THE EFFECT OF TEMPERATURE ON THE ECOLOGY, EVOLUTION, AND BIOGEOGRAPHY OF PHYTOPLANKTON

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ABSTRACT

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Temperature is a fundamental driver of biological dynamics, but we do not know how it shapes the physiology and ecology of any community across global temperature gradients. Here I examine the influence of temperature on phytoplankton, which are extremely sensitive to changes in environmental conditions and play a critical role in global food webs and biogeochemical cycles.

I address how global variation in temperature regimes has shaped distributions of phytoplankton temperature traits, identifying patterns of adaptation as well as differences in how major functional groups respond to environmental temperature gradients. I also show that due to the asymmetric cost of exceeding the optimal temperature and the traits of tropical species, ocean warming this century may drive a reduction in the diversity of tropical phytoplankton communities in the absence of evolutionary adaptation. Tropical phytoplankton species may persist, however, by poleward migration, bringing them into competition with temperate species.

Our study of the temperature traits of an invasive cyanobacterium supports the idea that rising temperatures will increase the probability of invasion by tropical and subtropical species into temperate environments. Predicting these invasions, however, is a challenge that requires us to model the phytoplankton community dynamics in complex natural environments. This will require a mechanistic understanding of how temperature interacts with important resources such as nutrients and light to influence growth. To address this, I

have developed and tested a model describing how temperature and nutrients interact to affect growth rates. Our experimental tests confirm a novel prediction: that optimum temperature for growth is a saturating function of nutrient concentration. Together, this work forms a foundation from which we can build predictive models of how environmental warming will affect population and community dynamics across broad spatial scales.

Dedicated to my parents and my wife.

ACKNOWLEDGEMENTS

As with any effort stretching over the better part of a decade, this work could not have been done without a great deal of help. I'm indebted to Elena Litchman, my advisor, for giving me the opportunity to pursue a career as an ecologist, free rein to pursue ideas I found interesting, and for guidance, criticism, and funding along the way. Chris Klausmeier, Colin Kremer, and Kyle Edwards taught me far more about ecological theory and analytical techniques than I could possibly have learned on my own; my thanks can't do justice to their patience and generosity with their time. Gary Mittelbach and Jen Lau critiqued my (initially extremely naïve) ideas and have shaped the way I think about ecology and evolution. I'm very grateful to Pam Woodruff and Ally Hutchens for helping tremendously with my laboratory work over the years, and tolerating my (hopefully) occasional fumbles in that regard. The KBS academic community – faculty, postdocs, and graduate students – all made me a far better scientist by forcing me to defend and refine my ideas on a regular basis. And my experimental work could not have been done without the assistance of a number of undergraduates who worked in our lab: Caitlin Ryan, Alexandra David, Marilyn Gould, and Lydia Auner.

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CHAPTER 1

INTRODUCTION

Temperature is one of the fundamental drivers of biological activity, influencing processes at multiple levels of organization, from sub-cellular to ecosystem. The principal mechanism underpinning its effects on organisms is the exponential increase in chemical reaction rates with temperature. In organisms, metabolic reactions are catalyzed by enzymes through conformational changes that require a balance between flexibility (in order to catalyze reactions through changes in shape) and rigidity (in order to maintain their specificity in binding to ligands) in order to perform their function. As both enzyme shape and flexibility are influenced by temperature, these may be evolutionarily optimized only for a subset of possible temperature conditions, setting up fundamental trade-offs in the ability to perform under different thermal regimes (Hochachka & Somero 2002). A similar trade-off is seen in cell membrane lipids, which need to maintain viscosity within a particular range in order to function. These lead to important consequences for the growth of organisms: the maximum possible rate of growth in ectotherms increases exponentially with temperature (Figure 1.1), and no organism can perform well across the full range of biologically relevant temperatures. A further constraint imposed on fitness in ectotherms is that it is a left-skewed functions of temperature (called a thermal reaction norm or thermal fitness curve), possibly due to the exponential relationship between both development rates and mortality rates; however, the mechanisms behind this form are not fully understood (Figure 1.1). Together, these constraints and trade-offs have shaped the evolution of life since its inception.

A number of the mechanisms by which temperature influences ecological and evolutionary patterns have been uncovered by the metabolic theory of ecology – these include

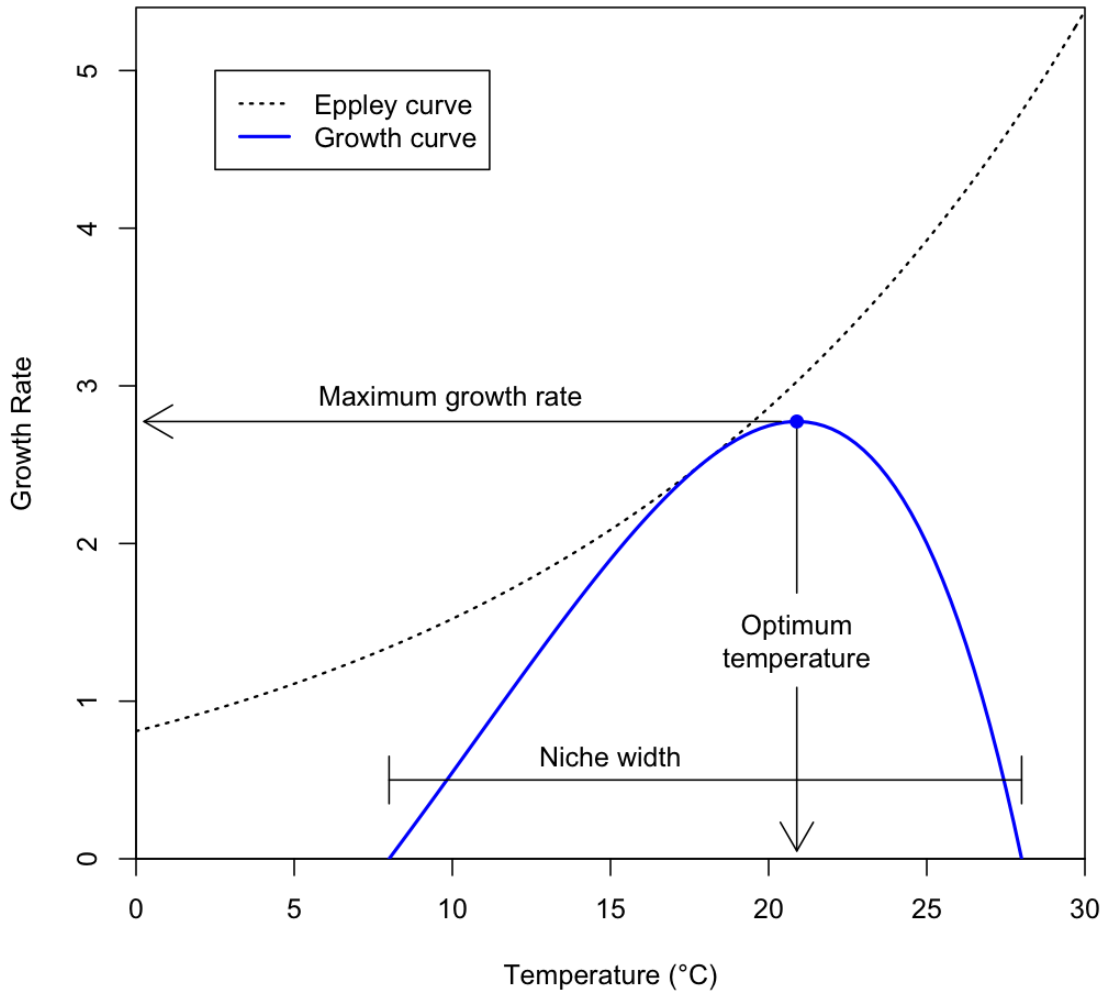

Figure 1.1 An example thermal tolerance curve, illustrating the skewness typical of all known ectotherms and some of the major traits. We also show the (empirically-estimated) upper limit on maximum population growth rate, which increases exponentially with temperature (Eppley 1972). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

the effects of temperature on growth, respiration, and mortality rates, equilibrium population sizes, lifetime lengths, and possibly speciation rates (Allen et al. 2006; Belgrano et al. 2002; Brown et al. 2004; Gillooly et al. 2001; Gillooly et al. 2002; Savage et al. 2004; West et al. 1997). However, by focusing on broad patterns driven by universal physical and chemical constraints, this line of research has ignored the fact that fitness declines above the optimum temperature,

possibly because there may be no single underlying cause driving variation in this decline. The fact of this decline and the asymmetry of the thermal reaction norm has important biological implications. This nonlinearity, in combination with spatial or temporal heterogeneity, may permit the co-existence of multiple species with different thermal strategies, contributing to the maintenance of biological diversity. Also, increases in temperature above the optimum of a species' exact a large fitness cost due to this skewness, suggesting that small amounts of environmental warming may threaten ectotherm populations, in the absence of behavioral change or evolutionary adaptation. These examples illustrate that a better understanding of the nonlinear effects of temperature on organisms can illuminate both fundamental theoretical questions as well as complex practical challenges in ecology.

The relative simplicity of phytoplankton life histories and environment, as well as their broad spatial distribution makes them an ideal system with which to study general ecological questions by connecting theory and data. However, they form an important topic of study in their own right. As a group, they are responsible for nearly half of global primary production, form the basis of aquatic food webs, and also influence global cycles of nitrogen, phosphorus and calcium, among other elements (Falkowski et al. 1998; Field et al. 1998). The practical challenge of modeling future biogeochemistry and climate change requires us to understand the drivers of phytoplankton growth.

In the following chapters, I describe my efforts to understand how global temperature variation coupled with evolutionary constraints has shaped phytoplankton physiology. This work focuses on four aspects:

1) How has global temperature variation influenced distributions of phytoplankton temperature traits in the oceans, and how will ocean warming affect phytoplankton communities? My

analysis of phytoplankton thermal reaction norm variation was strongly supported by eco-evolutionary modeling work by Colin Kremer (joint first author on this paper).

2) How have evolutionary constraints and differences in temperature regimes influenced phytoplankton temperature trait variation across environments (freshwater vs. marine) and latitude?

3) Do the temperature traits of a toxic, invasive phytoplankton species help to explain its recent invasion in temperate North American lakes?

4) How does temperature interact with nutrient availability to determine the growth of phytoplankton?

This work has involved a combination of data analysis and experiments to illuminate questions of theoretical and practical interest, and forms a foundation from which we may approach the problem of modeling how environmental change will affect future patterns of community composition, dynamics, and coexistence.

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CHAPTER 2

A GLOBAL PATTERN OF THERMAL ADAPTATION IN MARINE PHYTOPLANKTON

ABSTRACT

Rising ocean temperatures will alter the productivity and composition of marine phytoplankton communities, thereby affecting global biogeochemical cycles. Predicting the effects of future ocean warming on biogeochemical cycles depends critically on understanding how existing global temperature variation affects phytoplankton. Here we show that variation in phytoplankton temperature optima over 150 degrees of latitude is well explained by a gradient in mean ocean temperature. An eco-evolutionary model predicts a similar relationship, suggesting that this pattern is the result of evolutionary adaptation. Using mechanistic species distribution models, we find that rising temperatures this century will cause poleward shifts in species' thermal niches and a sharp decline in tropical phytoplankton diversity in the absence of an evolutionary response.

INTRODUCTION

Marine phytoplankton are responsible for nearly half of global primary productivity (Field et al. 1998). They play essential roles in food webs and global cycles of carbon, nitrogen, phosphorus, and other elements (Falkowski et al. 1998; Redfield 1958). Empirical studies have shown that recent ocean warming has driven changes in productivity (Behrenfeld et al. 2006), population size (Boyce et al. 2010), phenology (Edwards & Richardson 2004), and community composition (Morán et al. 2010). Global ocean circulation models predict further temperature-driven reductions in phytoplankton productivity this century, with consequent decreases in

marine carbon sequestration (Bopp et al. 2001; Steinacher et al. 2010). The main mechanism these studies have identified is indirect: rising temperatures drive an increase in ocean stratification, which in turn leads to a decrease in nutrient supply to surface waters. However, most models do not consider the direct effects of rising temperatures on individual phytoplankton species, which experience sharp declines in growth rate above their optimum temperatures for growth. They may, therefore, underestimate the effects of warming on ecosystems.

METHODS

To understand how ocean warming will directly affect marine and estuarine phytoplankton, we examined growth responses to temperature in 194 strains belonging to more than 130 species from the major phytoplankton groups (see supplementary methods in Appendix 1). Temperature-related traits, such as the optimum temperature for growth and the thermal niche width, are among the most important in ectothermic species, especially given predictions of global warming (Kingsolver 2009). We estimated these traits from >5000 growth rate measurements, synthesized from 81 papers published between 1935 and 2011. The strains were isolated from 76°N to 75°S, giving us exceptionally broad coverage of the latitudinal and temperature gradients (Figure A1.1 in Appendix 1).

Growth responses to changes in temperature are characterized by thermal tolerance curves (reaction norms). Two features of these curves are common to all ectotherms: unimodality and negative skewness, i.e. a sharper decline in fitness above the optimum temperature than below (Figure A1.2 in Appendix 1) (Eppley 1972; Kingsolver 2009). The latter condition makes ectotherms living at their optimum temperature more sensitive to warming than cooling, with important consequences for their performance in the environment (Martin & Huey 2008).

Furthermore, there is an exponential increase in the maximum growth rate attainable with increasing temperature (across species). These curves may be described using three principal traits: maximum growth rate, optimum temperature for growth, and thermal niche width (the temperature range over which growth rate is positive). We estimated these traits for each strain by fitting a thermal tolerance function to the data (Norberg 2004) and examined their relationships with environmental and taxonomic covariates (see supplementary methods in Appendix 1).

RESULTS AND DISCUSSION

Our analysis revealed large-scale patterns in thermal traits. First, strains exhibited a clear latitudinal trend in the optimum temperature for growth (Figure 2.1, R^2=0.55, p<0.0001), demonstrating the existence of a global pattern in a key microbial trait. Second, optimum temperature was even more strongly related to mean annual temperature at the isolation location (Figure 2.2A, R^2 = 0.69, p<0.0001), suggesting that temperature is a major selective agent and that adaptation to local environmental conditions occurs in marine microbes despite the potential for long-distance dispersal through ocean currents. In contrast, the width of the thermal niche was unrelated to temperature regimes. Third, strains from polar and temperate waters had optimum temperatures that were considerably higher than their mean annual temperatures, while tropical strains had optima closer to or lower than the mean temperatures (Figure 2.2A). Finally, variation in optimum temperature and niche width was not explained by taxonomic differences above the level of genus, indicating that thermal adaptation is not highly phylogenetically constrained in this group (Tables A1.1 and A1.2 in Appendix 1).

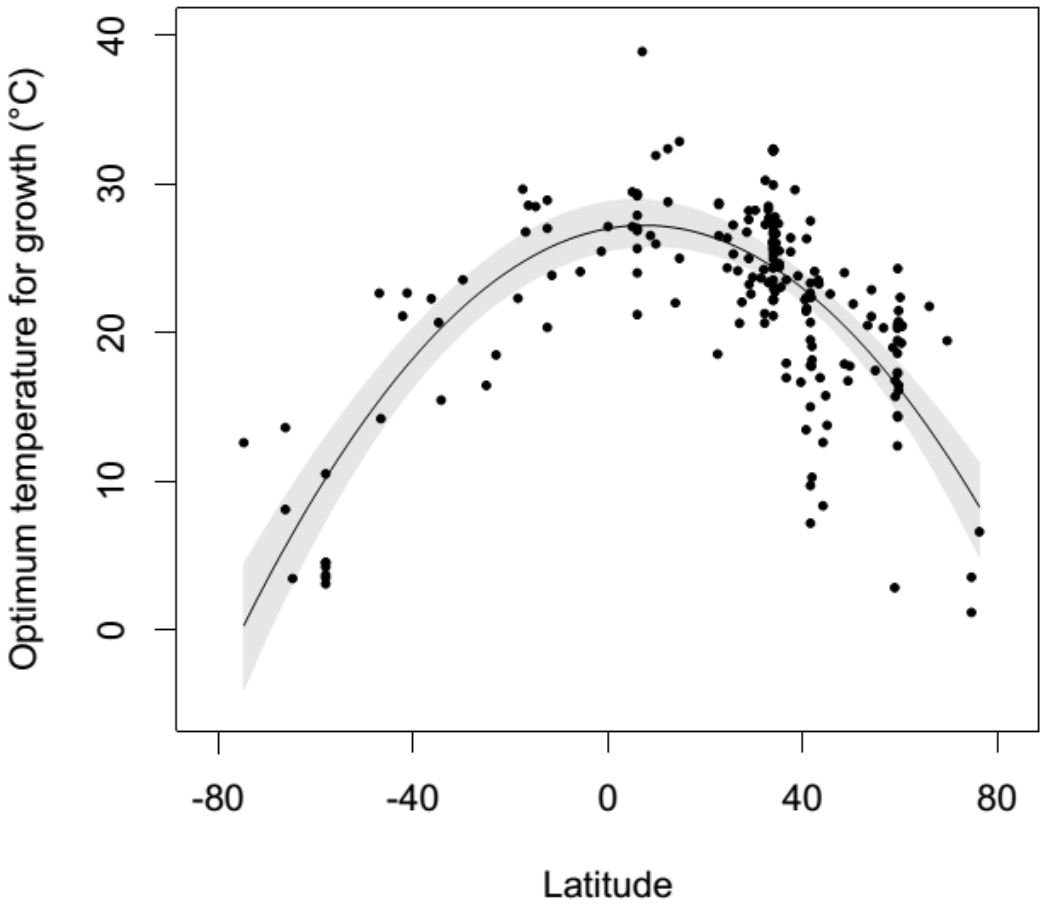


Figure 2.1. Latitudinal gradient in the optimum temperature for growth of marine and estuarine phytoplankton strains (n=194, R^2=0.55, p<0.0001). Each point represents the optimum temperature for growth of a single strain, estimated by fitting a thermal tolerance function (Norberg 2004) to the data. The regression line (black) is shown, along with 95% confidence bands (grey). Confidence bands account for asymmetric uncertainty in trait estimates using a bootstrapping algorithm (see supplementary methods and Figure A1.9 in Appendix1).

