, spatially averaging over the longitude range of 330 °to 335 °E, after verifying that temperature and nutrient conditions within this narrow range are relatively uniform. We selected the Atlantic transect for this analysis as it is less dominated by iron limitation compared to the Pacific and therefore offers a more representative environment for examining responses to nitrate limitation. We used historical and future SSP5-8.5 simulations from the CESM2-WACCM model (Danabasoglu et al., 2020) to construct present-day (1980–2000) and future (2080–2100) averages for temperature and nitrate concentrations, both averaged over the upper 20 m of the water column. Since changes in irradiance were not the focus of this study, we used a constant light dose of 300 $\mu mol\ photon\ m^{-2}\ s^{-1}$ as representative of average mixed layer conditions.

We parameterized the model to represent four phytoplankton functional types: *Prochlorococcus*, *Synechococcus*, warm-adapted diatom and cold-adapted diatom. We chose these four types given their importance to global primary production (Dutkiewicz et al., 2021) and their differences in three key properties: 1) nutrient uptake efficiency (Edwards et al., 2012), 2) thermal traits (Anderson et al., 2021), and 3) heat-mitigation capacities (Drábková et al., 2007). Given the wide thermal range of diatoms, we simulated two phenotypes to capture cold- and warm- adapted species. Parameter values were chosen based on previous literature and details are given in the Supplementary Methods (Table S3). To confirm that our parameter choices mimic the general physiology of the four types, we compared the emergent maximum growth rates (μ_{max}) and growth (left) slopes of the thermal curves from our model against data from 167 species previously compiled by Anderson et al. (2021). Diatom species within this dataset with a T_{opt} lower than 20 °C were assigned as cold-adapted. All *Prochlorococcus* and *Synechococcus* species in the dataset had a T_{opt} higher than 20 °C. To make this comparison (Figure 3A), we fitted the data to the Norberg equation. We used a log-likelihood function to fit the parameters of the Norberg equation for each experimental dataset from Anderson et al. (2021), then estimated the upper and lower temperature values at which the modeled growth rate was 20 % of its maximum value following Anderson et al. (2021). We calculated the lower and upper slopes of the thermal curve by dividing the difference between the maximum growth rate and 20 % of the maximum

growth rate value by the difference between the thermally optimum temperature and the identified temperatures at which growth rate was 20 % of maximum.

We also compared the emergent averaged cell size and nutrient uptake affinities from our model between cyanobacteria and diatoms to make sure these reflected differences observed empirically (Fig. 3B). Finally, we also evaluated if the highest optimal growth rate between cyanobacteria and diatom at any given latitude, as predicted by the proteome model, reflected in higher biomass for that group using simulations from a global model that also considered the different thermal dependencies between these two functional types (Anderson et al., 2024) (Fig. S4).

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Author contributions

S.G.L. and N.M.L. conceptualized this study and wrote the initial manuscript. S.G.L. developed the model, run simulations, and performed data analyses. L.B. developed code to improve the optimization algorithm. A.K. developed code to perform the analyses with the empirically derived thermal growth data. L.F. provided CESM2-WACCM model output. M.A.G. analyzed the proteomic dataset. H.A., H.V.M., C.L., E.L. and other authors revised the initial draft contributing to the final version of this manuscript.

Competing Interests

The authors declare that they have no competing interests.

Data Availability Statement

The model was written in Julia (Bezanson et al., 2017) and the code developed in Leles and Levine (2023) is deposited on Zenodo (DOI: `https://doi.org/10.5281/zenodo.8125732`). The model code used to run the analyses described in the present study are available on GitHub (`https://github.com/LevineLab/ProteomePhyto/tree/main`) and will be added permanently to Zenodo after acceptance. The CESM2-WACCM model output used in this study is available via the Earth System Grid Federation (`https://esgf-node.ipsl.upmc.fr/projects/esgf-ipsl/`).

Code Availability

The model code used to run the analyses described in the present study are available on GitHub (`https://github.com/LevineLab/ProteomePhyto/tree/main`) and will be added permanently to Zenodo after acceptance.

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Supplementary Materials for

New niches for larger phytoplankton in a warmer, more resource-limited ocean

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- Supplementary Methods
- Supplemental Figures
- Supplemental Tables

Supplementary Methods

Model equations

The model is fully defined below. A list of the optimized variables is given in Table S1. Detailed description for each of the metabolic pathways are described in Leles and Levine (2023) except for additions/modifications to the model which are then described here. Descriptions of the constant parameters are given in the following section as well as Tables S2 and S3.

C1-C8: Steady-state growth of proteins

$$\frac{d(p_p + p_{dp})}{dt} = \phi_p v_{ri} \frac{1}{\eta_p} - \mu(p_p + p_{dp}) = 0$$
$$\frac{dp_{ru}}{dt} = \phi_{ru} v_{ri} \frac{1}{\eta_{ru}} - \mu p_{ru} = 0$$
$$\frac{dp_{tr}}{dt} = \phi_{tr} v_{ri} \frac{1}{\eta_{tr}} - \mu p_{tr} = 0$$
$$\frac{dp_{ri}}{dt} = \phi_{ri} v_{ri} \frac{1}{\eta_{ri}} - \mu p_{ri} = 0$$
$$\frac{dp_{gl}}{dt} = \phi_{gl} v_{ri} \frac{1}{\eta_{gl}} - \mu p_{gl} = 0$$
$$\frac{dp_{lb}}{dt} = \phi_{lb} v_{ri} \frac{1}{\eta_{lb}} - \mu p_{lb} = 0$$
$$\frac{dp_{ld}}{dt} = \phi_{ld} v_{ri} \frac{1}{\eta_{ld}} - \mu p_{ld} = 0$$
$$\frac{dp_{re}}{dt} = \phi_{re} v_{ri} \frac{1}{\eta_{re}} - \mu p_{re} = 0$$

C9-C12: Steady-state growth of other macromolecules

$$\frac{dc_{ic}}{dt} = v_{ru} - v_{ri}\eta_{aac} - v_{lb} - v_{gl} - \mu c_{ic} = 0$$
$$\frac{dc_{in}}{dt} = v_{tr} - v_{ri}\eta_{aan} - \mu c_{in} = 0$$
$$\frac{dc_{lm}}{dt} = \alpha_{lm} v_{lb} \frac{1}{\eta_{lic}} - \mu c_{lm} = 0$$
$$\frac{dp_{ox}}{dt} = \alpha_{tag} v_{lb} \frac{1}{\eta_{lic}} - \mu p_{ox} = 0$$

