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ORIGINAL ARTICLE

Thermal performance of marine diatoms under contrasting nitrate availability

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Environmental factors that interact with increasing temperature under the ongoing global warming are an urgent issue determining marine phytoplankton's performance. Previous studies showed that nutrient limitation alters phytoplankton responses to temperature and may lower their temperature optima (T_{opt}), making them more susceptible to high temperatures. The generality of this relationship is unknown, as very few species were tested. Here we investigated how growth rate depended on temperature at two contrasting nitrogen concentrations in six marine diatoms isolated from different thermal environments, including the tropics. Low nitrate had a significant effect on thermal performance in five of the six species. The effect size was larger around the optimum temperature for growth, resulting in flattened thermal performance curves but no shift in T_{opt} . While that trend is independent of the thermal regime from which each species was isolated, the implications for the phytoplankton response to global warming may be region dependent.

KEYWORDS: phytoplankton; temperature; climate change; thermal performance curve; driver interaction

INTRODUCTION

Temperature and nutrient availability are critical environmental factors determining phytoplankton growth. Scientists have long explored the phytoplankton responses to each driver, both in freshwater and marine species. Both factors influence most physiological processes in a nonlinear way.

Warming increases phytoplankton growth until a maximum is reached (optimum temperature, T_{opt}), and then growth decreases steeply as temperature increases above the optimum (Eppley, 1972), resulting in a left-skewed thermal performance curve (TPC). Still, most scientific efforts have focused on temperatures below the optimum when studying cellular processes related to growth, such

as metabolic rates. Previous research has shown that, for example, warming increases rates of photosynthesis (Coles and Jones, 2000; Geider, 1987; Li *et al.*, 2018; Rhee and Gotham, 1981; Toseland *et al.*, 2013). However, when studies include temperatures above the optimum, photosynthesis then exhibits a unimodal relationship with temperature (Baker *et al.*, 2016; Neori and Holm-Hansen, 1982), similar to growth.

Nutrient limitation, such as by nitrogen (N), also changes key physiological functions. Phytoplankton cells exposed to low N downregulate photosynthetic processes (Berges *et al.*, 1996; Juergens *et al.*, 2015; Lampe *et al.*, 2019; Li *et al.*, 2018; Longworth *et al.*, 2016; Muhseen *et al.*, 2015 and references therein; Schmollinger *et al.*, 2014) and decrease chlorophyll-*a* cell content (Geider, 1987; Juergens *et al.*, 2015; McCarthy *et al.*, 2017; Park *et al.*, 2015) while increasing the intracellular N recycling (Hockin *et al.*, 2012; Lampe *et al.*, 2019; Longworth *et al.*, 2016) and lipid storage (Chen *et al.*, 2014; Longworth *et al.*, 2016; Muhseen *et al.*, 2015). As a result, N availability restricts phytoplankton growth (Caperon, 1967; Monod, 1949).

Both temperature and N availability vary widely in the ocean, leading to phytoplankton experiencing different combinations of these environmental factors that affect their growth and metabolism (Hutchins and Fu, 2017). Consequently, more studies are beginning to focus on the interactions between the two drivers.

On the one hand, temperature changes seem to alter nitrate uptake dynamics, but the results are contradictory. Some studies reported an increase of N requirements or nitrate uptake with warming (Berges *et al.*, 2002; Lewington-Pearce *et al.*, 2019; Reay *et al.*, 1999), whereas others showed a decrease in nitrate uptake or associated enzymes with increasing temperature (Lomas and Glibert, 1999 and references therein; Parker and Armbrust, 2005), or no change with temperature (Baker *et al.*, 2016; Berges *et al.*, 2002).

On the other side, phytoplankton temperature sensitivity seems to decrease under nutrient limitation (Lee *et al.*, 2019; Marañón *et al.*, 2018; Serra-Pompei *et al.*, 2019) at the below-optimum temperatures. Previous studies showed a decrease of the optimal temperature for growth in a marine diatom (Thomas *et al.*, 2017) and several freshwater species (Bestion *et al.*, 2018) under nutrient limitation. However, it is not known how general these effects are, as very few species were tested.