This strong trait-environment relationship suggests that microbes are adapted to the temperatures that they experience locally. However, this pattern could also occur through a correlated response to selection on other traits. To test whether the observed pattern arose as an adaptive response to variable thermal regimes, we used an eco-evolutionary model (Abrams 2001; Geritz et al. 1998) to predict the optimum temperatures that maximize fitness at each isolation location. The model allows us to study the effects of thermal adaptation alone by forcing all other aspects of strains to be identical. Purely theoretical applications of such

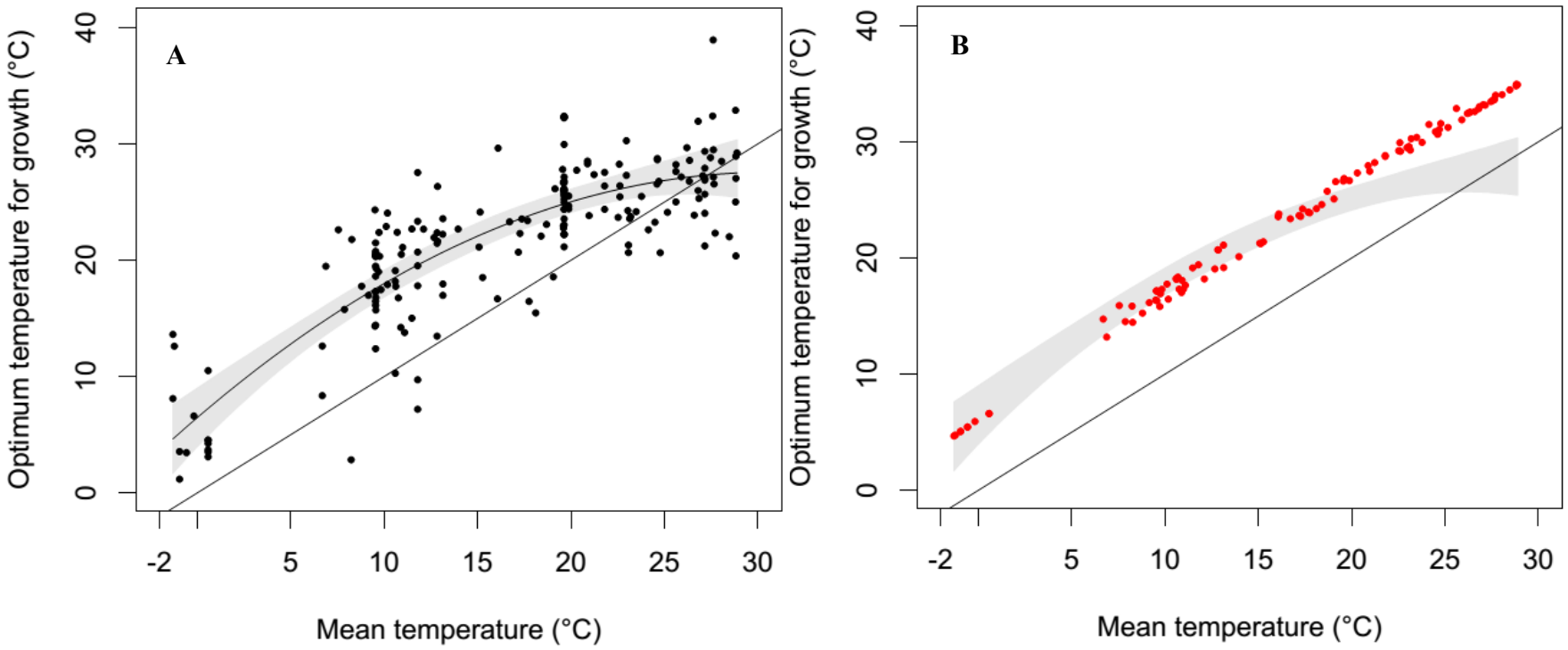


Figure 2.2. Optimum temperatures for growth across a gradient of ocean temperature. A) Optimum temperature of phytoplankton strains is well explained by variation in the mean annual temperature at their isolation locations (n=194, R^2=0.69, p<0.0001), indicating adaptation to local environmental conditions. The 1:1 line (black, straight), regression line (black, curved) and 95% confidence bands (grey) from bootstrapping are shown. The regression line shown is for the best model (Table A1.4), which posits a quadratic relationship between mean temperature and optimum temperatures. B) The eco-evolutionary model predicts evolutionarily stable optimum temperatures (red points) for each isolation location that are several degrees higher than the mean environmental temperatures (i.e. above black line) and agree well with the data, except in the warmest waters. The confidence band from A is shown in grey for comparison.

eco-evolutionary models have been extensive, but they have rarely been compared to quantitative field data (Stegen et al. 2012).

In the model, strains differ only in their thermal tolerance curves (characterized by their optimum temperature) while competing for a single nutrient. The growth rates of all strains are bounded by an exponential function that increases with temperature, an empirical relationship known as the Eppley curve (Eppley 1972). We require that each individual strain's thermal tolerance curve touch the Eppley curve at a single point, forcing maximum growth rate to become a function of optimum temperature. Niche widths are held constant across strains, as we found no significant relationship in our dataset between niche width and environmental or taxonomic covariates (Tables A1.1 and A1.2 in Appendix 1). Given these constraints, we allow optimum temperatures of a set of strains to evolve in response to deterministic temperature regimes. These regimes were based on model fits to a 30 year sea surface temperature time series at every isolation location (Reynolds et al. 2007, see supplementary methods in Appendix 1). For each environment, we used an evolutionary algorithm based on quantitative genetics to identify evolutionarily stable states (ESSs) (Abrams, 2001, see supplementary methods in Appendix 1). At an ESS, the strains that persist (defined by their traits) cannot be invaded by any other strain. These temperature optima serve as a theoretical prediction of the best strategy (or strategies) at each isolation location, which we can then compare to our data as a test of thermal adaptation.

Our eco-evolutionary model predicts that optimum temperatures should increase with mean temperature and exceed it by several degrees (Figure 2.2B, Figure A1.3 in Appendix 1). This is in agreement with the observed pattern (Figure 2.2A) and bolsters the case that this relationship arises from adaptation to mean temperature. However, in regions with the highest mean temperatures (the tropics), the model predicts optima that are significantly higher than

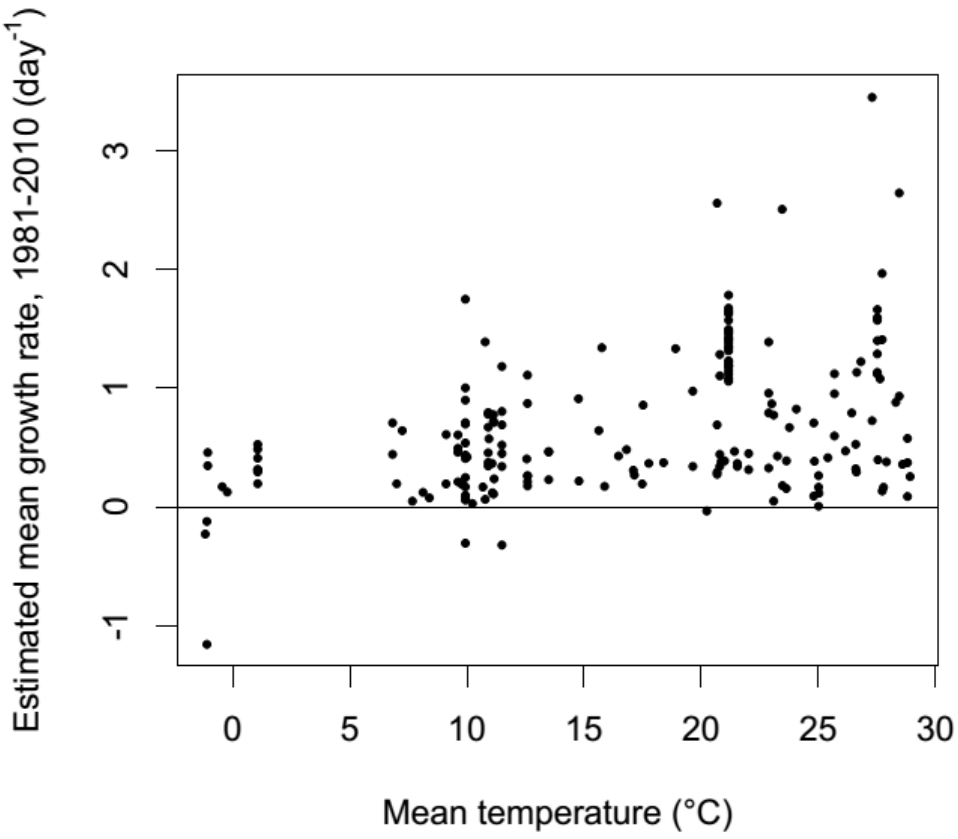


Figure 2.3. Estimated mean daily growth rates of all strains at their isolation locations, between 1980 and 2010. These estimates were based on monthly temperature records (Reynolds et al. 2007) and each strain's thermal tolerance curve and depend on the assumption that growth was limited solely by temperature. Even warm-water strains have mean growth rates exceeding zero (the horizontal line), indicating that they are capable of persisting in their environment though their optima are below what our model predicts to be most adaptive.

those observed. Though this discrepancy suggests that tropical strains may be less well-adapted to their environmental temperatures, we estimated that these strains are capable of persistence under the temperature regimes they experience (Figure 2.2B, 2.3) (Reynolds et al. 2002). The difference may be a result of interactions between temperature and other factors, constraints on thermal adaptation at high temperatures, or adaptation to laboratory temperatures prior to measurement. Examining model predictions across a range of assumed niche widths reveals that

wider niches lead to larger differences between predicted optima and the mean annual temperatures and a decrease in the number of co-existing strains (Figure A1.3 in Appendix 1). These results illustrate that temperature variation can support species co-existence, although it cannot fully explain the levels of trait diversity observed in the data.

Phytoplankton strains may be adapted to their current conditions, but could be negatively affected by warming oceans. Moving from the eco-evolutionary model to purely physiological mechanistic species distribution models (SDMs), we then examined whether changing environmental temperatures could alter species ranges and global diversity patterns. These models use physiological trait measurements to predict species abundances across environmental gradients (Kearney & Porter 2009) but do not account for species interactions or evolution. We generated growth rate predictions across the ocean for each strain represented in our dataset based on their thermal tolerance curves and a ten-year temperature time series (see supplementary methods in Appendix 1). If the ten-year mean growth rate of a strain was positive at a location, the location was deemed to fall within its range. We repeated this using both historical (1991-2000) and future (2091-2100) temperature regimes, the latter predicted by a global climate model (Delworth et al. 2006; IPCC Fourth Assessment Report 2007; Nakicenovic et al 2000; Reynolds et al. 2002; see supplementary methods in Appendix 1). These estimates indicate that ocean warming is likely to drive poleward shifts in strains' equatorial boundaries, though polar range boundaries remain approximately constant (Figure A1.4). Consequently, many strains are predicted to experience a reduction in range size (Figures A1.5, A1.6), potentially increasing extinction probabilities. Our SDMs assume that growth rates are limited solely by temperature but other factors, such as nutrient availability, could also be incorporated if

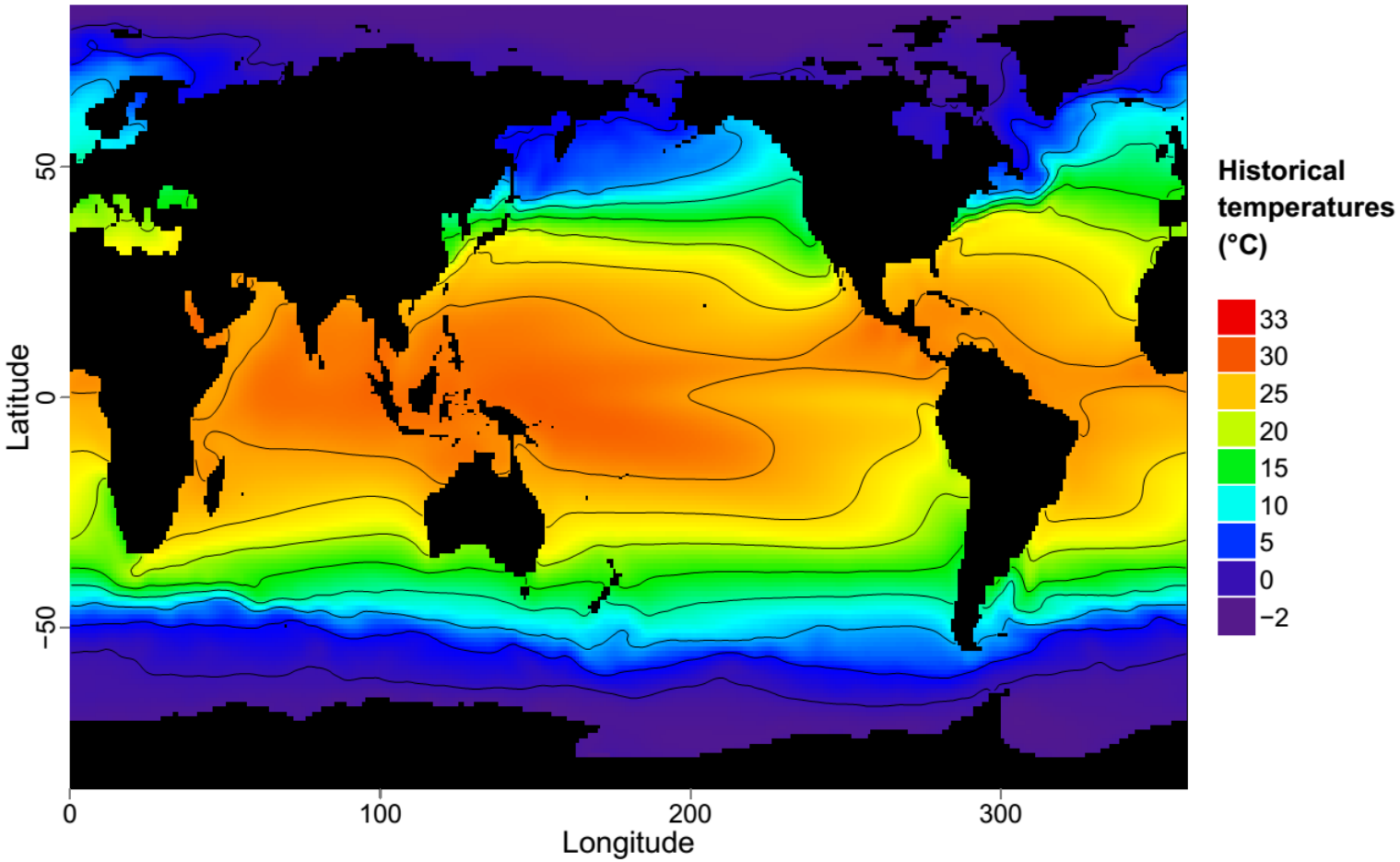
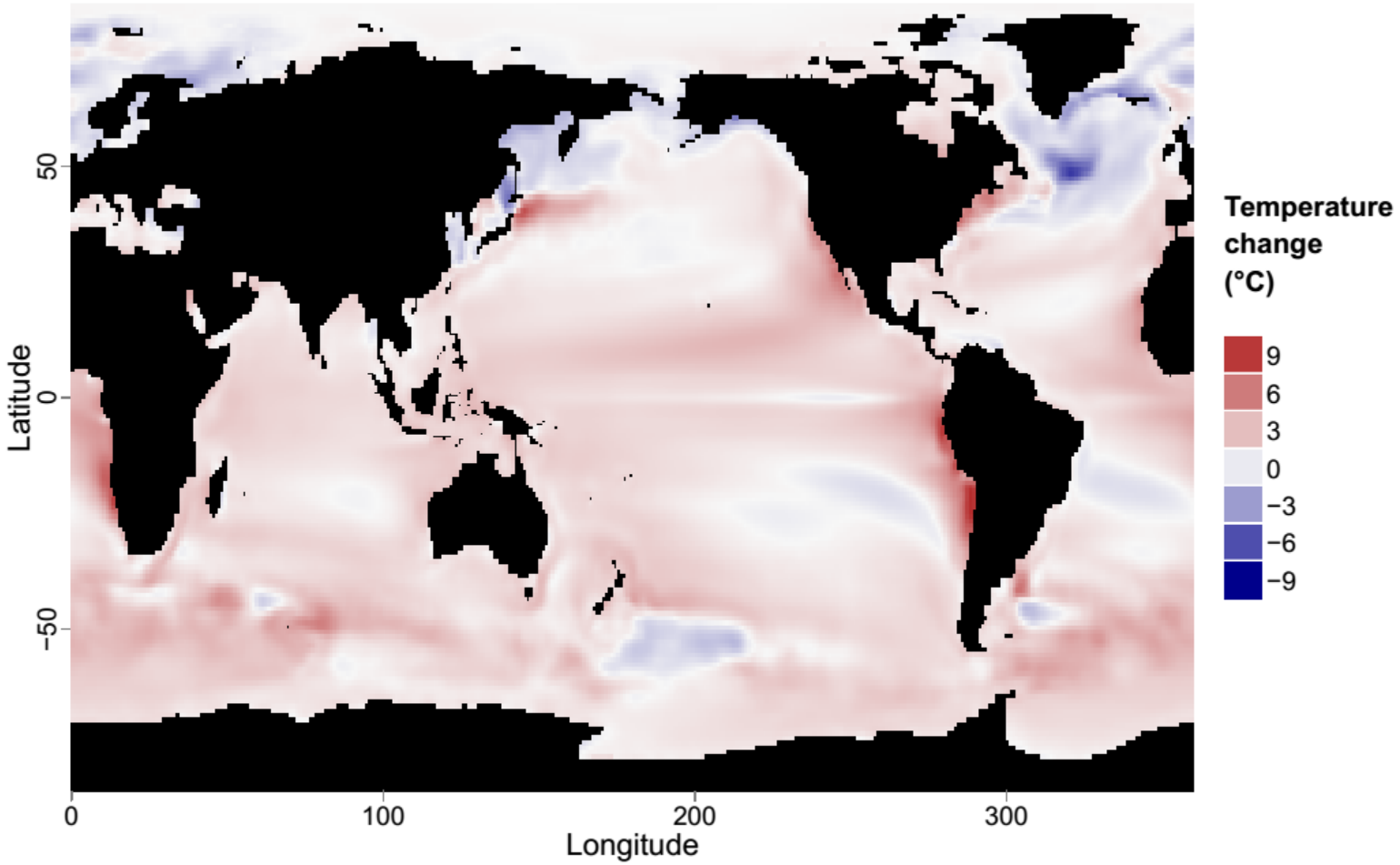