C13: Proteome investments, in which i represents protein pools

$$\sum \phi_i = 0.5$$

C14: Fractions of the lipid synthesis flux:

$$\alpha_{lm} + \alpha_{tag} + \alpha_{ld} = 1.0$$

C15: Fraction of damaged photosystems (a function of heat damage and repair)

$$\frac{p_{dp}}{p_{dp} + p_p} = \frac{k_d p_p \frac{\alpha}{p_{ox}}}{k_{re} p_{re}}$$

C16: Constraint on the maximum concentration of stored lipids that mitigate damage

$$p_{ox} \leq \alpha_{min} + \alpha$$

C18: Membrane integrity

$$\frac{p_{tr}}{c_{lm}} \leq M_{min} + M_{max} \gamma_{Tm}$$

C19: Lipid degradation:

$$v_{ld} = \alpha_{lb} v_{lb}$$

C20-22: Energy metabolism

$$e_p v_p \geq e_{ru} v_{ru}$$

$$e_{cost} \leq (e_p v_p - e_{ru} v_{ru})$$

where, $e_{cost} = e_{tr}v_{tr} + e_{ri}v_{ri} + e_{lb}v_{lb} + e_d v_d + e_f v_{re}$

$$f_{dr}e_{cost} \leq e_{gl}v_{gl} + e_{ld}v_{ld}$$

C23: Space constraint

$$1 = (p_{tr}s_{tr} + c_{lm}s_{lm})\beta$$

C24: Density constraint, in which k indicates all pools of the cell except for photosystems, lipid storage and membrane components

$$\sum c_k \frac{1}{\eta_k} \leq D_{max}\frac{V}{V_{tot}}$$

where, $\frac{V}{V_{tot}} = 1 - (p_p + p_{dp})V_p - (c_{li} + c_{tag})V_{li} - \frac{V_{nu}}{V_{tot}}$

Reaction rates are defined as following:

$$v_p = k_{ref_p} p_p \frac{I}{I + K_p}$$
$$v_{ru} = k_{ref_{ru}} \gamma_{Ta} p_{ru} \frac{DIC}{DIC + K_{ru}}$$
$$v_{tr} = k_{ref_{tr}} \gamma_{Ta} p_{tr} \frac{DIN}{DIN + K_{tr}}$$
$$v_{ri} = k_{ref_{ri}} \gamma_{Ta} p_{ri} \frac{c_{ic}}{c_{ic} + K_{ic}} \frac{c_{in}}{c_{in} + K_{in}}$$
$$v_{gl} = k_{ref_{gl}} \gamma_{Ta} p_{gl}$$
$$v_{lb} = k_{ref_{lb}} \gamma_{Ta} p_{lb} \frac{c_{ic}}{c_{ic} + K_{lb}}$$
$$v_{ld} = k_{ref_{ld}} \gamma_{Ta} p_{ld}$$
$$v_d = k_{ref_d} \gamma_{Td} p_p \frac{\alpha}{p_{ox}}$$
$$v_{re} = k_{ref_{re}} \gamma_{Ta} p_{re} \frac{p_{dp}}{p_{dp} + p_p}$$

Temperature effects:

$$\gamma_{Ta} = exp(\frac{E_a}{R}(\frac{1}{T_{ref}} - \frac{1}{T}))$$
$$\gamma_{Td} = exp(\frac{E_d}{R}(\frac{1}{T_d} - \frac{1}{T}))$$
$$\gamma_{Tm} = (\frac{T_{max} - T}{T_{max} - T_{min}})$$

Number of molecules of nitrogen per amino acid:

$$\eta_{aan} = \eta_{aac} \sum \phi_i q_i$$

Estimated storage pools that fuel dark respiration:

$$c_{gu} = v_{gl}\frac{1}{\eta_{guc}}td$$
$$c_{li} = v_{ld}\frac{1}{\eta_{lic}}td$$

Cell nitrogen to carbon quota (q_{cell}), in which i represents protein pools and η_i represents the molecular weight of proteins in units of aa protein^{-1}:

$$q_{cell} = \frac{\sum c_i\eta_i\eta_{aac}q_i + c_{in}}{\sum c_i\eta_i\eta_{aac} + c_{ic} + (c_{lm} + c_{li} + c_{tag})\eta_{lic} + c_{gu}\eta_{guc}}$$

Constraining the minimal model cell size

The proteome allocation model solves for the optimal concentration of different macromolecules in the cell given a specific set of environmental conditions. We impose a constraint on the maximum intracellular density of macromolecules (D_{max}) such that, if the optimal metabolic strategy involves building more proteins and the cell is at D_{max}, the cell must increase its cell size. The volume of the cell is defined by the volume occupied by photosystems and stored lipids as well as all other macromolecules, following Eqs. 41-43 in Leles and Levine (2023). These constraints effectively keep cell size from increasing indefinitely in our model. In order to constraint the minimal size of our modeled cell, we require the model to account for the intracellular space taken up by the cellular nucleus (V_{nu}). Thus we modify Eq. 41 from Leles and Levine (2023):

$$V_{tot} = V + (n_p + n_{dp})V_p + (n_{li} + n_{tag})V_{li} + V_{nu}, \tag{5}$$

in which V_{tot} is the total cellular volume, V is the volume occupied by all macromolecules with the exception of photosystems, stored lipids, and nucleus. n_p and n_{dp} are the total number of functional and damaged photosystems, respectively, and n_{li} and n_{tag} are the total number of stored lipids required to regulate oxidative stress and to fuel

respiration, respectively. V_p and V_{li} correspond to the volume of photosystem and stored lipid molecules, respectively.

Parameter values

The parameters used to define the different phytoplankton functional types can be found in Table S3. Below we describe in detail our choices for each of these parameters. All other parameter values of the model were kept the same and can be found in Table S2; for detailed description on the choice of these parameter values and respective references please refer to the supplemental methods in Leles and Levine (2023).

E_a: this parameter sets the slope at which metabolic rates in the model increase exponentially as a function of temperature. To parameterize E_a, we derived the growth (left) slope of the thermal growth curves for the different functional types from empirical data available for 167 species of phytoplankton based on the compilation in Anderson et al. (2021) and validated our model against the averaged values obtained from empirical observations (Fig. 3A).

T_d: similarly, the heat-stress temperature was defined based on averaged values from the same dataset compiled in Anderson et al. (2021).

K_{tr}: the half-saturation for nitrogen uptake was derived from Figure 1B in Edwards et al. (2012).

V_p: the average volume occupied by a photosystem molecule is $3 \times 10^{-5} \mu m^3$ for *Thalassiosira pseudonana* (Arshad et al., 2021) and between 1×10^{-6} - 2×10^{-5} μm^3 for the cyanobacteria *Synechococcus* (BioNumbers: ID103908 and ID103909).

V_{nu}: the nucleus volume was derived from Malerba and Marshall (2021), in which the nucleus volume was given for several species of phytoplankton.