Here we present the temperature performance curves (TPCs, growth rate variation with temperature) for several tropical and cosmopolitan marine diatom species at two contrasting nitrate availabilities. Tropical species may be especially vulnerable to warming because their T_{opt} are often very close to the temperatures they inhabit and,

thus, any increase in ambient temperatures would result in steep declines of growth rates (Thomas *et al.*, 2012). Despite this, the TPCs for tropical diatoms are not measured as often as for temperate species. Therefore, it is important to investigate whether nutrient limitation may further exacerbate the sensitivity of tropical diatoms to rising temperatures.

METHOD

Culture conditions

We used monospecific cultures of six marine diatoms isolated from different thermal environments. The diatoms used, *Chaetoceros simplex* Ostensfeld strain CCMP 200 (warm water) and *Skeletonema tropicum* Cleve strain CCMP 2157 (tropical), were obtained from the National Center for Marine Algae and Microbiota (NCMA). The other four species were obtained from the Theriot Lab diatom collection at University of Texas, Austin: *Astrosyne radiata* Ashworth and Lobban (tropical), *Perideraion elongatum* R.W.Jordan, Y. Arai and Lobban (tropical), *Hemiaulus sinensis* Greville (temperate) and *Coscinodiscus concinnus* W. Smith (cosmopolitan).

All cultures were grown in 50 mL polycarbonate culture flasks, at 100 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ cool white fluorescent light on a 14/10 hour day/night cycle. We gently inverted and randomly repositioned flasks daily. Maintenance temperatures before the experiments were 25°C (*C. simplex*, *S. tropicum* and *H. sinensis*), 27°C (*A. radiata* and *P. elongatum*) or 29°C (*C. concinnus*). We used nutrient-enriched artificial L1 seawater medium (884 $\mu\text{M NO}_3^-$) from NCMA, modified from Guillard and Hargraves (1993), at pH of 8–8.2 and 33 psu salinity. Only temperature and the nitrate concentration were modified during subsequent experiments, establishing two experimental N-availability conditions: high N (884 $\mu\text{M NO}_3^-$) and low N (5 $\mu\text{M NO}_3^-$).

The low N concentration used in our experiments is at the upper limit of the values for the half saturation constants (K_s) reported in the literature for diatoms of the same or related genera; they range from 0.1 to 5 $\mu\text{M NO}_3^-$ for N uptake (Edwards *et al.*, 2012; Eppley and Thomas, 1969; Ganguly *et al.*, 2013; Litchman *et al.*, 2006; Nishikawa *et al.*, 2010).

TPC assays

First, we maintained the cultures for 5 days in nitrogen-free medium, to deplete intracellular stored nitrogen. Then TPCs were characterized following Aranguren-Gassis *et al.* (2019). Briefly, for each species, six replicate

populations were acclimated at the assay temperatures (10, 16, 20, 25, 29, 31 and 34°C), three in the high N (884 $\mu\text{M NO}_3^-$) and the other three in the low N (5 $\mu\text{M NO}_3^-$) medium. After acclimation, for each replicate, we estimated daily growth rate using *in vivo* chlorophyll-*a* fluorescence until the cultures arrived at the stationary phase, using SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA). This method is commonly used to estimate phytoplankton growth (Aranguren-Gassis *et al.*, 2019; McCarthy *et al.*, 2017; Pearce *et al.*, 2019; Thomas *et al.*, 2017). For each species, we started the growth experiments adjusting the amount of culture inoculated to the experimental flasks so that the initial fluorescence would be similar for all temperatures in both nitrate treatments. We established a limit of 40% of the final volume for the amount of inoculated culture. For 28 out of 42 pairs of populations (high vs. low N treatments for the same species at the same temperature), their initial fluorescence values were not statistically different ($P > 0.05$, *t*-student or Wilcoxon test, depending on the normality of the samples). Some problems with dilution precision and very low cell concentrations in several low N populations resulted in significant differences between populations in 14 pairs. For most of them, biomass at high N was higher than at low N.

Calculations and statistical analyses

Growth rates were calculated from linear regressions of log-biomass estimates (*in vivo* fluorescence) vs. time (days). First, we visually selected the period where growth rate was exponential, close to log-linear (Supplementary Figs S1 and S2). When positive growth was detected, we selected at least three data points during the exponential phase. When there was no clear exponential phase, we selected as many data points as possible to characterize the general trend of the population. The population growth rate (day^{-1}) is the slope of the resulting regression.