Figure 2.4. Changes in temperature drive changes in the potential diversity of phytoplankton, as predicted by mechanistic species distribution models. (A) Mean annual temperature across the oceans over historical (1991-2000) temperature regimes. (B) Change in mean annual temperature (°C) between historical (1991-2000) and predicted future temperature regimes (2091-2100). (C) Changes in temperature drive changes in the potential diversity of phytoplankton, as predicted by mechanistic species distribution models. Percent change in potential diversity between historical and predicted future temperature regimes. Potential diversity is reduced sharply in the tropical oceans, despite these regions experiencing relatively small increases in temperature.

Figure 2.4 (cont'd)



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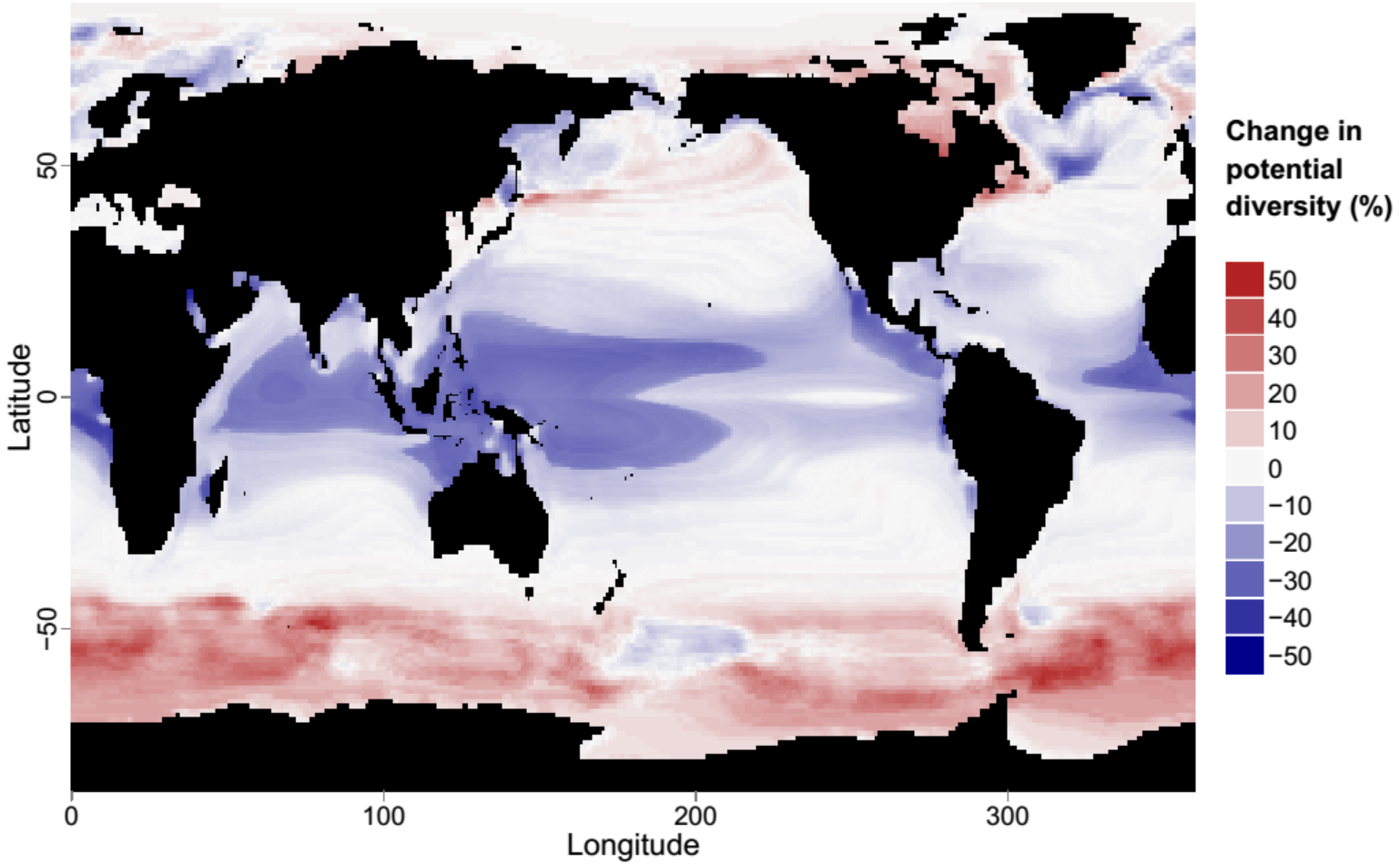

relevant trait data were available. When the range shifts of all strains are considered in the aggregate, they can be used to predict global patterns of phytoplankton diversity change as a result of ocean warming (Figure 2.4) (McKenney et al. 2007). In order to do this, we calculated 'potential diversity', defined as the number of phytoplankton strains (out of the 194 in our dataset) theoretically capable of growing at a location, assuming that temperature is the sole limiting factor (Figures A1.7, A1.8). A comparison of potential diversity patterns under both historical and future temperature regimes shows that temperature change may drive a large reduction in tropical phytoplankton diversity over the course of this century. Approximately a third of contemporary tropical strains are unlikely to persist there in 2100 (Figure 2.4C), despite a change in mean temperature of only ~2°C (Figures 2.4A, 2.4B). High latitudes may experience small increases in potential diversity, as a result of poleward shifts in strain ranges. Rising temperatures have the strongest effect on tropical strains because tropical optima are close to current mean temperatures (Figure 2.2A) and thermal tolerance curves are negatively skewed. Small increases in temperature can therefore lead to sharp declines in growth rate. A decrease in diversity is likely to have a strong impact on tropical ecosystems, as biodiversity loss is a major cause of ecosystem change (Hooper et al. 2012). One possible consequence is a decrease in tropical primary productivity, which could occur through two distinct mechanisms: the loss of highly productive species or a decrease in complementarity (Reich et al. 2012; Tilman et al. 1996).

Our findings lend support to the hypothesis that tropical communities are most vulnerable to increases in temperature (Deutsch et al. 2008). However, the existence of high genetic diversity within species, as has been noted in some cases (Härnström et al. 2011), may prevent the loss of entire species. Adaptation to changing temperatures may mitigate some of the

predicted losses in diversity, particularly in rapidly reproducing taxa such as phytoplankton. Evolution of thermal tolerance has been examined in a few taxa, including phytoplankton (Bennett & Lenski 2007; Huertas et al. 2011; Knies et al. 2006), but we currently lack the information necessary to accurately model the consequences of evolutionary change on ecosystem processes (Angilletta 2003; Chown et al. 2010). In the case of phytoplankton, we need estimates of rates of adaptation to high temperature stress in a variety of taxa, as well as an examination of the evolutionary constraints and trade-offs that may be associated with this. Characterizing these constraints will allow us to make improved forecasts of species survival and may prove critical for understanding the fate of tropical communities and oceanic ecosystems.

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LITERATURE CITED

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CHAPTER 3

ENVIRONMENT AND EVOLUTIONARY HISTORY DETERMINE THE GLOBAL BIOGEOGRAPHY OF PHYTOPLANKTON TEMPERATURE TRAITS

ABSTRACT

Characterizing worldwide patterns in functional traits can reveal signals of historical selection, as well as environmental constraints on community composition and species distributions. These patterns reflect a combination of adaptation to local conditions, species interactions, and both ecological and evolutionary constraints. Here we examine global variation in five important temperature-response traits in phytoplankton, a group of autotrophs critical to aquatic food webs and biogeochemical cycling. We show systematic variation across latitude, environment type (marine vs. freshwater), and functional group in all of these traits - optimal temperature for growth, maximum persistence temperature, minimum persistence temperature, temperature niche width, and maximum growth rate. Our results indicate that niche partitioning or evolutionary constraints have contributed to differences in trait-environment relationships between different functional groups. Understanding how evolutionary history and environment interact to determine trait distributions will improve our ability to model community re-organization as a result of environmental change.

INTRODUCTION

Functional traits characterize how organisms interact with their environment and each other, providing a strong foundation for predictive community ecology (Lavorel & Garnier 2002; McGill et al. 2006; Litchman & Klausmeier 2008; Webb et al. 2010). They have been shown to explain major ecological phenomena such as seasonal succession patterns (Edwards et al. 2013) and invasibility in novel environments (Funk & Vitousek 2007; Schmidt & Drake 2011). Current

trait distributions reflect a history of environmental selection and evolutionary constraints: selection drives species to optimize their traits for their local environment, but this is limited by trade-offs, genetic constraints, gene flow, and dispersal limitation. By comparing trait-environment relationships across groups with differing evolutionary histories, we can uncover the evolutionary constraints and ecological interactions underlying community composition and dynamics. This will also improve our ability to predict the ecological and evolutionary consequences of environmental change. As many systems will experience novel patterns of environmental covariation in the future (Williams et al. 2007), predictions based on statistical associations between current species distributions and environmental conditions are inherently limited in their power. By contrast, a mechanistic understanding of the biological constraints and trade-offs that underpin current distributions would allow us to model community change in novel conditions. In this paper, we present the results of a comprehensive study of global patterns of temperature response traits in phytoplankton, comparing differences across latitude between marine and freshwater environments and major functional groups.

Phytoplankton communities play a critically important role in global biogeochemical cycles and aquatic food webs (Falkowski et al. 1998; Field et al. 1998) and are extremely sensitive to changes in environmental conditions. They are a diverse group of autotrophs composed of evolutionarily distinct (though not always monophyletic) functional groups which differ strongly in the roles they play in biogeochemical cycling and food webs (Reynolds 2006). Cyanobacteria are the only prokaryotic group and form relatively poor food for herbivorous zooplankton. Uniquely among the phytoplankton, some cyanobacteria possess the ability to fix atmospheric nitrogen. Diatoms are disproportionately responsible for primary production and carbon sequestration among the phytoplankton (Field et al. 1998; Nelson et al. 1995). They are

the primary group involved in silicon cycling, and their cells are among the largest in the community. Green algae are fast growers that are abundant in high-light environments (Edwards et al. 2012; Reynolds 2006). The marine coccolithophores form calcium carbonate plates and may affect weather and climate patterns through their production of dimethyl sulfoniopropionate, a precursor to cloud condensation nuclei (although this has been challenged) (Charlson et al. 1987; Franklin et al. 2010; Quinn & Bates 2011). Closely related to the coccolithophores, the non-calcifying haptophytes are a diverse group of mixotrophs that form a large proportion of the open ocean phytoplankton community (Liu et al. 2009). Dinoflagellates are large-celled, motile mixotrophs, some of which are responsible for the formation of toxic red tides in coastal ecosystems (Reynolds 2006). This functional diversity is reflected in broad differences between the ecological strategies and traits of different groups (Edwards et al. 2012; Litchman et al. 2007). However, it is not known whether functional groups differ in their response to temperature, though there are large differences between individual species. This leaves unclear how temperature variation affects existing communities, and how warming will drive community re-organization: through species replacement within functional groups or broader changes to community structure. Earlier work on seasonal succession patterns and physiology have led to the conclusion that cyanobacteria are adapted to high temperatures (e.g. Kosten et al. 2012; Robarts & Zohary 1987). But a recent analysis of freshwater isolates found no evidence for differences in temperature traits between groups (Lürling et al. 2013). Notably, no study has compared these traits across environmental gradients, leading to a possible bias in earlier results.

Temperature has strong effects on fitness and drives changes in communities across both time and space (Ettinger et al. 2011; Kingsolver 2009; Kordas et al. 2011; O'Connor et al. 2009; Poloczanska et al. 2013). The effects of temperature on ectotherms such as phytoplankton are

characterized by thermal reaction norms, functions that describe how fitness changes with temperature. These are unimodal and negatively skewed (Figure A2.1 in Appendix 2); the major biological consequence of this asymmetry is that an increase in temperature above the optimum leads to a much larger decline in fitness than a decrease of an equal magnitude (Kingsolver 2009; Martin & Huey 2008). The reaction norms may be described by different combinations of parameters, which constitute the temperature-response traits we are interested in. We consider five traits here - optimum temperature for growth, maximum persistence temperature (Tmax, the temperature above which population growth rate becomes negative), minimum persistence temperature (Tmin, the temperature below which population growth rate becomes negative), temperature niche width (the range of temperatures over which population growth rate is positive), and maximum population growth rate (Figure A2.1 in Appendix 2). We note that not all of these parameters are needed to characterize a single reaction norm and that other parameterizations are also used (e.g. see Dell et al. 2011; Ratkowsky et al. 1983; Schoolfield et al. 1981). Variation in these parameters has been used to identify signals of adaptation to local temperatures as well as examine susceptibility to environmental warming (Clusella-Trullas et al. 2011; Deutsch et al. 2008; Sunday et al. 2011; Thomas et al. 2012). This body of work has shown that species are adapted to current temperature regimes across broad temperature gradients and that changes in these regimes are likely to drive community re-organization.