α: the maximum concentration of antioxidants (i.e. stored lipids) in the cell (not used to fuel dark respiration) was constrained based on the average fraction of the cellular volume that is occupied by lipids, i.e. 20% Finkel et al. (2016), resulting in a maximum value of 2×10^7 molecules μm^{-3}.

e_d: we assumed that the rate of heat-damage scales with $c_{tag_{max}}$ so that the cost is higher for cyanobacteria based on their higher sensitivity to heat stress (Drábková et al., 2007), as following: $e_d = \frac{2\times10^7}{c_{tag_{max}}}$.

Settings for the sensitivity analyses

The baseline parameters used for the sensitivity analyses (Figs. 2D-G) were the same as those set for the warm-adapted diatom type, with the exception of the following parameters:

K_{tr}: 10.0 μM

E_a: variable, 10 values from 0.55 to 1.05 eV

α: variable, 10 values from 6×10^6 to 2.4×10^7 molecules μm^{-3}.

We changed the value of K_{tr} to represent cells that have higher nutrient affinity than our modeled diatoms, but lower nutrient affinity than our cyanobacteria types (Table S3). By varying E_a and α we were able to capture phenotypes with diverse strategies, from varying degrees of thermal dependency, as well as cells with different abilities to mitigate oxidative stress caused by heat-damage.

The phenotypes shown in Fig. 2A assumed a fixed α of 2×10^7 and variable E_a while the phenotypes shown in Fig. 2B assumed a fixed E_a of 0.55 and variable α. The two example phenotypes shown in Fig. 2C differed in the following parameters:

Nutrient-efficient: $E_a = 1.05$ and $\alpha = 6 \times 10^6$

Thermal-tolerant: $E_a = 0.61$ and $\alpha = 2.4 \times 10^7$.

The environmental conditions for these analyses were set as following:

Temperature: from 18 to 35 °C, every 0.25 °C

Dissolved Inorganic Nitrogen (DIN) concentrations: replete (20 μM) and limited (0.1 μM) conditions

Irradiance: 300 $\mu mol\ photon\ m^{-2}\ s^{-1}$.

Improving optimization algorithm

The proteome model solves for the optimal steady-state solution which maximizes growth rates. However, sometimes the optimizer can get stuck in a local minimum instead of finding the global minimum. This can occur for many reasons including the optimizer 'timing out' due to the optimization surface being too flat or poor initial conditions. As this work required solving for 10,000 combinations of trait values (model parameters) and environmental conditions, we needed to develop a pipeline for ensuring that the optimizer indeed found the global minimum. We used an iterative approach based on the assumption that the model solutions would be either continuous or exhibit a relatively small number of states across the tested environmental conditions.

We first conducted a set of simulations across the environmental gradient (e.g., temperature) using several random initial guesses. We used the optimized solutions from these initial runs to identify 'anomalous' solutions that might be local minima. Specifically, we used a moving average approach relying on tangent line calculations to evaluate the rate of change between consecutive solutions across a temperature gradient. An abrupt change in the tangent between consecutive data points resulted in the solution being flagged as potentially 'anomalous'. Solutions which violated biologically realistic bounds for key variables were also flagged. We used the following bounds for each of the key variables: growth rates (0.001-2 $day^{-}1$), cell length (0.1-30 μm), and nitrogen to carbon quota of the cell (0.008-0.5 mol N:mol C). We then identified, for each 'anomalous' solution, the closest preceding and succeeding 'good' solution (i.e., solutions not flagged as anomalous). The initial conditions (starting guesses) for the optimizer were then derived by extrapolating between these closest good solutions using a linear interpolation. We performed two re-optimization passes using this strategy. For each pass, the new solutions were compared against the original 'anomalous' solution and the one with the maximum growth rate was selected (the optimizer cost function maximizes growth rates). This allows us to reduce the impact of local minimum and ensure the results presented were indeed the optimal (global) solution.

Model comparison against proteomic data

We compared the proteomic shifts predicted by the model against proteomic data previously published in the literature for diatom and cyanobacteria species grown under controlled growth conditions. We also compared the shifts in response to warming predicted by the model to data from an experimental evolution study (Aranguren-Gassis et al., 2019). This dataset provided robust evidence supporting the trade-off between investing in transporters versus gly-

colysis and heat-mitigation strategies (Table S4). The GO terms used for this comparison are given in Table S4. We were unable to find any previous studies that reported the proteomic response of phytoplankton to the multi-stressor growth condition of warming and nutrient limitation. Therefore, we compared the model against other datasets to evaluate the metabolic responses to only nutrient limitation (Table S5). The list of proteins/gene terms used for this comparison is specified below:

Nutrient trasporters: nitrate/ammonia transporters (Remmers et al., 2018), nitrate/ammonium assimilation (Remmers et al., 2018), phosphate-binding protein (Domínguez-Martín et al., 2017), nitrogen assimilation enzymes (nitrate reductase and glutamate synthase) (Yang et al., 2014), nitrate/nitrite transporters (Chen et al., 2018), and phosphate transporters (Chen et al., 2018).

Photosystems: proteins from Domínguez-Martín et al. (2017) are described in their Table 1 and correspond to Cytochrome b559 subunit beta, Photosystem II reaction center protein L, Cytochrome b6-f complex subunit 4, Cytochrome b559 subunit alpha, Divinyl chlorophyll a/b light-harvesting protein PcbE, Divinyl chlorophyll a/b light-harvesting protein PcbD. Others included Fucoxanthin chlorophyll a/c protein (Yang et al., 2014). See also Figure 5B for detailed proteins in Chen et al. (2018) and Figure 5 and Supplementary file I in Remmers et al. (2018).

Rubisco: Ribulose bisphosphate carboxylase and other proteins related to carbon fixation; see Figure 5B for detailed proteins in Chen et al. (2018) and Figure 5 and Supplementary file II in Remmers et al. (2018).

Ribosomes: ribosomal proteins (small and large units); see Figure 5D in Chen et al. (2018).

Glycolysis: KEGG pathways related to Glycolysis/Gluconeogenesis, which includes Embden-Meyerhof pathway (glucose to pyruvate), glycolysis, pyruvate oxidation and gluconeogenesis; see Table S5 in Chen et al. (2018).

Lipid synthesis: KEGG pathways associated with Glycerophospholipid metabolism (see Table S5 in Chen et al. (2018)), and Glycerolipid metabolism, Fatty acid metabolism and elongation (see Supplementary file II in Remmers et al. (2018)).