For TPC fitting and comparison, we used Generalized Additive Models (GAM), with a smoother for the growth rate by temperature term, and the main effect for the high N vs. low N treatments. Both the growth rate and the temperature were compared in different treatments using factorial analysis of variance. The T_{opt} for growth was calculated from the GAM fits and also with a double exponential model (Supplementary Figs S3–S6) to compare with previous studies.

We then calculated the difference between the growth rates at high N and low N conditions, as an indicator of the effect size of N supply on the growth response to temperature.

All analyses were performed using the R statistical environment v. 3.3.2. (R Core Team 2016-10-31).

RESULTS

For all six diatoms studied, the effect of temperature on growth rate was significant when growing at high N conditions, resulting in a typical TPC (Fig. 1). When growing under low N conditions, the temperature effect on growth rate becomes insignificant only for *P. elongatum* and *C. concinnus*, for which the maximum growth rates were low. T_{opt} varied between 18°C for *C. concinnus* and 30°C for *A. radiata*, with no general trend of lower T_{opt} under low N conditions. Only in a single species, *H. sinensis*, the T_{opt} under low N concentration was lower than under high N concentration regardless of the calculation method (Supplementary Table S4).

Tropical species (*A. radiata*, *P. elongatum* and *S. tropicum*) had higher T_{opt} than the cosmopolitan and temperate species (*H. sinensis* and *C. concinnus*) (Supplementary Table S4). They also tended to have a narrower thermal niche (Fig. 1), but it was not possible to test this statistically because of the poor estimates of niche width for some species.

Maximum growth rates were higher for populations growing under high N conditions for all six diatoms, and the effect of lower N concentration on growth was statistically significant for all species but *S. tropicum*. The difference between the high N and low N TPCs depended on the species and was larger near the optimum temperature for all the species except for *H. sinensis* (Fig. 2).

DISCUSSION

Our results suggest that the effect of low nitrate concentration on the diatoms' response to temperature is species specific but tends to be larger close to the optimum temperature, regardless of the species origin.

The decrease in the maximum growth rate observed under low N concentration agrees with previous findings for *Thalassiosira pseudonana* (Thomas *et al.*, 2017). However, in contrast with previous studies (Bestion *et al.*, 2018; Boyd, 2019; Thomas *et al.*, 2017), we did not find a decrease in the optimum temperature for growth under low nutrient availability. The lack of a detectable shift in T_{opt} in our observations could possibly be because the degree of N deprivation during our experiments was not as strong as in previous studies (Thomas *et al.*, 2017). The nitrate concentration used for the low N populations in the present work (5 $\mu\text{M NO}_3^-$) is a realistic concentration that diatoms encounter in natural environments, similar