We systematically analyzed variation in these five temperature traits in 442 phytoplankton isolates belonging to approximately 252 species, distributed widely across latitude (76°N to 78°S, Figure 3.1), environment type (marine and freshwater), and taxonomy (primarily cyanobacteria, diatoms and green algae, but also dinoflagellates, coccolithophores, non-calcifying haptophytes, desmids, chrysophytes, and raphidophytes), using data extracted from

published papers. This greatly expands on previous work showing that distributions of optimum temperature in marine phytoplankton indicate that they have adapted to local temperature conditions (Thomas et al. 2012). We expect that trait-environment relationships will be driven by variation in local temperature regimes; we use latitude and environment type as proxies here because temperature estimates are unavailable for most freshwater bodies. Specifically, we expect that patterns of trait variation will be driven by the following global temperature patterns in aquatic environments: 1) Environmental temperatures (mean, maximum, and minimum) decline from the equator to the poles (Figure A2.2 in Appendix 2). 2) Temperature variability is highest at mid-latitudes and lowest in the tropics and at the poles (Figure A2.2 in Appendix 2). 3) Freshwater environments are smaller and therefore more thermally variable than marine environments at the same latitude, experiencing higher maximum temperatures and lower minimum temperatures over the course of a year.

As a result of these three trends, we predicted the following: 1) Optimum temperature, Tmax, and Tmin will all decline with distance from the equator. 2) Tmax will be higher, Tmin will be lower, and niche widths will be broader in freshwater taxa. 3) Optimum temperature will also be higher in freshwater environments, due to greater thermal variability in addition to the asymmetric fitness costs of exceeding the temperature optimum. 4) Niche widths will be wider at intermediate latitudes than at the tropics or near the poles. We have contrasting hypotheses regarding maximum growth rate, which may be selected across a latitudinal gradient in two different ways. Metabolic constraints lead to an exponential increase in the maximum attainable growth rate with temperature (Figure A2.3 in Appendix 2; also see Bissinger et al. 2008; Eppley 1972). Therefore, species with higher temperature optima have a higher upper limit on their maximum growth rates, leading to the prediction that maximum growth rate will peak in the

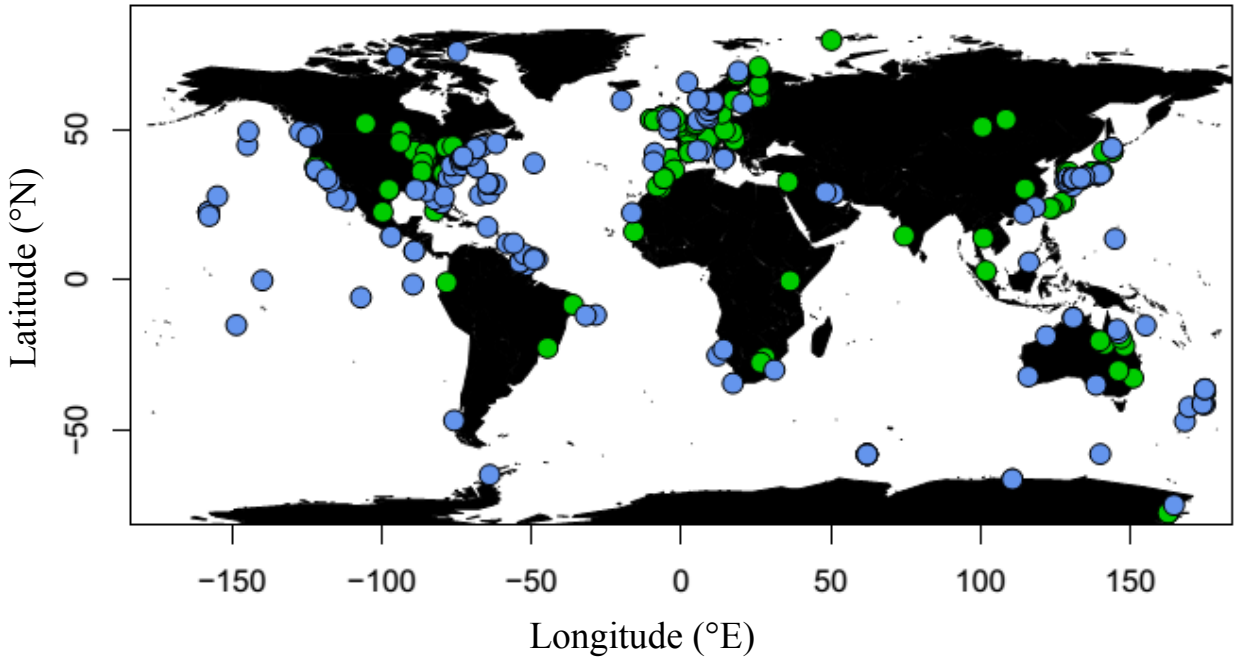


Figure 3.1. Isolation locations of 394 phytoplankton strains in our dataset (an additional 48 strains were from unknown locations). Freshwater locations are marked in green and marine locations in blue. Freshwater locations range from 68°N to 78°S and marine locations from 76°N to 75°S.

tropics. On the other hand, selection to grow quickly is likely to occur in environments that experience pulses of nutrients, a characteristic of temperate environments by virtue of their water columns being less stable (an indirect effect of temperature). If this is a more important factor, maximum growth rates should peak at temperate latitudes. We tested these competing hypotheses for this trait. Due to the contrasting previous results on functional group differences, we did not have strong hypotheses about how evolutionary history would interact with environmental drivers, but chose to explore these possibilities.

METHODS

Data collection, quality control, and temperature trait estimation followed Thomas et al. (2012), with minor differences. The methods are reiterated and differences described in detail in the supporting information. Aside from the five traits already described, we also investigated

variation in skewness, but found no systematic variation across latitude or functional groups. A small difference between environments was largely driven by differences in the average niche width between environments (Figure A2.4 in Appendix 2). A summary of our methods for this trait can also be found in the supporting information (Appendix 2).

Model comparison and parameter estimation

We quantified the effects of functional group, environment type (marine vs. freshwater) and latitude on each trait. In order to detect differences between traits in marine and freshwater environments, we first constructed models to explain variation in the traits of the three functional groups well- represented in both environments - cyanobacteria, green algae, and diatoms. Other functional groups were represented almost exclusively in one environment, and were not included initially. If no effect of environment type on a particular trait was detected, we re-ran the analyses after dropping the environment term and including data on the additional functional groups. If differences between environments were detected, we constructed separate models for each of the additional groups. These models used the structure of the best model identified with the three major groups, but excluded the unnecessary environment and group covariates. Finally, if no relationship between latitude and a particular trait was detected, we included additional data from isolates with unknown isolation locations.

We fit mixed models to the trait data, including a random intercept effect based on species identity to account for variance caused by measurements on multiple strains of the same species. Examinations of within-species variation of the traits showed no systematic variation across latitude, indicating that a random slope term was unnecessary. To determine the importance and significance of covariates, we first fit a full model (containing all interactions between latitude, group, and environment) to data for each trait. However, we chose to only

include terms that were biologically plausible in the model. In particular, we excluded a linear latitude term for models involving optimum temperature, Tmax, and Tmin, as we have strong reason to believe that these will peak around the equator, driven by the latitudinal trend in mean, maximum, and minimum temperature (Figure A2.2 in Appendix 2). However, we included the linear parameter in model comparison for the other parameters, as a peak away from the equator was plausible in these cases. Starting with this full model, successive comparisons were made between complex models and simpler, nested models generated by removing one term at a time beginning with the most complex interaction. At each step of this process, complex and nested models were compared using a likelihood ratio test (LRT). The significance of the LRT was determined using a parametric bootstrapping approach (with 10000 samples), instead of the typical χ^2 distribution approximation, avoiding assumptions of large sample sizes and asymptotic normality (Halekoh & Højsgaard 2013). When a term had a non-significant bootstrap p-value (> 0.05) it was dropped and the next term of the resulting model was tested. After considering all interaction terms, we also examined the importance of main effects, retaining them only if they were significant or included in a significant interaction. We examined the fit of the resulting best model to the trait data, checking for outliers, influential points, and deviations from normality assumptions. When identified, problematic points were removed and the model comparison was re-run. In all cases, the best model remained the same and parameter estimates remained similar after the removal of these points; we therefore retained these points in our analyses and present our original findings. With the final model for each trait determined, we obtained 95% confidence intervals on all parameter estimates using parametric bootstrapping. Lastly, we calculated the marginal and conditional R^2 of the final models, which quantify the explanatory power of the fixed effects and the combined fixed and random effects respectively (Nakagawa &

Schielzeth 2013). Conditional and marginal R^2 values for the best models for all traits, and bootstrap p-values for their parameters are shown in Table A2.1 in Appendix 2.

All analyses were performed in the R statistical environment (R Core Team 2013). Thermal reaction norm fitting and trait estimation was performed using the *bbmle* package (Bolker & R Development Core Team 2012). Mixed model fits, parameter estimation, and confidence interval estimation were performed using the *lme4* package (Bates et al. 2013), and diagnostic tests on these fits were performed using the package *HLMdiag* (Loy 2013). Parametric bootstrapping to determine significance of model terms in the model comparison procedure was performed using the *PBmodcomp* function in the package *pbkrtest* (Halekoh & Højsgaard 2013). Conditional and marginal R^2 were calculated using the package *MuMIn* (Barton 2013).

RESULTS

1. Optimum temperature for growth

Optimum temperatures for growth are highest in equatorial waters and decline towards the poles, but they vary across environments and functional groups (Figure 3.2, Tables A2.1, A2.2 in Appendix 2). In freshwater environments, optima are approximately 4°C higher across latitudes. Furthermore, while all three functional groups possess similar optima in the tropics (confidence intervals on all group main effects overlap zero), the rate of decline with latitude differs between groups. Diatom optima decline fastest, while cyanobacteria show little variation with latitude. The marginal R^2 (Nakagawa & Schielzeth 2013) of the model, which is the variance explained by the fixed effects in the model, is 50%. The conditional R^2, or the variance

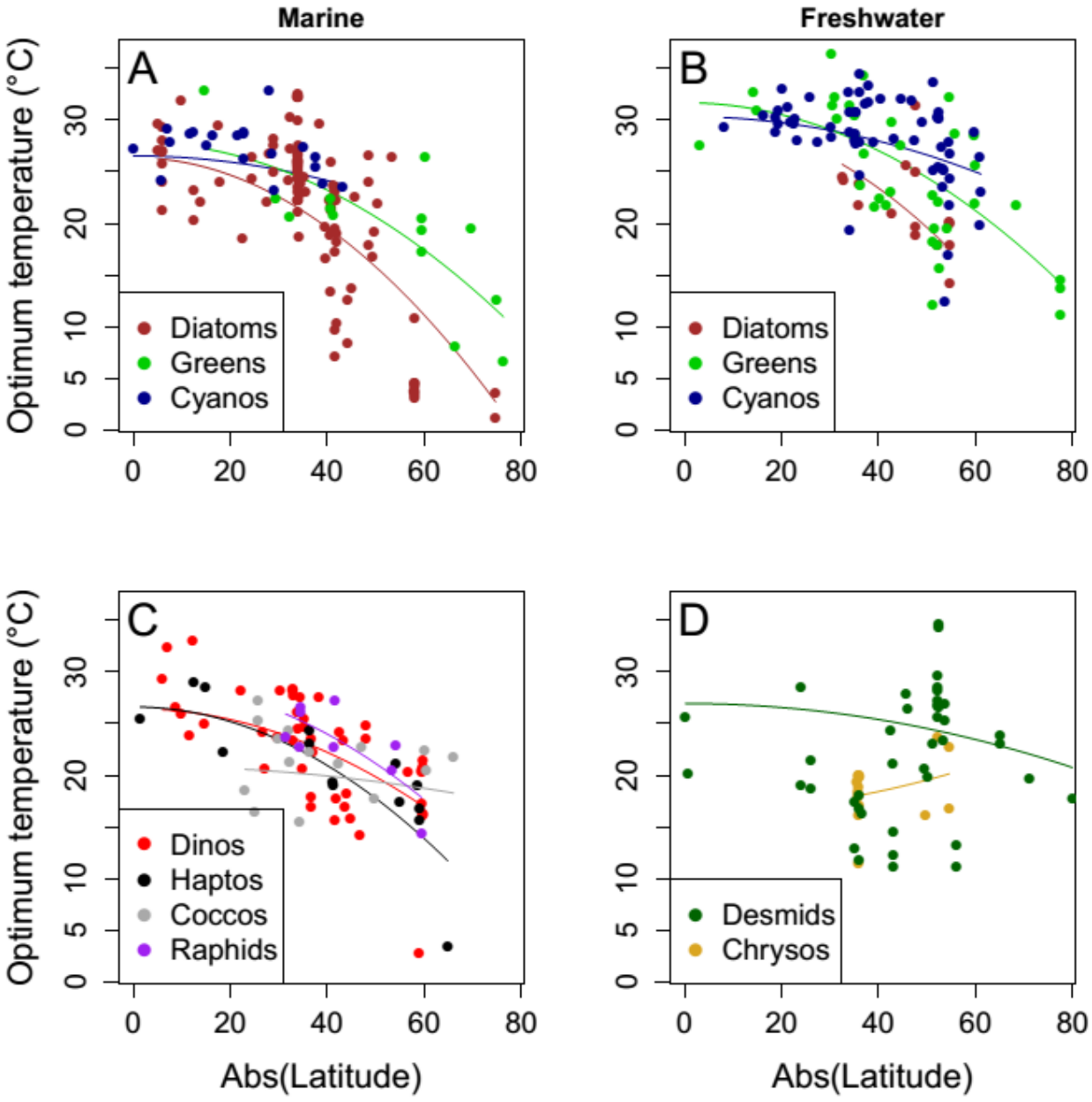


Figure 3.2. Optimum temperature for growth decreases towards the poles, but this decline differs between functional groups and environments. The best model was identified using groups common to both environments, shown in A and B. Groups for which we had data largely from one environment are shown in C and D; model parameters for these groups were estimated separately.

explained by fixed and random effects together, is 83%. The optimum temperatures of dinoflagellates, non-calcifying haptophytes, and raphidophytes also decline with latitude, but we are unable to detect similar trends in the coccolithophores, chrysophytes and desmids (95% confidence intervals on quadratic term overlap zero). The desmids in particular exhibit extremely consistent optima across latitude (Figure 3.2, Table A2.2 in Appendix 2).

2. Maximum persistence temperature (Tmax)

Tmax shows patterns similar to those of optimum temperature: it is highest at the equator and declines towards the poles, freshwater values are approximately 4°C higher than marine ones across latitudes, and functional groups differ in the rate of change in Tmax with latitude (Figure 3.3, Tables A2.1, A2.3 in Appendix 2). However, we find additionally that Tmax is highest at low latitudes among the green algae. The model's fixed effects explain 46% of the variance in the data, while the fixed and random effects together explain 90% of the variance.

The results for the other groups are also similar to those of optimum temperature for growth: Tmax of dinoflagellates, non-calcifying haptophytes and raphidophytes declines with latitude (Figure 3.3, Table A2.3 in Appendix 2), while desmids show little variation in Tmax with latitude. Coccolithophores and chrysophytes do not show a latitudinal trend either, possibly due to a lack of data.