Supplemental Figures

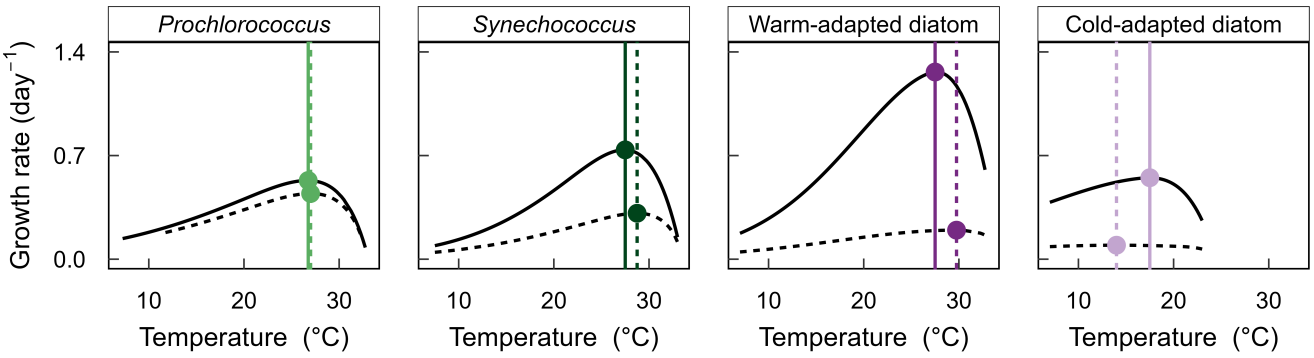

Figure S1: **Emergent thermal growth curves for the different phytoplankton functional types under nitrogen-replete (solid lines; 20 μM) and nitrogen-limiting (dashed lines; 0.1 μM) conditions.** Symbols and vertical lines indicate temperature optima (T_{opt}) in both nutrient regimes. Colors indicate the different phytoplankton functional types following Figs. 3 and 4.

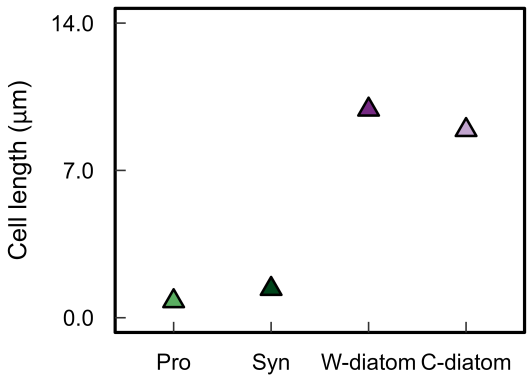

Figure S2: **Cell diameter under nitrogen replete (20 μM) conditions.** Data are given at T_{opt} for each functional type as following: Pro: *Prochlorococcus*, Syn: *Synechococcus*, W-diatom: warm-adapted diatom, C-diatom: cold-adapted diatom. Colors indicate the different phytoplankton functional types following Figs. 3 and 4.

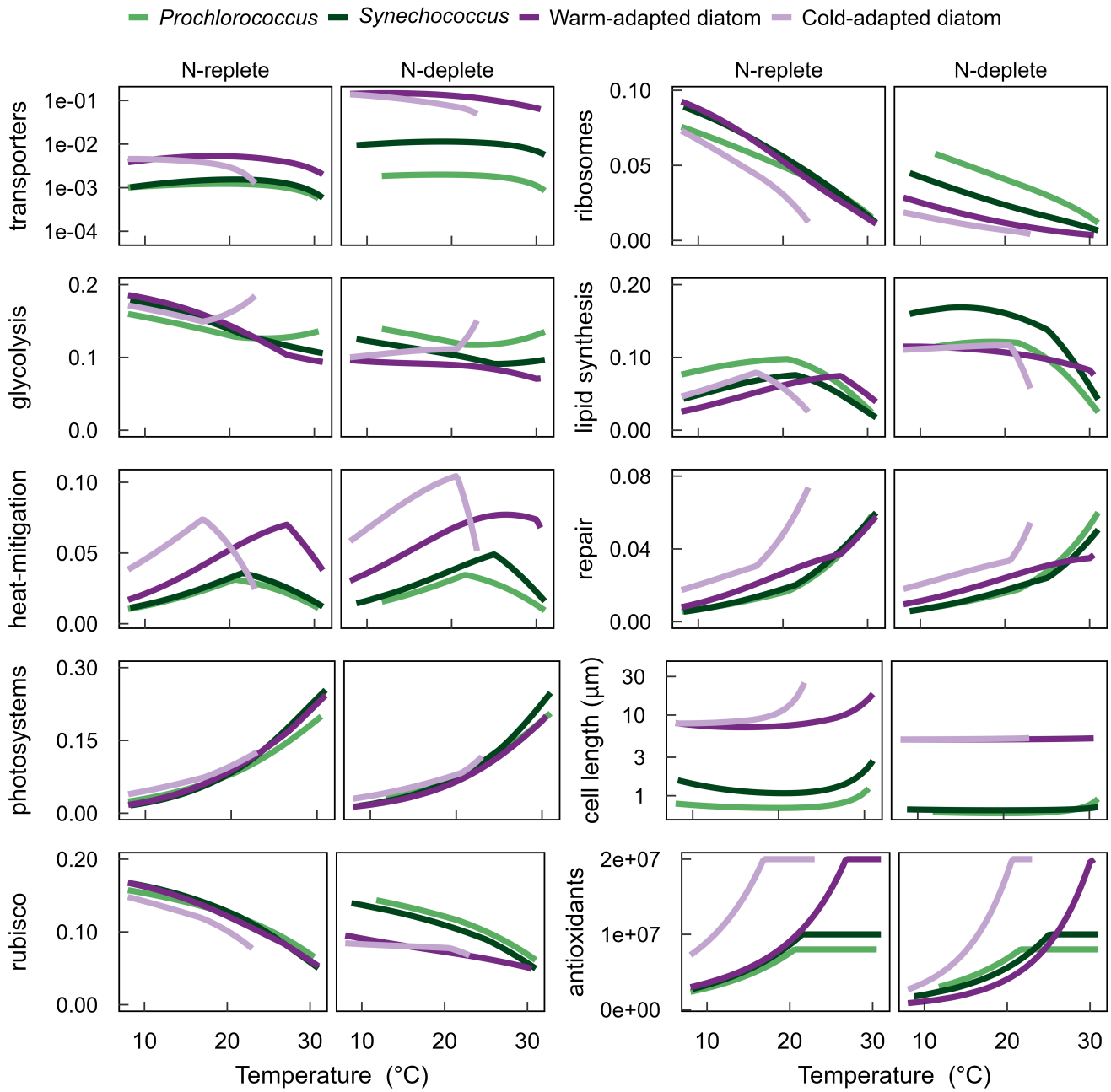

Figure S3: **Optimal relative proteome investments and cell size.** Model output as a function of temperature for N-replete (20 μM) and N-limited (0.1 μM) conditions are compared among four phenotypes corresponding to those illustrated in Figs. 3 and 4. Relative proteome investments vary between 0 and 1 and antioxidants are given in units of number of molecules μm^{-3}.