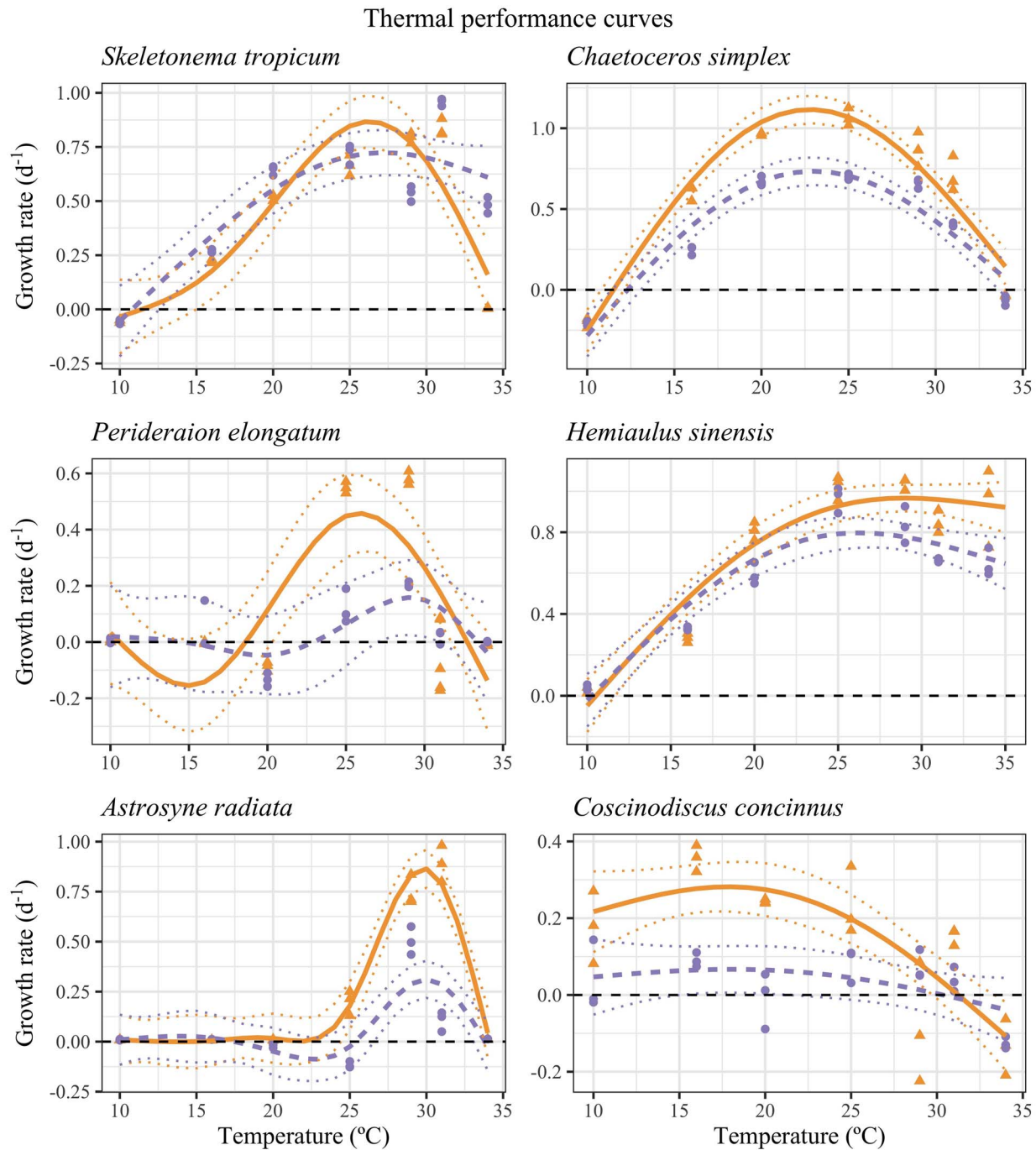


Fig. 1. TPCs under high N (yellow triangles with solid line) and low N (violet dots with dashed line) conditions. Regression lines are GAMs with standard error bands and the dashed black horizontal lines signal growth rate equals zero.

to the lowest levels with significant diatom presence at global scale (Bopp *et al.*, 2005; Boyer *et al.*, 2013). However, this N concentration may still be above the expected K_s for the diatom species we tested, especially for the tropical strains, as they may be adapted to lower N conditions.

The values of the K_s for N uptake of the same diatom genera range from 0.1 to 5 $\mu\text{M NO}_3^-$ under a variety of growth conditions, which may affect the K_s values (Edwards *et al.*, 2012; Eppley and Thomas, 1969; Ganguy *et al.*, 2013; Litchman *et al.*, 2006; Nishikawa *et al.*,