3. Minimum persistence temperature (Tmin)

Tmin is also highest at the equator and declines towards the poles across groups, which differ in the rate of decline with latitude and estimated equatorial Tmin (Figure 3.4, Tables A2.1, A2.4 in Appendix 2). Across environments, Tmin differs strongly between marine and freshwater cyanobacteria; freshwater cyanobacteria have considerably lower Tmin values. We have

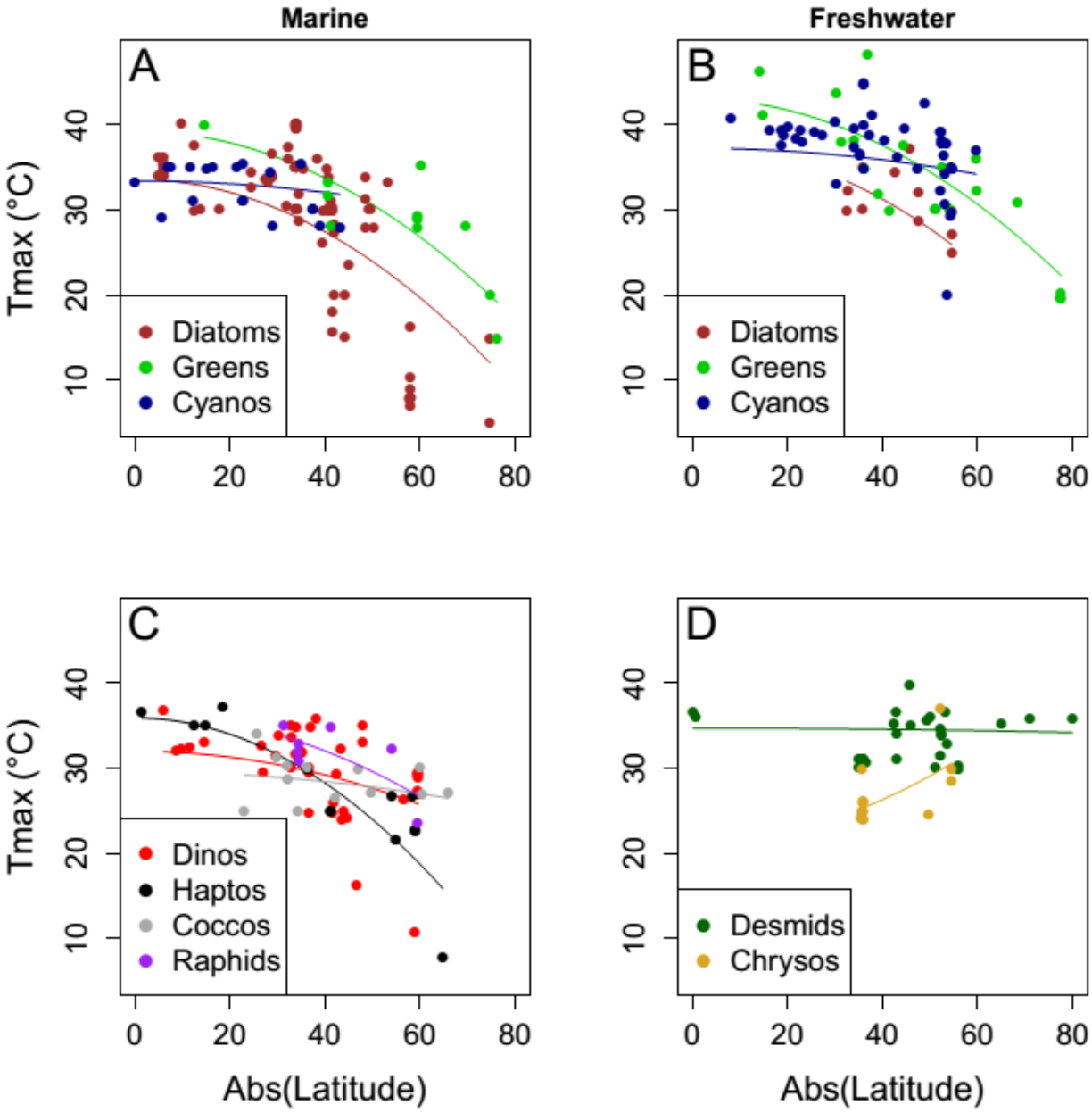


Figure 3.3. Maximum persistence temperature (Tmax) decreases with increasing latitude, but there are also strong differences between functional groups and environments. The best model was identified using groups common to both environments, shown in A and B. Groups for which we had data largely from one environment are shown in C and D; model parameters for these groups were estimated separately.

insufficient data among the freshwater diatoms with which to make cross-environment comparisons. Tmin is also higher among marine cyanobacteria than other marine functional

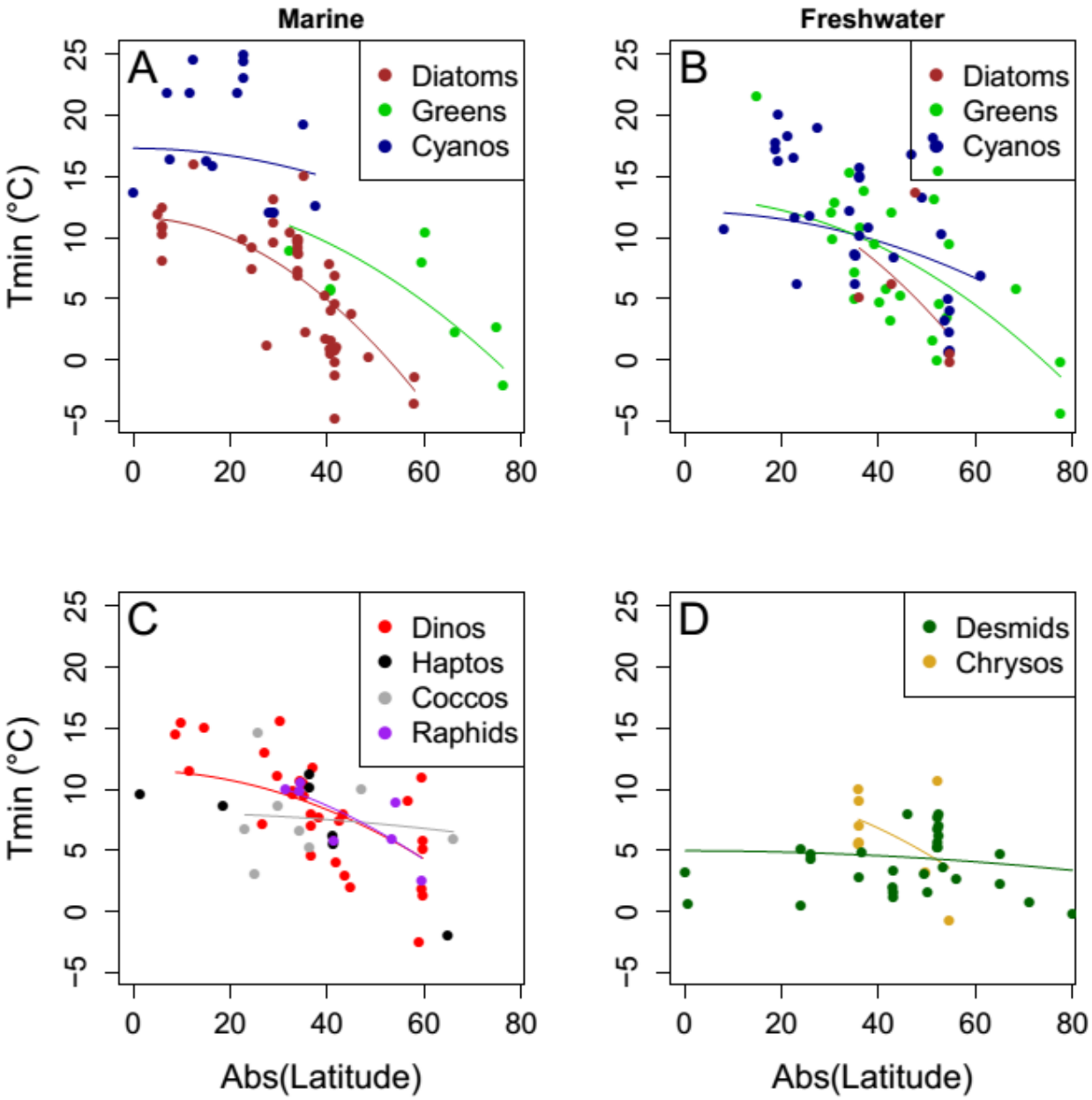


Figure 3.4. Minimum persistence temperature (Tmin) decreases towards the poles, but also differs between functional groups and environments. The best model was identified using groups common to both environments, shown in A and B. Groups for which we had data largely from one environment are shown in C and D; model parameters for these groups were estimated separately.

groups, while diatoms have the lowest Tmin across latitude. The model's fixed effects explain 49% of the variance in the data, while the fixed and random effects together explain 82% of the variance.

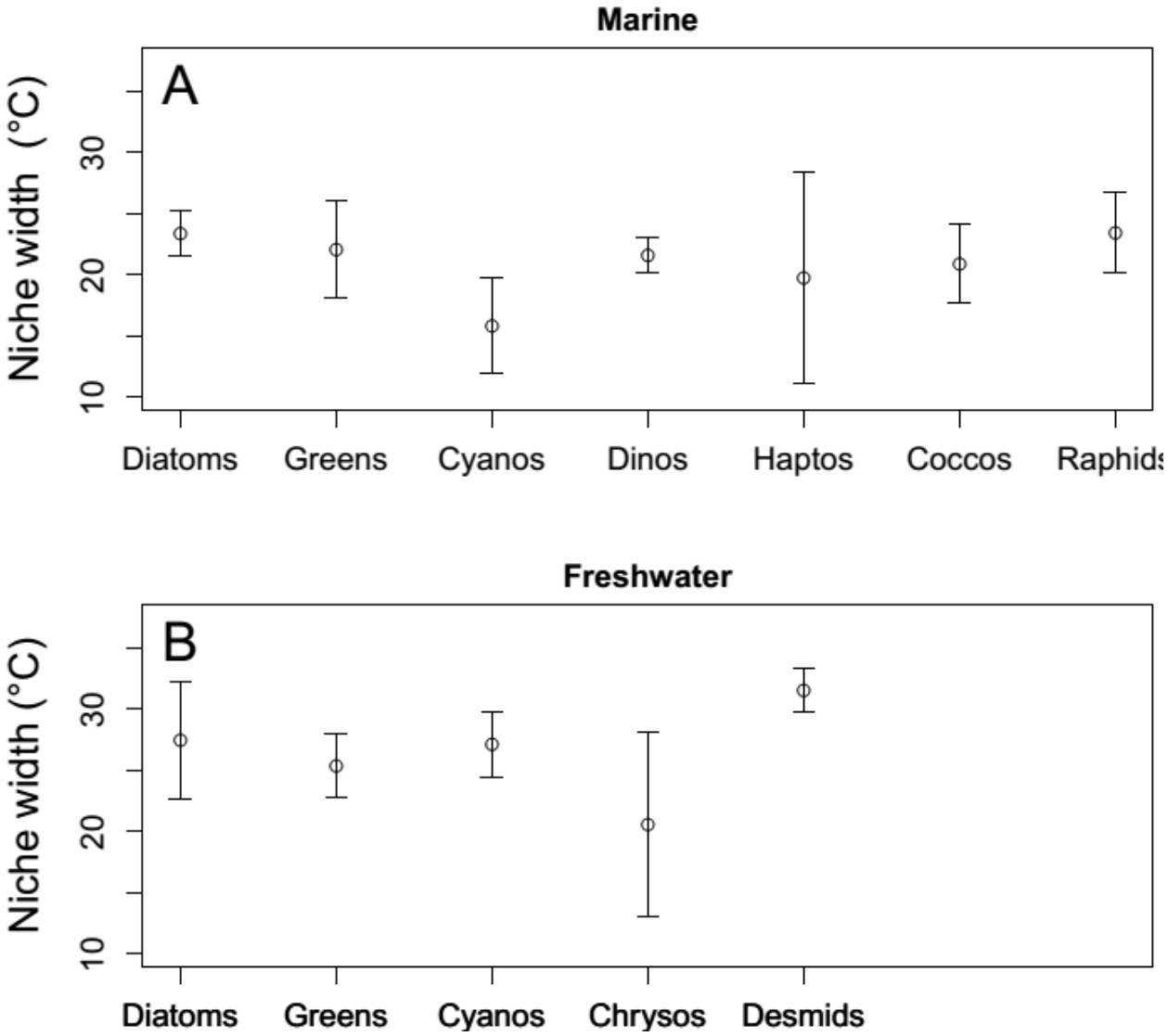
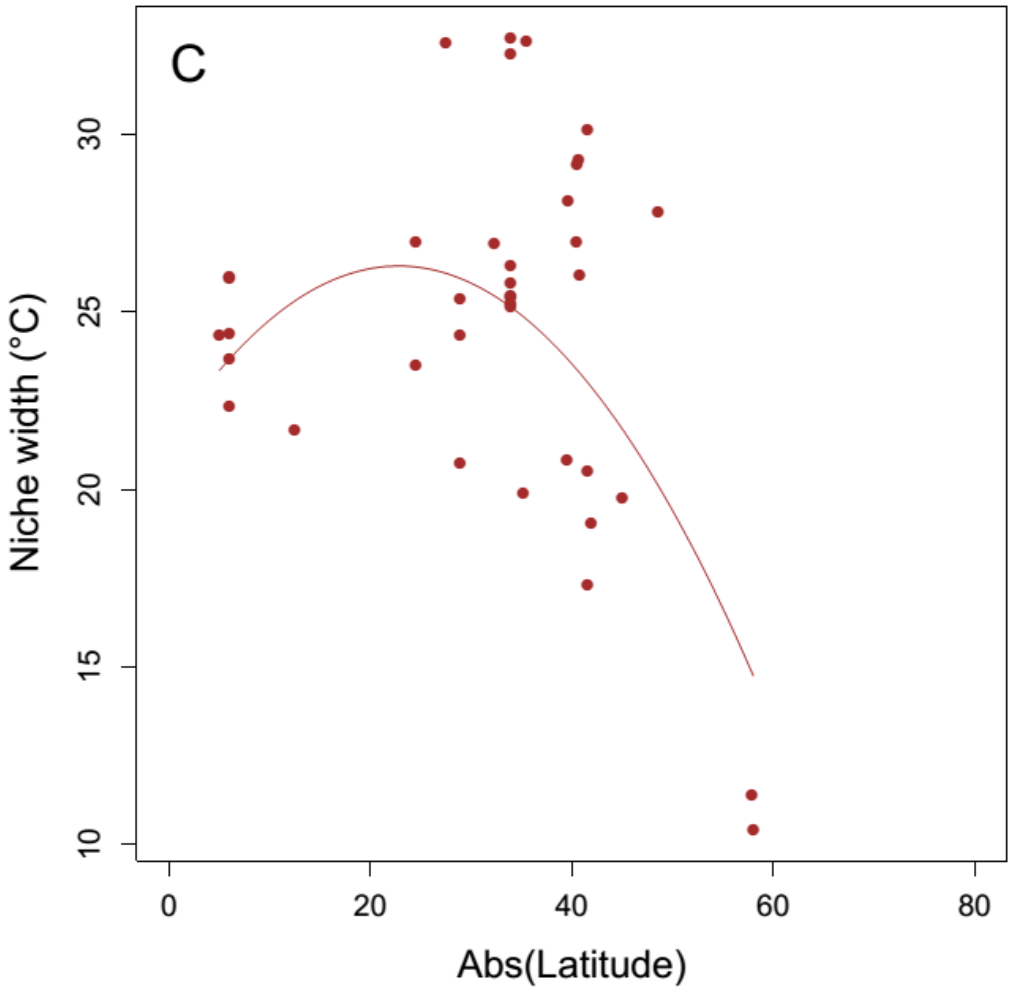


Figure 3.5. (A) and (B) Niche widths differed between functional groups and environments. Error bars are 95% confidence intervals. (C) Niche width across absolute (latitude) for marine diatoms, the group for which we had the broadest latitudinal distribution for this trait. A quadratic curve with a mid-latitude peak was the best statistical descriptor for this group, consistent with adaptation to environmental variability

Figure 3.5 (cont'd).



The results for the other groups are also similar to those of the previous two traits: Tmin declines with latitude in dinoflagellates, non-calcifying haptophytes and raphidophytes (Figure 3.4, Table A2.4 in Appendix 2), while showing little latitudinal variation in the desmids. We have insufficient data to detect latitudinal trends in Tmin in coccolithophores and chrysophytes.

4. Temperature niche width

We found that niche width was determined by functional group and environment, with an interaction driven largely by differences in cyanobacterial taxa in the two environments (Figure

3.5A and B, Tables A2.1, A2.5 in Appendix 2). Freshwater cyanobacteria had niches that were approximately 11°C wider on average than their marine counterparts. The mean niche widths for green algae and diatoms were 3-4°C wider in freshwater environments, but this difference was not statistically distinguishable from zero (Table A2.5 in Appendix 2). Most groups in both environments had mean niche widths between 20 and 30°C (Figure 3.5A and B, Table A2.5 in Appendix 2). The exceptions were the marine cyanobacteria (15°C) and the desmids (31.5°C). The model's fixed effects explain 35% of the variance in the data, while the fixed and random effects together explain 77% of the variance.

However, the data for most groups were distributed over relatively narrow ranges of absolute latitude, making it difficult to detect this pattern across groups. We therefore examined latitudinal variation in niche width in the marine diatoms, for which we have the most data and across the broadest latitudinal range. In this group, we found strong evidence for a peak in niche width at intermediate latitudes (Figure 3.5C).