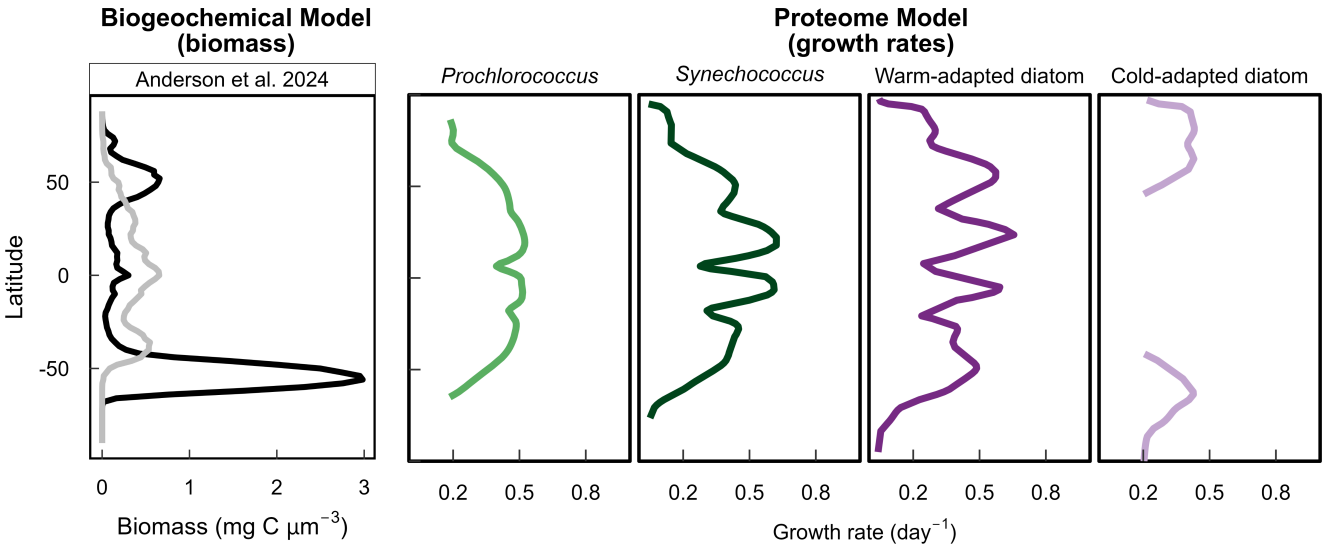

Figure S4: **Mean phytoplankton biomass across a latitudinal gradient from the DARWIN model.** Integrated biomass over the upper 240 m averaged over 10 years is given for diatoms (black line) and cyanobacteria (gray line). Simulated data were obtained from Anderson et al. (2024). Also shown are the optimal growth rates from the proteome model for the different functional types.

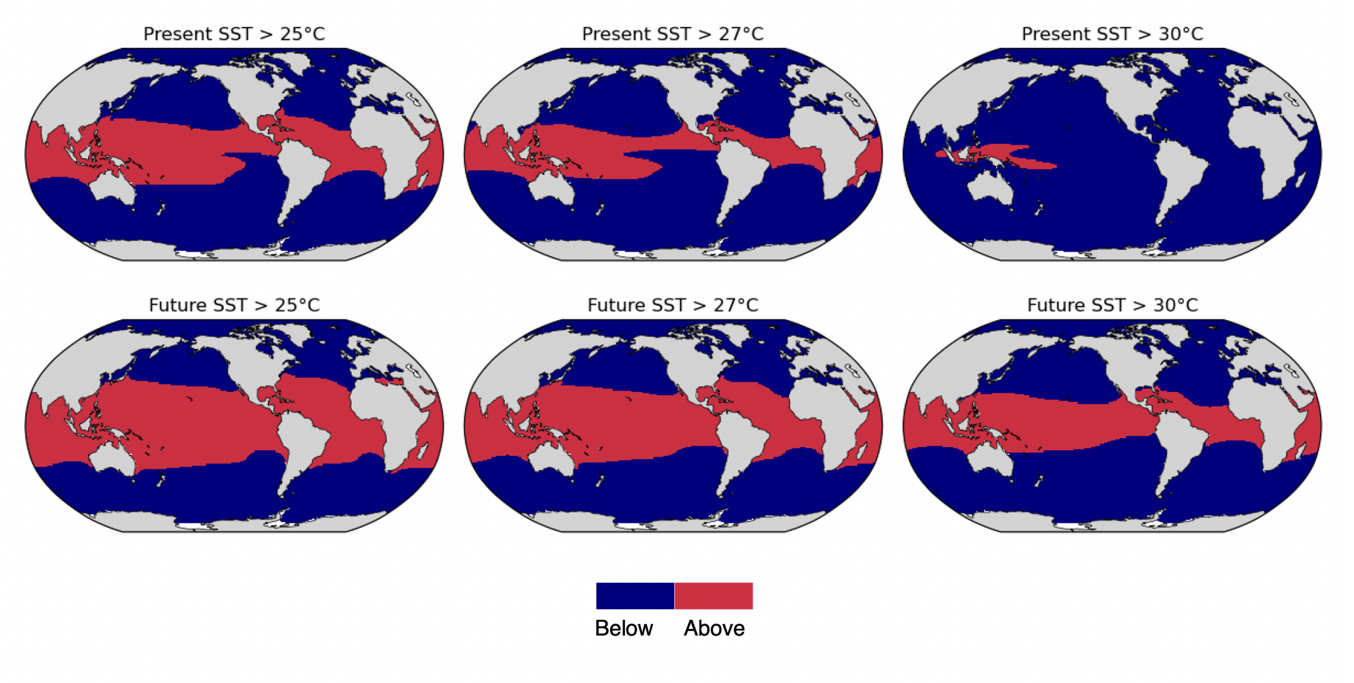

Figure S5: **Projections of oceanic regions experiencing different surface sea temperature (SST) thresholds under present-day and future climate scenarios from the CESM2-WACCM model under SSP5-8.5.** Under the future climate scenario, the model predicts a global increase in surface waters experiencing temperatures above: 25°C from 37.7% to 52.9%, 27°C from 26.9% to 46.3%, and 30°C from 2.7% under present day to 34.5% by the end of the century. Temperature thresholds were chosen based on the T_{opt} of the different modeled phytoplankton functional types (Fig. S1).