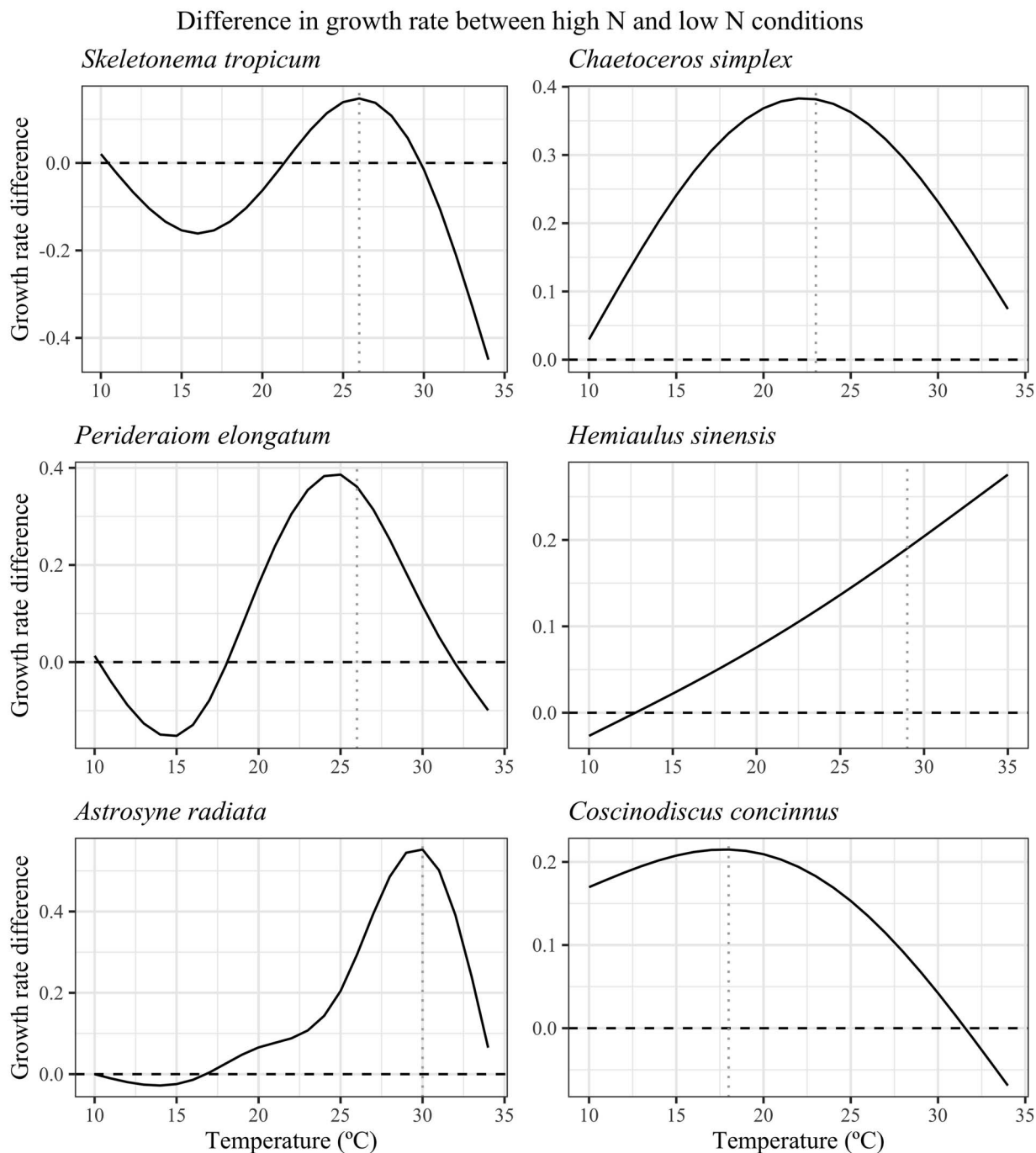


Fig. 2. Effect of low nitrate on the growth rate of six marine diatom species. Solid black lines are the GAM fits for the difference between the growth rates under high N and low N conditions estimated from GAM fitting (Fig. 1). Dashed black horizontal lines show zero difference, and the dotted gray vertical lines show the optimum temperature for growth at high N conditions.

2010). Assuming that K_s for uptake is similar to K_s for growth (Eppley and Thomas, 1969), the diatom cultures may not be strongly limited by N in our experiments. Considering that growth conditions were the same for all cultures during the experiments, this underscores the

species-specific differences in the possible effects of low nutrient concentrations on TPCs. While some species may be limited by the low N concentrations similar to that used in our experiment and may experience a decrease in their T_{opt} , other species adapted to low N may not be

affected as strongly and could have the same T_{opt} across N concentrations. Therefore, to make realistic predictions of how nutrient limitation may affect thermal performance of species, studies should include the information on nutrient requirements for those species.