5. Maximum growth rate

As we could not detect any influence of environment type on growth rate, we modeled all functional groups together instead of focusing on the three major groups. We found strong evidence for differences in maximum growth rate between groups and for a latitudinal trend in maximum growth rate peaking at the equator (Figure 3.6, Tables A2.1, A2.6 in Appendix 2). The estimated growth rate at the equator varied approximately threefold between the fastest growth group (diatoms) and the slowest (desmids). Latitude had a considerably weaker effect. In the diatoms, the estimated maximum growth rate in the tropics is approximately 50% greater than at the poles. As the data were modeled on a log scale, this latitudinal difference is of a far smaller

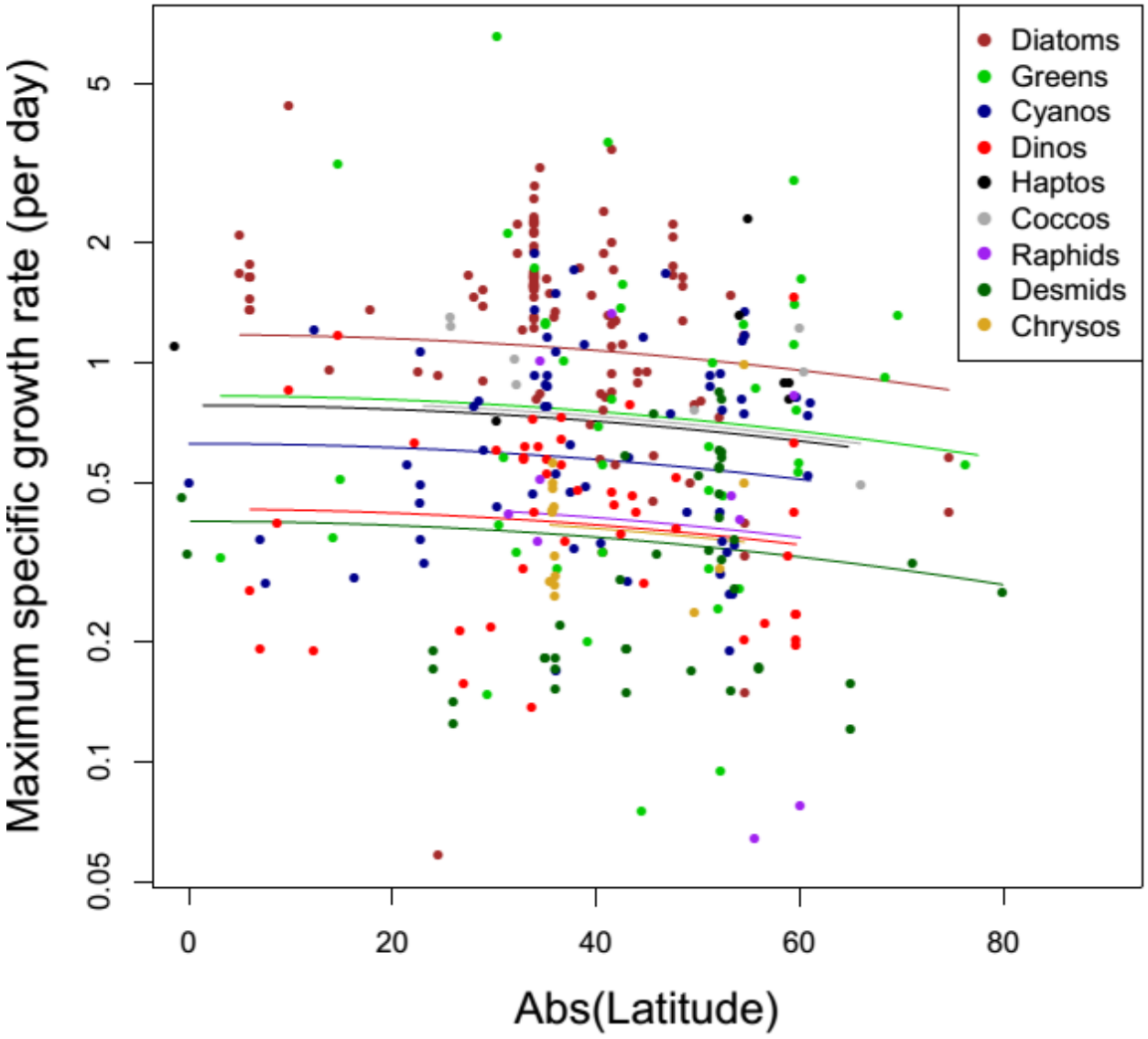


Figure 3.6. Maximum growth rate differs between functional groups and decreases towards the poles. As there were no detectable differences between environments, all groups were modelled together.

magnitude for slower growing groups. The model's fixed effects explain 29% of the variance in the data, while the fixed and random effects together explain 61% of the variance.

DISCUSSION

Our results show that major phytoplankton functional groups differ considerably in their temperature trait distributions, and in how these traits vary across latitudinal gradients and

between environments. Such variation in traits can emerge as a consequence of selection by local environments, evolutionary constraints, and niche partitioning. Our findings parallel recent studies showing that selection has shaped distributions of temperature traits in a variety of terrestrial and marine taxa. Optimum temperature has been shown to vary strongly across latitudinal gradients in terrestrial insects and marine phytoplankton (Deutsch et al. 2008; Thomas et al. 2012). Tmax is thought to be strongly phylogenetically conserved, and a recent synthesis examining trait variation in terrestrial organisms found strong evidence for its conservation across lineages (Araújo et al. 2013). Earlier studies had found contrasting patterns: some showed weak or no variation in Tmax across latitudinal and temperature gradients (Hoffmann et al. 2013; Sunday et al. 2011), while others showed increases in Tmax with distance from the equator (Huey et al. 2009), a pattern that is opposite to our expectation and finding in this study. Our results are the first demonstration of strong selection on Tmax across broad temperature gradients, indicating that phylogenetic conservation in this trait is likely not strong in phytoplankton. In contrast, Tmin is thought to be more evolvable, and multiple studies have shown that it increases with mean environmental temperature and latitude in terrestrial ectotherms (Araújo et al. 2013; Hoffmann et al. 2013; Huey et al. 2009; Sunday et al. 2011). Through examining trait-environment relationships such as these across taxonomic and functional groups, we can better understand the historical determinants of current ecological patterns, identify constraints in adapting to environmental conditions, and predict how future environmental change is likely to affect communities.

Marine and freshwater environments pose contrasting selection regimes that we predicted would cause specific differences between temperature trait distributions in these environments. We found strong evidence for these differences, driven by the greater thermal variability of

freshwater environments in combination with the asymmetric fitness cost of exceeding the optimum temperature (Figure A2.1 in Appendix 2). Optimum temperatures and Tmax are higher in freshwater environments across latitudes and taxa (Figures 3.2 and 3.3). Tmin is also lower and niche widths broader in freshwater cyanobacteria than their marine counterparts; we did not find evidence for this in other groups, however (Figures 3.4 and 3.5). One caveat to these results is that if strains were isolated more frequently during the warm summer months, the higher temperatures in freshwater systems during this period would exaggerate optimum and Tmax differences between environments; however, our data sources rarely recorded this information. We had no theoretical reason to expect differences in maximum growth rate between environments and found no evidence for this. Together, these results link differences in environmental variation between two distinct habitats to variation in organismal physiology within functional groups present in both, providing a strong case for selection as a mechanism. Though previous studies have uncovered important differences between marine and freshwater phytoplankton communities, such as nutrient limitation (Elser et al. 2007) and trophic cascades (Sommer & Sommer 2006), few have examined how environmental differences have selected on the physiology of taxa in both environments. Two recent studies have shown that phytoplankton nutrient competitive abilities and cell sizes differ in a manner consistent with differing nutrient, mixing and grazing regimes (Edwards et al. 2011), and that the prevalence of larger cell sizes in marine diatoms is linked to differences in nutrient pulse frequency and mixed layer depth (Litchman et al. 2009). Our results further demonstrate how selection shapes physiology in different environments by examining an additional axis of environmental variation.

Latitudinal differences in temperature regime were the strongest driver of trait variation, suggesting that selection has played a dominant role in determining trait variation. Optimum,

Tmax, and Tmin all declined strongly with latitude across functional groups and in both environments (Figures 3.2-3.4). Within some groups, optimum and Tmax changed by more than 20°C across the full gradient, in contrast to other studies suggesting that Tmax is strongly phylogenetically conserved (Araújo et al. 2013). The decline in Tmin across latitude was much smaller, which may indicate either weaker selection on this trait or the lack of a physiological cost to having a low Tmin. Our results also show that these traits are strongly correlated with each other. Notably, optimum is more strongly correlated with Tmax (r=0.88) than with Tmin (r=0.65), possibly lending support to the hypothesis that Tmin is an evolutionarily labile trait, even if it does not experience strong selection (Figures A2.5 and A2.6 in Appendix 2, Araújo et al. 2013). These trait correlations, when combined with strong environmental correlations (environments with high mean temperatures also have high maximum and minimum) preclude any possibility of separating out the identifying whether selection acts independently on these traits or whether they are driven by physiological correlations; both processes may play a role. These three traits were weakly correlated with niche width, which was not strongly driven by latitude, most taxa having niche widths in the 20°-30°C range (Figures 3.4, A2.6 in Appendix 2), including those from relatively constant environments. However, only a fraction of the taxa in our dataset were measured across a sufficient temperature range to estimate this trait, and so we may be limited by a lack of data. Examination of the best-represented group in our dataset, the marine diatoms, shows strong evidence of a peak in niche width at temperate latitudes, with declines of up to 10°C on either side (Figure 3.5C). This is consistent with the climate variability hypothesis, which proposes that species living at high latitudes must have broader environmental tolerances in order to survive the greater level of environmental variability (Stevens 1989).

Latitude also plays a weak but distinct role in explaining variation in maximum growth rate. We tested contrasting hypotheses for how this trait would vary across latitude and found evidence that growth rates in all groups are highest in the tropics (Figure 3.6), providing support for the notion that low temperatures limit growth rates at higher latitudes.

Functional groups showed large differences in all traits as well as their trait-environment relationships, indicating that evolutionary constraints or niche partitioning have shaped these patterns globally. Our results also suggest that the impression of high temperature-adapted cyanobacteria and cold-adapted diatoms (Paerl & Huisman 2009; Robarts & Zohary 1987; Shatwell et al. 2008) may be an oversimplification and illustrate the importance of incorporating spatial gradients when comparing traits under selection. In the case of optimum temperature, all groups possess similar values in the tropics but exhibit a pronounced divergence with latitude (Figure 3.2). Diatom optima are indistinguishable from other groups in the tropics, but are lower than cyanobacteria and greens at higher latitudes. Groups also show differences in rates of Tmax and Tmin decline with latitude; cyanobacteria exhibit the smallest change in traits across latitude of the major groups in all cases (Figures 3.3 and 3.4).

These different patterns in the group trait-environment relationships may be driven by two possible causes, which are not mutually exclusive: 1) evolutionary constraints in the ability to adapt to low temperatures among the cyanobacteria, as indicated by the very small change in their traits with latitude, and 2) convergent evolution of temperature traits in the tropics and partitioning of the temperature niche at high latitudes. Some evidence for the first explanation may be found in the predominance of benthic polar cyanobacteria with high optimum temperatures. Tang et al. (1997) measured thermal reaction norms in 27 polar cyanobacteria and found that all possessed optima between 15°C and 35°C. Nadeau & Castenholz (2000) found that

the majority of 30 polar strains had optima >20°C, though they found optima as low as 8°C in a few. Since benthic species may face different temperature environments and selection pressures, measurements on high-latitude planktonic taxa will be needed to test this. Alternately, stable thermal environments in the tropics may drive convergence to a single best temperature strategy, while strongly seasonal temperate environments might permit co-existence of multiple temperature strategies. Tropical oceans typically experience approximately 5°C in annual temperature variation (Figure A2.2 in Appendix 2, Reynolds et al. 2007), making it likely that taxa will be selected upon to possess similar optima and Tmax. As further evidence for this proposition, the differences in optima of the major groups at high latitudes reflects patterns of temperate seasonal succession (Alvain et al. 2008; Reynolds 2006; Sommer et al. 1986). Our results therefore suggest that trait divergence at temperate latitudes has occurred by partitioning the temperature niche, possibly as a secondary effect of other constraints such as seasonality in nutrient or light environment. Groups may have originally experienced different abundance peaks because of differences in nutrient requirements (diatoms perform well in pulsed environments) or ability to survive in stratified waters (cyanobacteria are less dense), which would have then led to adaptation to the temperatures correlated with those particular conditions. These different explanations lead to very different predictions for the effects of community re-organization as a result of climate change. While diatoms have been thought to be vulnerable to the direct effects of warming, their temperature traits in the tropics indicate that as a group, they will be able tolerate higher temperatures. This may entail species turnover within the group or evolutionary adaptation, however. This has large implications for models of aquatic ecosystem productivity and carbon sequestration, as diatoms are disproportionately responsible for these in phytoplankton communities (Field et al. 1998; Nelson et al. 1995).

Functional groups were also the major determinant of niche width (Figure 3.5) and maximum growth rate (Figure 3.6). Differences between group niche widths were not large, however, with the marine cyanobacteria and freshwater desmids being the only groups that differed strongly from the rest. Maximum growth rates differed very strongly between groups (Figure 3.6), as has been found in earlier trait compilations (Edwards et al. 2012). Diatoms are the fastest-growing group, while desmids, dinoflagellates, chrysophytes and raphidophytes are among the slowest growing groups. These differences in growth rate distributions reflect differences in competitive strategy and influence their occurrence and abundance patterns. To take one example, diatoms flourish in fluctuating nutrient environments, where their high growth rates allow them to make rapid use of nutrient pulses.

Our work points towards the need for ecologists to consider how traits are shaped by both selection and evolutionary constraint in order to predict how communities will reassemble in changing environments. In order to understand and predict community change, we will need a better understanding of variation in trait-environmental relationships within a community (Litchman et al. 2012). Studies examining spatial patterns in temperature traits have already highlighted the vulnerability of tropical organisms to environmental warming in both terrestrial and marine environments (Dillon et al. 2010; Sunday et al. 2012; Tewksbury et al. 2008; Thomas et al. 2012). Future efforts could advance the goals of understanding community assembly and predicting community change by considering these physiological findings in the context of ecological interactions and evolution. Parametrizing predictive models will require data gathered over broad ranges of environmental conditions to capture evolutionary constraints or nonlinear trade-offs. Measurements at the most extreme portions of a group's range (as defined by environmental parameters or parameter combinations) will therefore be most valuable. In the

phytoplankton, more work is needed to measure the physiological traits of polar cyanobacteria and tropical diatoms, as well as the understudied picoeukaryotes that play a large role in global primary productivity (Liu et al. 2009; Vaulot et al. 2008; Worden et al. 2004). Our study shows how we can use measurements of trait-environment relationships such as these to elucidate how environmental variation, evolutionary history, and selection interact to determine physiological patterns, thereby informing predictions of future ecological and evolutionary change.

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LITERATURE CITED

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CHAPTER 4

INTERACTIVE EFFECTS OF TEMPERATURE, NITROGEN AVAILABILITY, AND TOXICITY ON THE GROWTH OF INVASIVE AND NATIVE CYANOBACTERIA

ABSTRACT

Rising temperatures are expected to favor the growth of cyanobacteria in temperate lakes, increasing the frequency of harmful algal blooms. However, warming may change the composition of cyanobacterial communities by favoring species adapted to higher temperatures. In order to predict future community and bloom dynamics, it is important to understand how different bloom-forming species respond to temperature. *Cylindrospermopsis raciborskii* is a toxic nitrogen-fixer that may benefit from lake warming. Having recently invaded temperate waters from the subtropics, it may be adapted to higher temperatures than native cyanobacteria, implying that warming should increase its growth rate relative to natives. We compared the effect of temperature on the growth of three *C. raciborskii* strains to four strains of *Microcystis aeruginosa*, a bloom-forming native cyanobacterium with which it may compete for resources in temperate lakes. Because temperature interacts with nitrogen availability to affect growth, we also compared the effects of temperature on growth in nitrogen-free conditions in *C. raciborskii* to one strain of *Anabaena flos-aquae*, another nitrogen-fixer. All three *C. raciborskii* strains had optimum temperatures that were higher than toxic *M. aeruginosa* strains, but lower than the single non-toxic *M. aeruginosa* strain and *A. flos-aquae*. Higher temperatures provided *C. raciborskii* with a dramatic growth advantage over *M. aeruginosa*, at least over the toxic strains we tested, indicating that warming could favor its proliferation. Differences between *C. raciborskii* strains were consistent with adaptation to different N. American temperature regimes, suggesting that local adaptation may have played a role in its recent spread. Nitrogen-deprivation

reduced growth rates of *C. raciborskii* and *A. flos-aquae* at all temperatures, but did not drive any consistent trend across temperatures. Additionally, we found suggestive evidence that toxic strains of cyanobacteria respond to temperature differently than non-toxic strains. Specifically, we show that in both *M. aeruginosa* and *C. raciborskii,* the normalized growth rates of toxic strains are higher than those of non-toxic strains below the optimum temperature, while the converse is true above it.