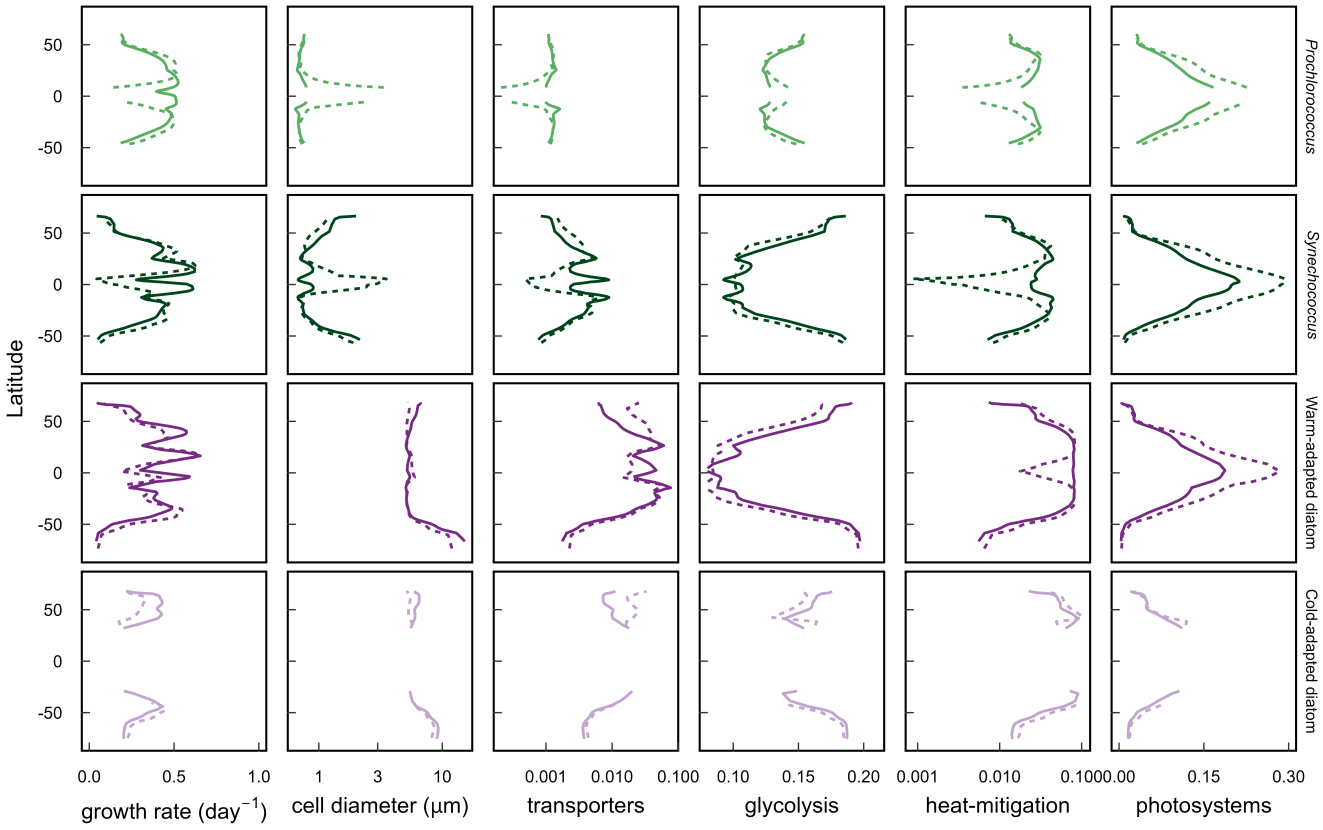

Figure S6: **Phytoplankton optimal metabolic strategies across a latitudinal transect in the Atlantic Ocean based on temperature and nitrogen concentrations averaged over the upper 20 m of the water column.** Simulations were performed for present-day (1980-2000; solid lines) and future (2080-2100; dashed lines) projections of nitrate concentrations and temperature using model output from the the CESM2-WACCM model under SSP5-8.5 (Fig. 4B). Results are given for four phytoplankton functional types for optimal growth rates, cell diameter, and relative proteome investments that vary between 0 and 1. Note that no data is shown when growth rates were equal to zero.

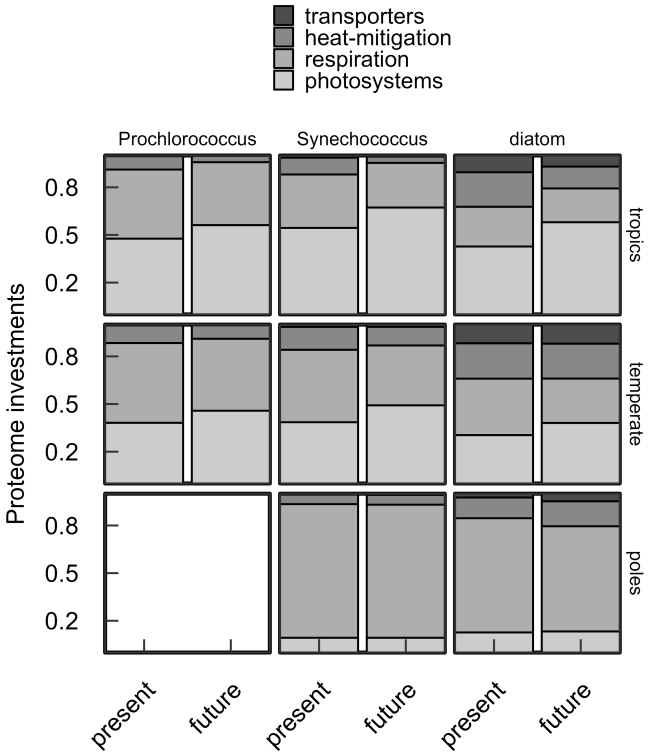

Figure S7: **Metabolic strategies based on phytoplankton functional type across major oceanic regions.** Scaled relative proteome investments (varies between 0 and 1) for present-day and future climate projections across three distinct regions of the ocean: tropics (22°S-22°N), temperate (25°-45°) and poles (50°-70°). For full latitudinal simulations, see Fig. S6.