The higher T_{opt} for tropical species in our study are in line with previous findings that showed the T_{opt} correlates with the average temperatures of the environment from which strains or species are isolated (Thomas *et al.*, 2012).

The unimodal shape of the TPCs is the result of several cellular processes, such as photosynthesis, respiration and nutrient metabolism, with different sensitivities to temperature (Barton *et al.*, 2018, 2020; Baker *et al.*, 2016). The interaction of both drivers (warming and N reduction) may generate trade-offs at the cellular and metabolic level (Aranguren-Gassis *et al.*, 2019; Marañón *et al.*, 2018) that affect the population growth rate. Photosynthesis/primary production and respiration play a crucial role in growth rate variations (Barton *et al.*, 2018; Marañón *et al.*, 2018), because the balance between the two processes determines the amount of carbon available for growth. A decrease in N availability usually has a strong effect on primary production (see references above), altering the growth response at different temperatures. At low temperatures, even under low N availability, there may be enough N to sustain cellular photosynthetic rate determined by that low temperature, ensuring enough available carbon for growth. But with warming, cellular N demand increases with increasing photosynthesis rate, and N becomes the most influential limiting factor for growth (Rhee and Gotham, 1981), impeding cell's growth response to higher temperature (Marañón *et al.*, 2018, Padfield *et al.*, 2015) and flattening the TPC closer to the optimum.

The available information on how metabolic rates respond to temperatures above the optimum is scarce. Previous research showed that optimum primary production and respiration temperatures are usually higher than the growth optima (Baker *et al.*, 2016; Barton *et al.*, 2020), and that production declines more sharply (Barton *et al.*, 2020), driving the steep decrease of growth. When N is scarce, the maximum photosynthesis rate may decrease, altering the growth response at temperatures just above the optimum. As a result, the maximum effect of the decrease in N concentration is observed around the optimum temperature for growth, flattening the TPC. Such flattening of the low N TPCs, where the maximum growth is reduced, can be interpreted as a lower sensitivity to temperature, because the variation of the growth rate with temperature becomes smaller. Hence, our results agree with the decrease of temperature sensitivity under nutrient limitation observed by other authors (Lee *et al.*, 2019; Marañón *et al.*, 2018; Serra-Pompei *et al.*, 2019).

At the same time, our results highlight the importance of considering temperature when quantifying the effect of nitrate limitation on phytoplankton growth.

In addition to photosynthesis and respiration, N uptake also depends on temperature. The enzyme nitrate reductase (NR) reduces nitrate to nitrite and, thus, controls the first step of incorporating N into cells. The NR's optimum temperature ranges between 10 and 20°C for phytoplankton (Berges *et al.*, 2002; Gao *et al.*, 2000; Gleich *et al.*, 2020; Kristiansen, 1983). However, the precise nature of the temperature dependence of N uptake remains contradictory. Some studies suggest that at low temperatures, nitrate affinity may decline (Reay *et al.*, 1999). Other studies did not find strong temperature dependence of N uptake (Baker *et al.*, 2016).

A decrease in N also affects cell metabolism, including the NR activity. While some studies observed that N-addition stimulated the NR synthesis and activity in phytoplankton (Berges, 1997; Hockin *et al.*, 2012; McCarthy *et al.*, 2017), others show NR activity increases with nitrate depletion (McKew *et al.*, 2015; Park *et al.*, 2015; Yang *et al.*, 2014). Future studies should simultaneously look at how temperature and N levels affect N uptake and metabolism.

Our observations indicate that, despite the considerable differences among the TPCs for several species, some generalities emerge, such as the larger effect of low nutrient concentration closer to the species' thermal optimum. However, this pattern may play out differently under warming, depending on the thermal regime. For those environments where species' optimum is close to the mean environmental temperature (tropical regions, Thomas *et al.*, 2012), the effect size of N availability may decrease, because warming will shift the mean environmental temperature away from their optimal temperature (Fig. 3). On the contrary, where species' optimum is higher than the mean environmental temperature (temperate regions, Thomas *et al.*, 2012), warming would drive that mean closer to the optimum, and the effect of nutrient availability may become grater (Fig. 3). Evolution may complicate those trends, as adaptation to warming tends to shift the optimum to higher temperatures (Aranguren-Gassis *et al.*, 2019; Listmann *et al.*, 2016; O'Donnell *et al.*, 2018), so that the effect of nutrient availability may depend on the distance between the mean environmental temperature and the optimum temperature for growth.