INTRODUCTION

Global environmental change has led to rising temperatures, which are a major source of stress in natural environments, having already affected most ecosystems on Earth (IPCC Fourth Assessment Report 2007). Other stressors such as changing nutrient deposition rates and the spread of invasive species interact with increasing temperatures, making predicting ecosystem responses difficult (Vitousek et al. 2002; Walther et al. 2009). In aquatic ecosystems, one of the major predicted consequences of warmer temperatures is an increase in frequency and severity of HABs (harmful algal blooms), which in lakes are caused mostly by toxic cyanobacteria (Paerl & Huisman 2009). These blooms can release toxins in high enough concentrations to pose a threat to human health, and may be harmful to algae, zooplankton, and fish, thereby having a negative impact on water quality and ecosystem functioning (Chorus & Bartram 1999). Rising temperatures may stimulate growth of toxic HAB species directly, because cyanobacteria are believed to have higher optimum temperatures for growth than other groups of algae (Robarts & Zohary 1987; Tilman & Kiesling 1984; but see Lürling et al. 2013) and indirectly through increased thermal stratification, as cyanobacteria can regulate their buoyancy and take advantage of the high stability of the water column (Jöhnk et al. 2008; Paerl & Huisman 2009).

Lake warming may stimulate growth not only of native species but also invasive cyanobacteria. These have the potential to alter community structure and dynamics in lakes as well as biogeochemical cycling (Litchman 2010). One such species is *Cylindrospermopsis raciborskii*, a nitrogen-fixing toxic cyanobacterium spreading in temperate regions across the world (Padisák 1997). Once restricted to the tropics and subtropics, it has increasingly been found in temperate regions, most recently in Europe and North America (Conroy et al. 2007; Hong et al. 2006; Kling 2009). It possesses a number of traits that likely make it an excellent competitor in lakes, including nitrogen fixation, low-light tolerance, buoyancy regulation and strong competitive ability for phosphorus (Istvánovics et al. 2000; Padisák 1997) which is thought to be atypical for nitrogen-fixers (Smith 1983). It produces a variety of toxins, some which have been shown to be allelopathic (Figueredo et al. 2007), while others have been implicated in human poisoning and cattle mortality events (Saker & Griffiths 2000). The reasons behind *C. raciborskii*'s recent appearance in temperate water bodies are as yet unclear, though climate change has been implicated (Briand et al. 2004; Wiedner et al. 2007). However, it is not clear whether rising temperatures will give it an advantage in competition with native species, including other HAB-forming cyanobacteria already adapted to local conditions. The effects of temperature on the growth of *C. raciborskii* and its native competitors are therefore factors that could determine its invasiveness in temperate regions.

One way to characterize the ability to compete with different species is to examine growth rates under different environmental conditions. Differences in species' growth rates at different temperatures have been experimentally shown to predict the outcomes of cyanobacterial competition (Chu et al. 2007; Fujimoto et al. 1997). *C. raciborskii*'s response to temperature has been examined in strains from Australia, Europe, Asia, Africa, and South

America (Briand et al. 2004; Chonudomkul et al. 2004; Mehnert et al. 2010; Saker & Griffiths 2000). However, despite its recent invasion into N. America and its potential to disrupt local lake ecosystems, little is known about the physiology of N. American strains of *C. raciborskii*, especially compared to its local competitors. Measurements of the temperature-related traits may help us predict the future pattern of invasion and possible ecosystem changes in temperate North American lakes.

To address this, we examined the effect of temperature on the growth rates of three N. American strains of *C. raciborskii*, two from Florida (subtropical) and one from Indiana (temperate). Since the species appears to have spread north from Brazil (Dyble et al. 2002), we also looked for differences in strain response consistent with local adaptation to new temperature regimes. Invasion into new environments is contingent on a species' ability to compete with natives, such as the common bloom-forming native cyanobacterium, *Microcystis aeruginosa*. Therefore, we compared the performance of *C. raciborskii* across temperatures with that of four strains of *M. aeruginosa*. Though *M. aeruginosa* is not a nitrogen-fixer and therefore not expected to compete strongly with *C. raciborskii* under highly N-limited conditions, the two species do co-occur and are in competition for other resources, such as light and phosphorus (Conroy et al. 2007; Kormas et al. 2011). Furthermore, *C. raciborskii* appears to be displacing *M. aeruginosa* in some tropical and subtropical lakes (Chapman & Schelske 1997; Saker & Griffiths 2001), indicating that this competition may be ecologically important in temperate lakes. We observed almost monospecific blooms of *C. raciborskii* in a lake in Michigan (Litchman *et al.*, unpublished data), a region that commonly experience blooms of *M. aeruginosa*.

Performance of *C. raciborskii* in lakes will also be affected by nitrogen concentration, as nitrogen-fixers are favored under N-limited conditions (Smith 1983). However, nitrogen fixation

requires an investment of cell resources (in production of enzymes, etc.) and, therefore, there is likely to be a fitness cost of N-fixation that may vary with temperature. At very high or low temperatures, cells might divert resources to protective machinery such as heat and cold shock proteins, potentially increasing the cost of N-fixation, thereby changing the shape of the temperature-growth curve. To understand how N-availability will affect *C. raciborskii*, we examined growth response to temperature under both N-replete and N-free conditions to determine if N-deprivation exacts a similar cost in nitrogen-fixers across temperatures, and if it had a consistent effect on maximum growth rate, optimum temperature for growth and temperature niche width. For comparison, we also estimated growth rates under N-free and N-replete conditions in a common HAB-forming N-fixer, *Anabaena flos-aquae*.

The public health consequences of HABs have made the ecology of cyanobacterial toxicity an important field of investigation, particularly given the predicted increase in their frequency. Though toxins have been shown to affect both grazers and competitors (Figueredo et al. 2007; Kearns & Hunter 2001; Leflaive & Ten-Hage 2007; Nogueira et al. 2004), it has been hypothesized that these are secondary to a more basic physiological role. Various hypotheses for their primary function include chelation of limiting nutrients such as metals, acting as exogenous stores of nutrients in the extracellular matrix, detoxification of other compounds, or attractants for bacterial symbionts (Paerl & Millie 1996). However, the existence of non-toxic strains of many toxic species suggests that their role is not vital under all conditions. This, along with the complexity of many toxin molecules also suggests that there may be a cost to investment in toxin production. This would lead to a trade-off between toxicity and other physiological parameters, which could have important implications for the success and spread of a species. Determining the nature of this trade-off and the axes involved may therefore help us predict how toxic the

blooms will be. Given current predictions for temperature increase, it is particularly important to determine if there is trade-off involving response to temperature. We examined the effect of strain toxicity on growth response to temperature in the case of *C. raciborskii* as well as *M. aeruginosa*. The former was done by re-analyzing previously published data on strains from Australia (Saker & Griffiths 2000). The effect of toxicity on *M. aeruginosa* was examined in this study, albeit weakly, as only one of the four strains measured did not produce toxins.

METHODS

Strains used:

We tested three strains of *Cylindrospermopsis raciborskii* (Indiana Lake Lemon, Florida D and Florida E, hereafter referred to as IN, FL-D, and FL-E respectively), four strains of *Microcystis aeruginosa* (Gull B-00, Gull K-00, Bear AC-02, Bear AG-02) and a single strain of *Anabaena flos-aquae* UTEX 1444 for growth responses to temperature. The three *C. raciborskii* strains and *M. aeruginosa* Bear AC-02 do not produce toxins, while the remaining three *M. aeruginosa* strains (Gull B-00, Gull K-00, Bear AG-02) and *A. flos-aquae* UTEX 1444 produce microcystin. The *C. raciborskii* and *M. aeruginosa* strains are recent isolates, increasing the likelihood that their growth responses are reflective of performance in natural environments. The *A. flos-aquae* strain has been maintained in laboratory culture since 1967, making it highly likely that adaptation to laboratory conditions has altered its physiology; we therefore do not compare *A. flos-aquae* with the other two species except in the context of nitrogen fixation.

Florida *C. raciborskii* strains were obtained from Dr. Julianne Dyble-Bressie, NOAA (isolated from Lake Dora, Florida) and the Indiana strain was obtained from Dr. Carole Lembi, Purdue University (isolated from Lake Lemon, IN). *M. aeruginosa* strains were obtained from Dr.

Alan Wilson, Auburn University and isolated from Gull Lake and Bear Lake in Michigan. *A. flos-aquae* UTEX 1444 was obtained from the UTEX Culture Collection of Algae.

Culture conditions

Non-axenic cultures of every strain were grown in autoclaved 250 ml conical flasks containing approximately 100 ml WC medium (Guillard 1975). Separate cultures of *A. flos-aquae* and the three *C. raciborskii* strains were maintained in N-replete (1 mmol N L^{-1}) and N-free WC medium, bringing the total number of cultures to 12. Each culture was maintained in a growth chamber at 20°C under cool white fluorescent lights (EcoLux 20W). All growth chambers used during the experiment were set to a 14:10 light/dark cycle, with a light intensity of approximately 100 μmol photons m^{-2} s^{-1}. This has been shown to be saturating for most phytoplankton species (Litchman 2000) and is consistent with past data on these species (e.g. Briand et al. 2004). Cultures were shaken every day by hand and diluted regularly to keep them in exponential growth phase.

Experiment

To measure the temperature response curves of all the strains in our study, we measured their population growth rates at six temperatures after acclimation to these conditions. Growth rates were estimated from measurements of chlorophyll-a fluorescence (excitation wavelength: 436 nm, emission wavelength: 680 nm) in 24-well microplates over 5 days using a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA). Before the experiment, we tested the efficacy of this method by showing that chlorophyll-a fluorescence correlated strongly with cell

density for all three species above a fluorescence value of 1 (relative fluorescence units, RFU), though the chlorophyll content per cell/colony differed between species.

Cultures were allowed to acclimate for a minimum of three days in growth chambers maintained at a light level of 100 μmol photons m^{-2} s^{-1} and 6 different temperatures (15°, 20°, 25°, 30°, 35° and 40° C). We began the assay by diluting the cultures to between 1 and 2 RFU in Greiner Bio-One CELLSTAR 24-well microplates (Monroe, NC). Each culture was transferred to two microwells on each of two microplates at every temperature (four replicates for every treatment combination). The microplates were then returned to the growth chambers and chlorophyll-a fluorescence was measured every 24 hours for five days. Before each measurement, microplates were agitated by the microplate reader to ensure that settling did not skew the results. Each well was divided into a 3x3 grid and 20 fluorescence measurements were made at each point, with the mean of all 180 measurements being used for further calculations. The microplates were returned to the growth chambers immediately after the measurements.

Calculation of specific growth rate

For each well, the linear regression of log-fluorescence against day was examined visually, and data points from the end of the growth period were removed if log-fluorescence plateaued before the end of the assay (i.e. culture was no longer experiencing exponential growth). This occasionally occurred when a culture became extremely dense or sparse, at which point it had either exhausted its nutrient supply or was beyond the range in which the instrument registered a linear relationship between chlorophyll fluorescence and biomass. The slope of the resulting regression was treated as the specific growth rate (day^{-1}) of the well. The initial cell

densities used appear to be too low for accurate measurement of negative population growth rates, as fluorescence levels quickly dropped below the lower detection limit for several cultures at 15°, 35°, and 40°C. Therefore, we have less confidence in these measurements than in those involving positive growth. Moreover, due to the rapid decline to below the detection limit, negative growth rate estimates are likely to be underestimates (i.e. the actual rates may be more negative). As this may be a source of bias, when growth rates were negative at both 35° and 40°C, the 40° measurements were excluded from further calculations of temperature traits and from the figures.

All growth rate measurements from our experiments are included in the supporting information.

Secondary analysis of published growth rate data

Growth rate data of seven other strains of *C. raciborskii* were extracted from the figures of Saker & Griffiths (2000) using the program g3data v1.5.1 (Frantz & Novak 2000).

Calculation of optimum temperature for growth:

The optimum temperature for growth of each strain was determined as in Thomas et al. (2012 and Boyd et al. (2013), according to the equation:

$$f(T) = ae^{bT}\left[1 - \left(\frac{T-z}{w/2}\right)^2\right] \quad (1)$$

where specific growth rate f depends on temperature, T, as well as parameters z, w, a, and b. w is the temperature niche width, while the other three possess no explicit biological meaning. We fit

(1) to the growth data for each strain using maximum likelihood to obtain estimates for parameters *z*, *w*, *a* and *b* (Table 4.1). We also estimated two further traits of interest - the optimum temperature for growth and maximum growth rate - by numerically maximizing the equation after estimating the parameter values. For each culture, equation (1) was fit to the data from all temperatures, except where growth was negative at both 35° and 40°. In these cases, the data for 40° degrees were omitted when determining the optimum temperature.

We also estimated confidence intervals on the optimum temperatures using a parametric bootstrapping approach. For each strain, we fitted (1) to the growth rate measurements and extracted the residuals from this fit. We then performed 1000 residual bootstraps, a procedure in which the residuals are randomly 'reassigned' to predicted values (each of which corresponds to a growth rate measurement) and added to them, thereby generating a slightly different thermal reaction norm. During each iteration, we refitted the function and re-estimated the reaction norm parameters (*z, w, a, b*) as well as the derived traits, maximum growth rate and optimum temperature for growth. Examining the distribution of these parameters and traits over the 1000 bootstraps allows us to quantify the uncertainty in our estimates, which we can then use to generate 95% confidence intervals and examine differences between strains.

Comparison of temperature response of strains with different toxicities

In order to visualize how strain toxicity affects temperature response in two different species, we calculated normalized growth rates (raw growth rate at each temperature divided by the maximum growth rate of the same strain) for every strain.

Data analysis was performed using R 2.15.2 (R Core Team 2012).

RESULTS

Growth in N-replete medium

The three species exhibited clear differences in their thermal niches. The growth rates of all three *C. raciborskii* strains were positive between 20° and 35° (Figure 4.1), while *A. flos-aquae* was able to grow from 15° to 40°. *M. aeruginosa* showed differences in temperature response between strains: Gull B-00 and Gull K-00 grew between 15° and 30°, Bear AG-02 grew only between 20° and 30°, while the non-toxic Bear AC-02 was similar to *C. raciborskii*, growing between 15° and 35°. The optimum temperatures for growth and maximum growth rate (estimated from the curve fits) also varied considerably between species and strains. Optimum temperatures of the *C. raciborskii* strains ranged between 29.8° and 32.5° (Figure 4.2 and Table 4.1), with the northern IN strain having the lowest optimum of the three, as well as the highest growth rates at 20° and 25° (Figure 4.1). *A. flos-aquae* had the highest optimum of all strains measured, of 36.4° (Figure 4.2). The three toxic strains of *M. aeruginosa* possessed very similar temperature curves and have optima between 28° and 29°, while the non-toxic Bear AC-02 has its optimum at 34° (Figure 4.2). Maximum growth rate differed between species and strains as well, ranging from 0.55 - 0.67 day^{-1} in *C. raciborskii*, 0.29 - 0.62 day^{-1} in *M. aeruginosa* and 1.48 day^{-1} in *A. flos-aquae* (Table 4.1). The growth rates obtained in this study agree well with previously measured rates for the same strains of *M. aeruginosa* (Wilson et al. 2006), confirming that the microplate-based method works well.