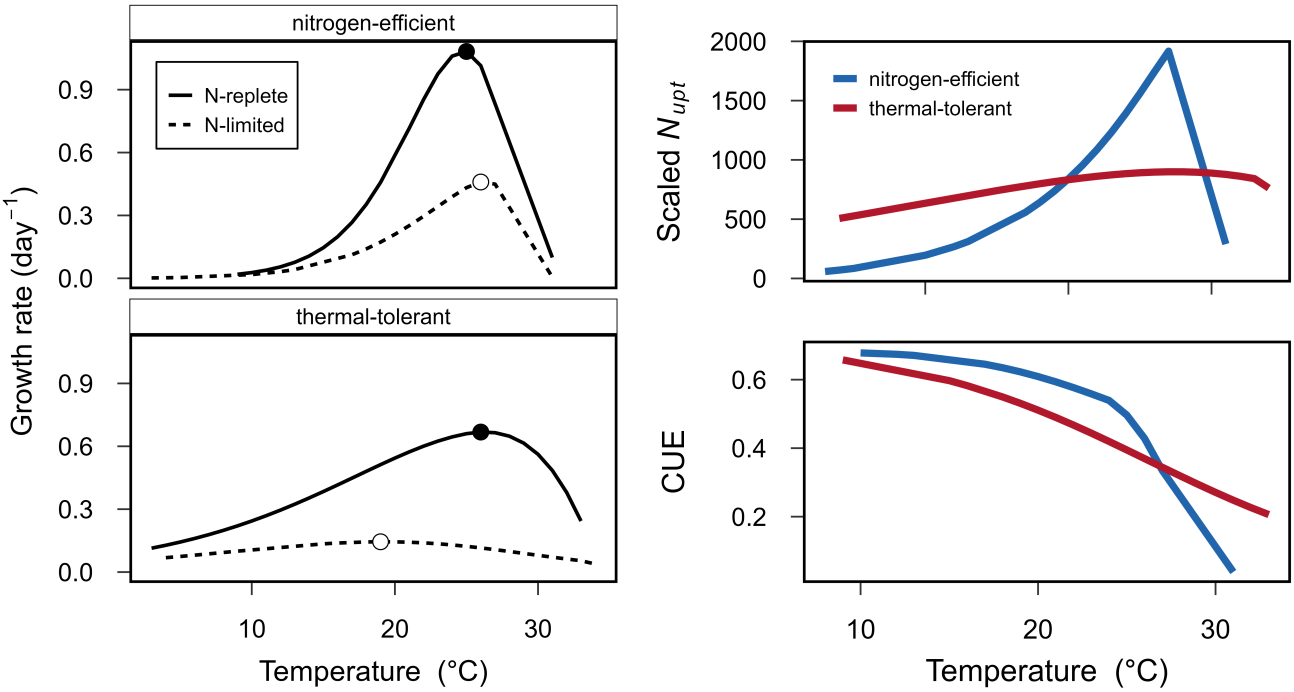

Figure S8: **Model runs where phytoplankton produce antioxidant enzymes to decrease oxidative stress instead of lipid storage in the form of TAGs.** (A) thermal growth curves indicate shifts in T_{opt} from nitrogen-replete to nitrogen-limited conditions as a result of different metabolic strategies. (B) model nitrogen uptake rates (N_{upt}) and Carbon Use Efficiencies (CUE) under nitrogen limitation. Carbon use efficiency is defined as 1 - respiration/photosynthesis.

Supplemental Tables

Table S1: **Optimized parameters.** Abbreviations, descriptions and units for the optimized variables. State variables correspond to the concentration of proteins and other macromolecules inside a phytoplankton cell.

	State Variables	
	Proteins	
p_p	Photosystems	molecules of p_p μm^{-3}
p_{ru}	rubisco	molecules of p_{ru} μm^{-3}
p_{tr}	Transporters	molecules of p_{tr} μm^{-3}
p_{ri}	Ribosomes	molecules of p_{ri} μm^{-3}
p_{gl}	Glycolysis	molecules of p_{gl} μm^{-3}
p_{lb}	Lipid Synthesis Pathway	molecules of p_{lb} μm^{-3}
p_{ld}	Lipid Degradation Pathway	molecules of p_{ld} μm^{-3}
p_{re}	Repair Proteins	molecules of p_{re} μm^{-3}
	Other macromolecules	
c_{ic}	Internal Carbon	molecules of C μm^{-3}
c_{in}	Internal Nitrogen	molecules of N μm^{-3}
c_{lm}	Membrane Lipids	molecules of c_{lm} μm^{-3}
p_{ox}	Antioxidants (as lipid storage)	molecules of p_{ox} μm^{-3}
	Parameters	
	Relative proteome investments in proteins	
ϕ_p	Photosystems	unitless
ϕ_{ru}	rubisco	unitless
ϕ_{tr}	Transporters	unitless
ϕ_{ri}	Ribosomes	unitless
ϕ_{gl}	Glycolysis	unitless
ϕ_{lb}	Lipid Synthesis Pathway	unitless
ϕ_{ld}	Lipid Degradation Pathway	unitless
ϕ_{re}	Repair Proteins	unitless
	Other parameters	
β	Volume to Surface Area ratio of the cell	μm
α_{lm}	Fraction of lipid synthesis flux used to build membrane lipids	unitless
α_{ld}	Fraction of lipid synthesis flux used to fuel lipid degradation	unitless
α_{tag}	Fraction of lipid synthesis flux used to build stored lipids	unitless

Table S2: **Parameter values.** Abbreviations, descriptions, values, and units. The values of tunable parameters are given in Table S3. All other parameter values were kept constant across our simulations. See Supplementary Text in Leles and Levine (2023) for references and more details regarding the source of the parameter values.

Maximum reference turnover rates (# molecules per protein per time)			
k_{ref_p}	Photosystems	7800	photons min^{-1}
$k_{ref_{ru}}$	rubisco	157	molecules of C min^{-1}
$k_{ref_{tr}}$	Transporters	120	molecules of N min^{-1}
$k_{ref_{ri}}$	Ribosomes	114	molecules of aa min^{-1}
$k_{ref_{gl}}$	Glycolysis	120	molecules of C min^{-1}
$k_{ref_{lb}}$	Lipid Synthesis	120	molecules of C min^{-1}
$k_{ref_{ld}}$	Lipid Degradation	120	molecules of C min^{-1}
k_{ref_d}	Heat damage	1000	molecules of p min^{-1}
$k_{ref_{re}}$	Repair Proteins	1000	molecules of p min^{-1}
Half saturation constants			
K_p	Photosystems	10	μmol photon m^{-2} s^{-1}
K_{ru}	rubisco	1	μM DIC
K_{tr}	Transporters	Table S3	μM DIN
K_{in}	Nitrogen Metabolism	10000	molecules of N μm^{-3}
K_{ic}	Carbon Metabolism	10000	molecules of C μm^{-3}
K_{gl}	Glycolysis	10000	molecules of C μm^{-3}
K_{lb}	Lipid Synthesis	10000	molecules of C μm^{-3}
K_{ld}	Lipid Degradation	10000	molecules of C μm^{-3}
Temperature effects			
E_a	Thermal dependency	Table S3	eV
$E_{a_{tr}}$	E_a of transporters	$E_a/2$	eV
E_d	Deactivation energy	1.4	eV
T_d	Stress temperature	Table S3	$°C$
T_{ref}	Reference temperature	20	$°C$
R	Boltzmann's constant	8.62×10^{-5}	eV/K
Molecular weights			
η_p	Photosystems	9082	aa/molecule of p
η_{ru}	rubisco	5933	aa/molecule of ru
η_{tr}	Transporters	1046	aa/molecule of tr
η_{ri}	Ribosomes	19393	aa/molecule of ri
η_{gl}	Glycolysis	15220	aa/molecule of gl
η_{lb}	Lipid Synthesis	13742	aa/molecule of lb
η_{ld}	Lipid Degradation	5128	aa/molecule of ld
η_{re}	Repair Proteins	6350	aa/molecule of re
η_{in}	Internal Nitrogen	14	Da
η_{ic}	Internal Carbon	12	Da
η_{aa}	Amino Acid	110	Da
η_{gu}	Glucose	180	Da
η_{li}	Lipid Storage	800	Da