CONCLUSION

The effect of nitrate availability on the marine diatoms' growth response to temperature is universally larger close

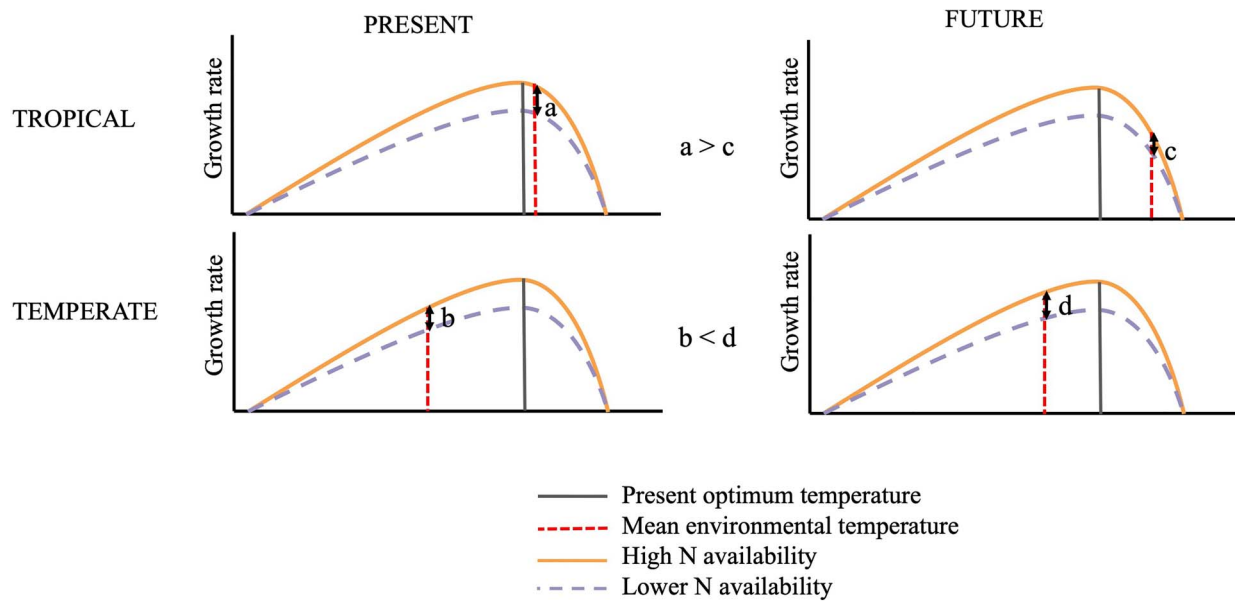


Fig. 3. Diagram illustrating how temperature increase may modify the effect of low N availability on phytoplankton growth, depending on the thermal regime. The curves represent the TPC for hypothetical phytoplankton species at high (yellow solid) and low (violet dashed) N availability. The dark gray solid vertical line is the optimum temperature for growth of the species (assuming no evolutionary shift) and the red dashed line is the ambient mean temperature that increase in the future scenario. Black arrows show the increase or decrease of growth rate when N availability changes under the corresponding ambient temperature. The letters a, b, c and d are the difference between growth rate at high N and low N availability at the mean environmental temperature (the length of the black arrows). The greater than and less than symbols denote whether present differences are higher or lower (respectively) than the corresponding future differences at the same thermal regime.

to their optimum temperature, for species with widely different temperature optima.

Nutrient supply variations in a warming world may affect marine diatoms differently, depending on the difference between the mean environmental temperature and their optimum temperature for growth and on the strength of nutrient deprivation.

DATA ARCHIVING

The data and scripts supporting the results will be publicly available in the GitHub repository upon publication: <https://github.com/MariaArangurenGassis/Thermal-performance-of-marine-diatoms-under-contrasting-nitrate-availability->.

SUPPLEMENTARY DATA

Supplementary data can be found at *Journal of Plankton Research* online.

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