Space and density constraints			
s_{tr}	Transporter surface area	1.26×10^{-5}	μm^2
s_{lm}	Lipid surface area	0.5×10^{-6}	μm^2
M_{max}	Transporter to lipid ratio	Table S3	unitless
V_p	Photosystem volume	Table S3	μm^3
V_{li}	Lipid volume	1.5×10^{-9}	μm^3
V_{nu}	Nucleus volume	Table S3	μm^3
D_{max}	Maximum density	1.8×10^{10}	Da μm^{-3}
α	Maximum antioxidant concentration	Table S3	antioxidants μm^{-3}
α_{min}	Minimum antioxidant concentration	200	antioxidants μm^{-3}
Carbon and Nitrogen quotas			
η_{aac}	Amino Acids Carbon	5	C/aa
η_{guc}	Glucose Carbon	6	C/molecule of gu
η_{lic}	Lipids Carbon	16	C/molecule of li
q_{pt}	Protein quota	0.20	N/C
q_p	Photosystems quota	0.10	N/C
q_{ri}	Ribosomes quota	0.33	N/C
Energy conversion factors			
e_p	Photosystems	1.0	ATP/photon
e_{ru}	rubisco	10	ATP/C
e_{tr}	Transporters	1.0	ATP/N
e_{ri}	Protein synthesis	48	ATP/aa
e_{gl}	Glycolysis	5.0	ATP/C
e_{lb}	Lipid Synthesis	3.9	ATP/C
e_{ld}	Lipid Degradation	6.6	ATP/C
e_{re}	Repair Proteins	4.5	ATP/photosystem
e_d	Heat damage	$2.0 \times 10^7/\alpha$	ATP/photosystem

Table S3: **Parameter values for the different phytoplankton functional types.** Parameter values used to parameterize the different phytoplankton functional types represented in our latitudinal simulations, i.e., *Prochlorococcus*, *Synechococcus*, warm-adapted diatom, and cold-adapted diatom. More details and sources can be found in the Supplementary Text.

Parameter	Pro	Syn	W-Diatom	C-Diatom	Unit	Description
E_a	0.85	1.2	1.2	0.65	eV	Thermal dependency
T_d	35	35	35	25	°C	Stress temperature
K_{tr}	0.1	2.0	60.0	60.0	μM	Half-saturation for nitrogen uptake
V_p	1.5e-5	1.5e-5	3e-5	3e-5	μm^3	Photosystem volume
V_{nu}	0.05	0.1	50	50	μm^3	Nucleus volume
M_{max}	1×10^{-5}	3×10^{-5}	4.5×10^{-4}	4.5×10^{-4}	unitless	Transporter to lipid ratio
α	8×10^6	1×10^7	2×10^7	2×10^7	ant μm^{-3}	Maximum antioxidant concentration

Table S4: **Comparison between modeled and observed proteomic shifts in response to heat-stress.** Thermal acclimation responses are shown where the arrows correspond to up-regulation (↑) or down-regulation (↓) of pathways from populations growing at temperature optimum to populations subjected to heat-stress. Observations (proteomic data) for the enriched gene ontology (GO) terms correspond to the ancestral populations from an experimental evolution study with the marine diatom *Chaetoceros simplex* growing at 25 °C (optimum) versus the ancestral populations growing at 31 °C (heat-stress) or the warm-evolved populations growing at 34 °C (heat-stress) (Aranguren-Gassis et al., 2024).

Model Variable	Model	GO terms	Ancestral	Evolved
Transporters	↓	nitrate/nitrite transport	↓	↓
		nitrate/nitrite transmembrane transporter activity	↓	↓
		nitrate reductase (NADPH) activity	not enriched	↓
		nitrate assimilation	not enriched	↓
Glycolysis	(↑)	glycolytic process	(↑)	(↑)
		fructose 1,6-bisphosphate metabolic process	(↑)	not enriched
		phosphopyruvate hydratase activity	(↑)	not enriched
		glucose 6-phosphate metabolic process	not enriched	(↑)
Heat-mitigation	(↑)	cell redox homeostasis	(↑)	(↑)
		superoxide dismutase activity	(↑)	not enriched
		removal of superoxide radicals	(↑)	not enriched
		cellular response to oxygen compound	(↑)	not enriched
Repair proteins	(↑)	cellular response to heat	(↑)	(↑)
		cellular response to unfolded protein	(↑)	(↑)
		heat shock protein binding	(↑)	(↑)
		protein folding chaperone	(↑)	(↑)
		misfolded protein binding	(↑)	(↑)
		positive regulation of DNA repair	(↑)	not enriched
		unfolded protein binding	(↑)	(↑)
		protein stabilization	not enriched	(↑)

Table S5: **Comparison between modeled (M) and observed (O) proteomic investment shifts in response to nutrient limitation.** Acclimation responses are shown where the arrows correspond to the up-regulation (↑) or down-regulation (↓) of pathways from nutrient-replete to nutrient- limiting or depleted conditions. Specific protein names used for the comparisons against each modeled pool are given in the Supplementary Methods.

Functional Type	Variable	M	O	Reference
Cyanobacteria	Transporters	(↑)	(↑)	Domínguez-Martín et al. (2017)
	Photosystems	↓	(↑)	Domínguez-Martín et al. (2017)
	Rubisco	↓	↓	Domínguez-Martín et al. (2017)
	Ribosomes	↓	↓	Domínguez-Martín et al. (2017)
	Glycolysis	↓	↓	Domínguez-Martín et al. (2017)
Diatom	Transporters	(↑)	(↑)	Yang et al. (2014); Chen et al. (2018); Remmers et al. (2018)
	Photosystems	↓	↓	Yang et al. (2014); Chen et al. (2018); Remmers et al. (2018)
	Rubisco	↓	↓	Chen et al. (2018); Remmers et al. (2018)
			(↑)	Yang et al. (2014)
	Ribosomes	↓	↓	Chen et al. (2018); Remmers et al. (2018); Yang et al. (2014)
	Glycolysis	↓	↓	Yang et al. (2014); Chen et al. (2018)
			(↑)	Remmers et al. (2018)
	Lipid synthesis	(↑)	(↑)	Yang et al. (2014); Chen et al. (2018)
			↓	Remmers et al. (2018)

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