Effects of N-deprivation at different temperatures

Growth in N-free medium reduced the growth rates of *C. raciborskii* and *A. flos-aquae* at most temperatures, by as much as 0.4 d^{-1} (Figures 4.3, 4.4). However, there were considerable

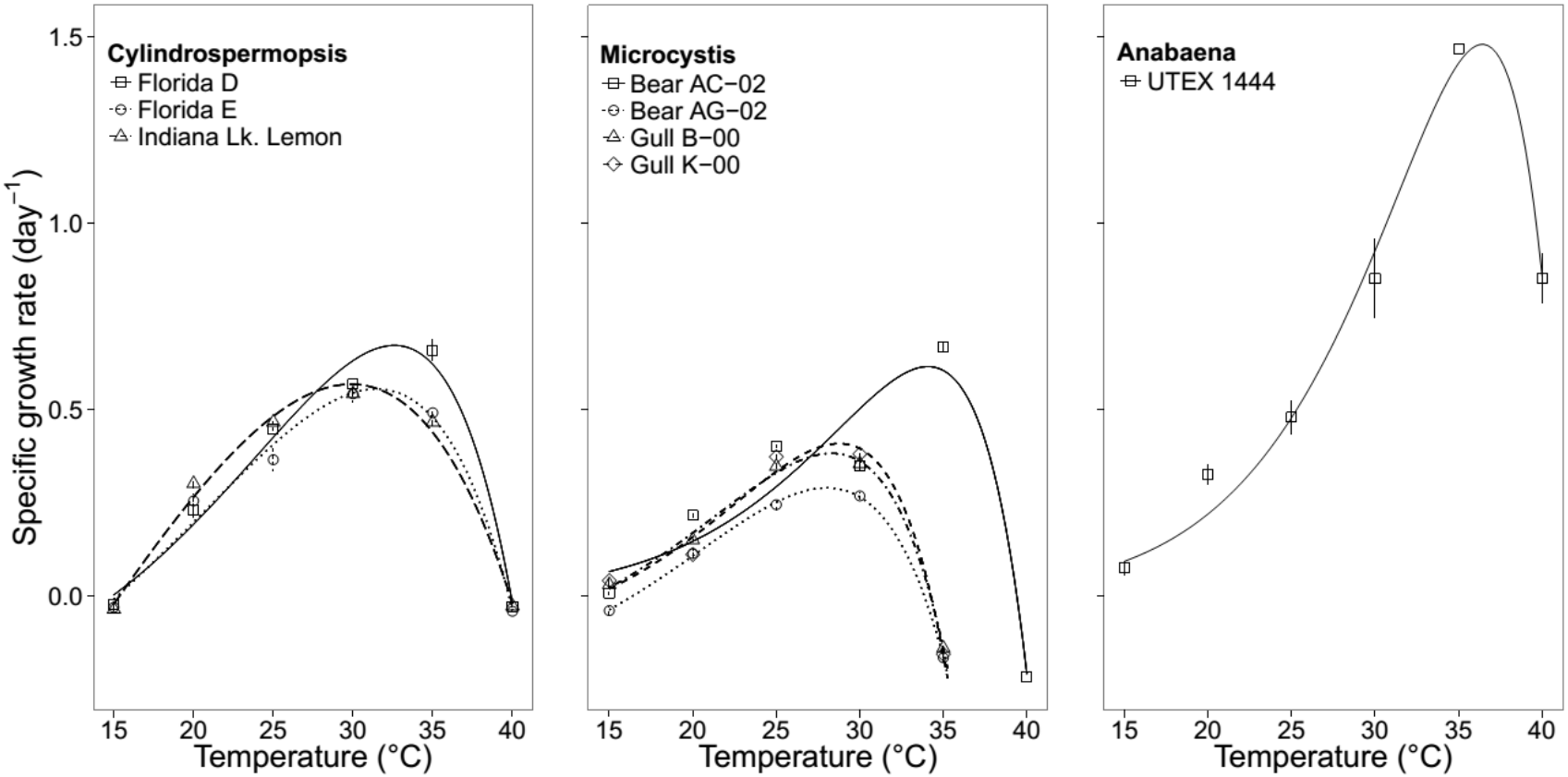

Figure 4.1. Specific growth rates (day^{-1}) of *C. raciborskii*, *M. aeruginosa* and *A. flos-aquae* between 15° and 40°C, as well as curve fits to the data based on equation (1). Error bars indicate standard errors from four replicates.

Species	Strain	Nitrogen added	*z*	*w*	*a*	*b*	Optimum temperature (°C)	Maximum growth rate (day^{-1})
Anabaena	UTEX 1444	Yes	14.09	54.65	0.23	0.03	36.4	1.48
Anabaena	UTEX 1444	No	15.37	52.59	0.02	0.11	35.9	1.07
Cylindrospermopsis	IN	Yes	27.60	24.53	0.06	0.08	29.9	0.57
Cylindrospermopsis	IN	No	27.74	22.77	0.19	0.03	33.2	0.52
Cylindrospermopsis	FL-D	Yes	27.39	25.05	0.10	0.06	32.6	0.67
Cylindrospermopsis	FL-D	No	28.16	20.88	0.06	0.06	29.6	0.41
Cylindrospermopsis	FL-E	Yes	27.49	24.60	0.01	0.18	31.5	0.56
Cylindrospermopsis	FL-E	No	28.35	22.00	0.01	0.15	31.6	0.34
Microcystis	Gull B-00	Yes	24.11	20.27	0.03	0.10	28.3	0.38
Microcystis	Gull K-00	Yes	24.13	20.29	0.02	0.11	28.8	0.41
Microcystis	Bear AC-02	Yes	16.29	46.24	0.01	0.16	34.1	0.62
Microcystis	Bear AG-02	Yes	25.11	17.52	0.03	0.09	28.0	0.29

Table 4.1. Fitted thermal tolerance curve parameters from equation (1) and derived traits estimated from the fits.

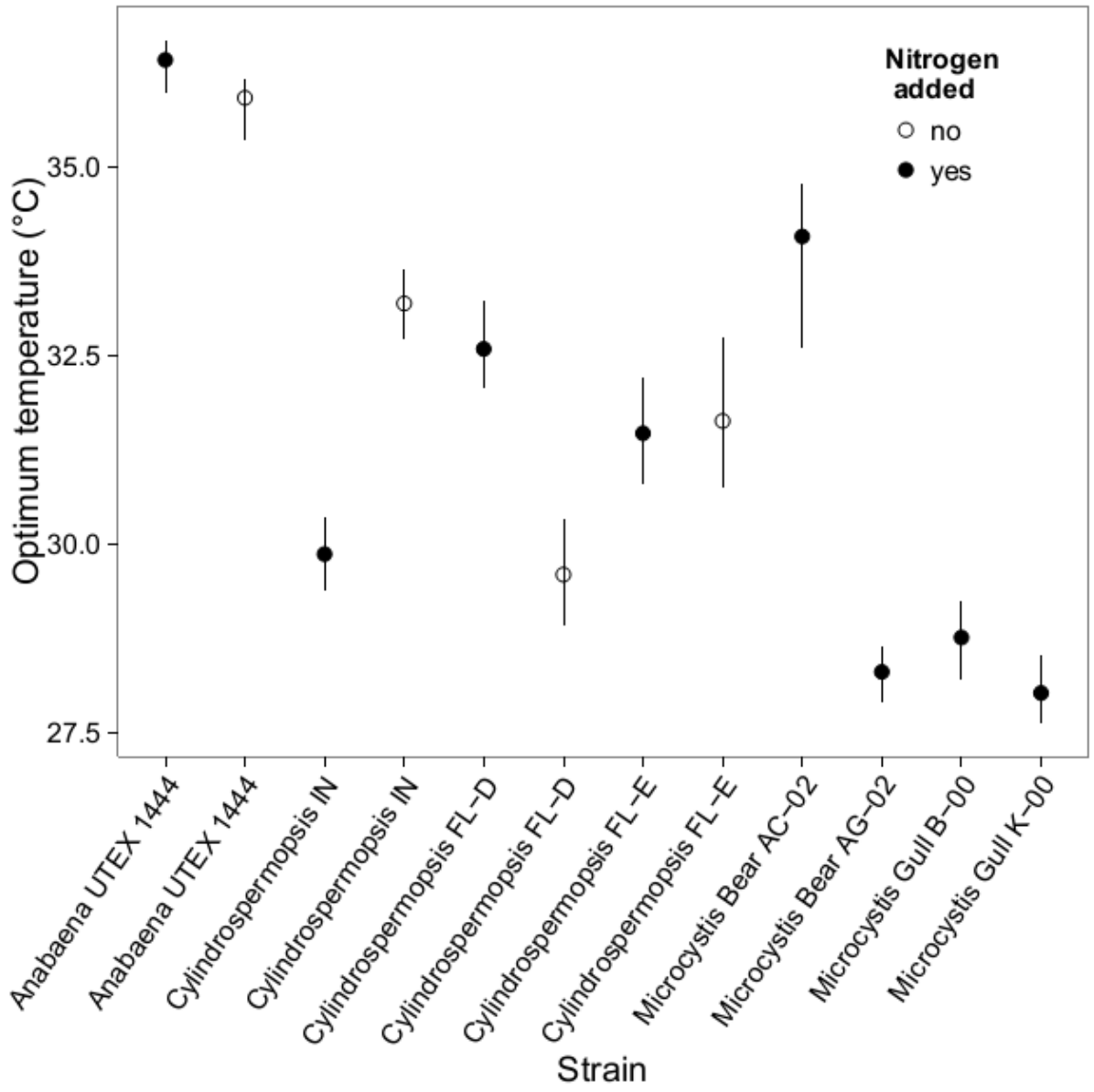

Figure 4.2. Optimum temperatures for growth (°C) of all strains. Optima of N-fixers are shown in both N-replete and N-free media. Error bars represent 95% confidence intervals estimated by parametric bootstrapping.

differences in its effects across strains and temperatures. *C. raciborskii* FL-E experienced little to no reduction in growth rate at 20° and 25°, while all other strains experienced small decreases ranging from 0.1 to 0.25 day^{-1}. All strains experienced similar reductions of 0.1 day^{-1} at 30°. The largest difference occurred at 35°, with *C. raciborskii* FL-D experiencing no detectable

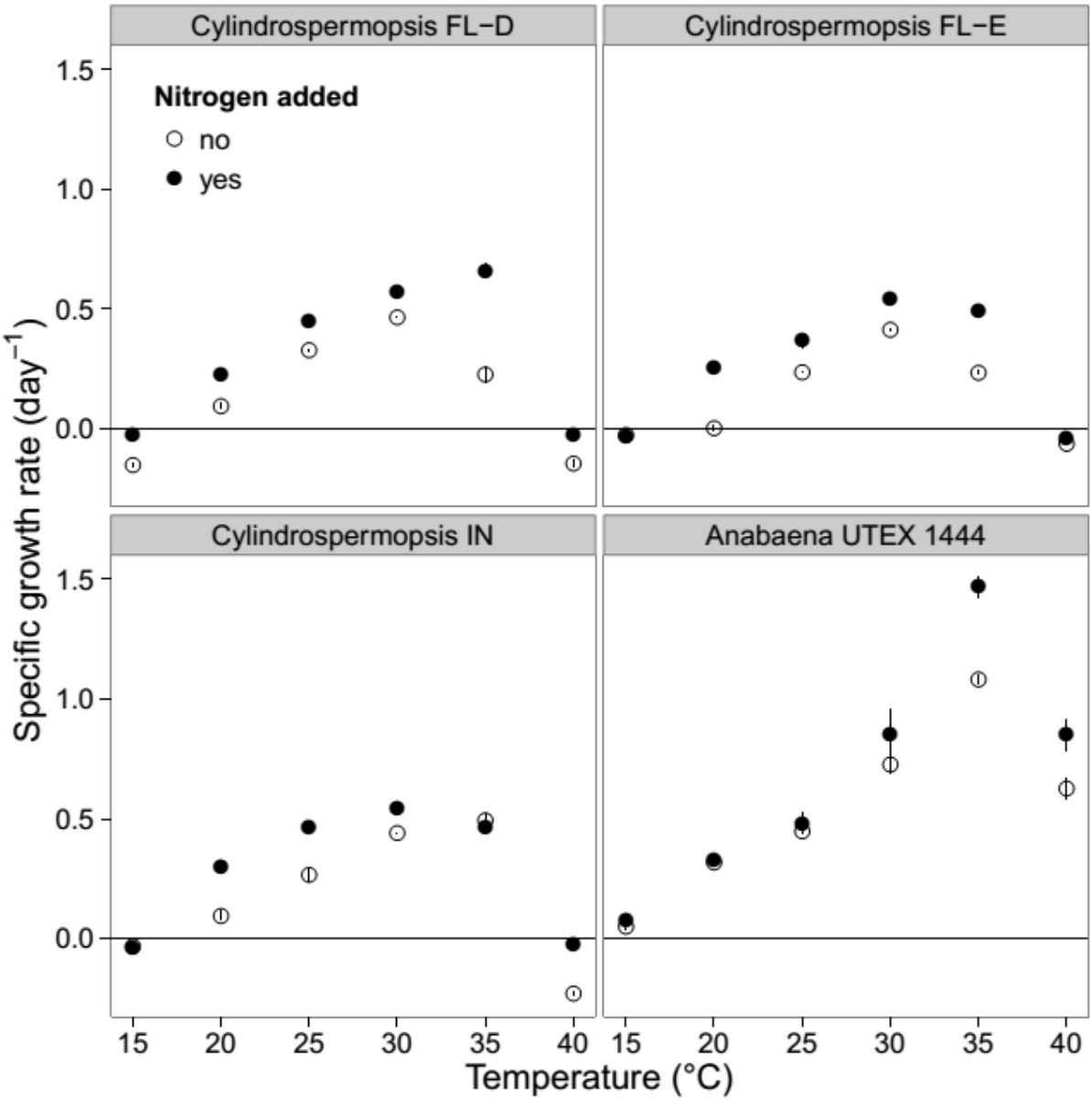

Figure 4.3 Growth rates of N-fixers under N-replete (filled points) and N-free (hollow points) conditions at all temperatures. Error bars indicate the standard error of the mean.

reduction in growth while the other three strains experienced reductions of 0.25 to 0.4 day^{-1}. Differences occurred at 15° and 40° as well, but since we have less confidence in negative growth rate measurements, we do not draw conclusions from them. N-deprivation did not have a consistent effect on the optimum temperature for growth. There was no detectable difference in

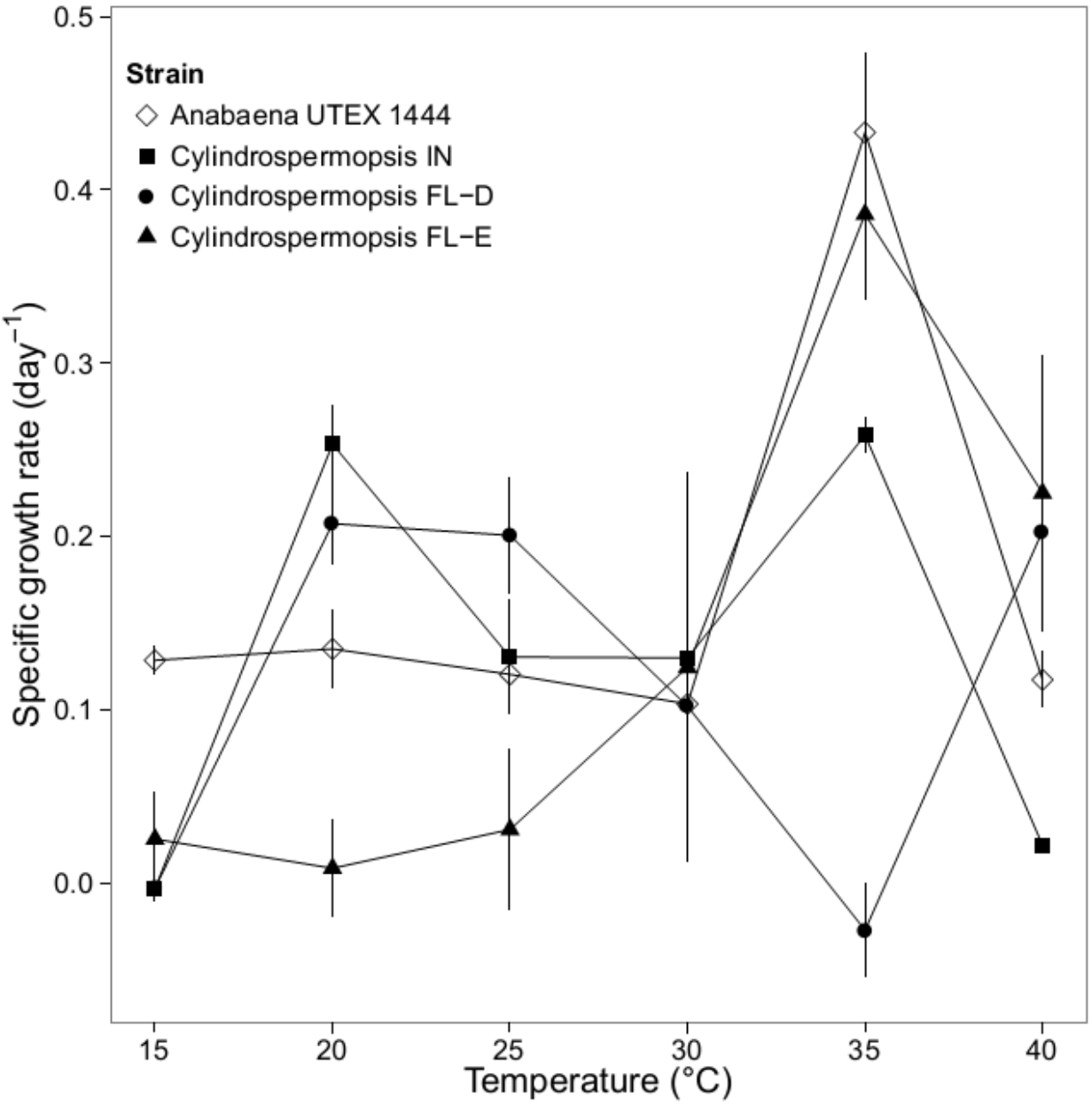

Figure 4.4. The difference in specific growth rate (day^{-1}) of N-fixers between N-replete and N-free conditions at all temperatures. Error bars indicate standard error of the difference between growth rates.

the case of *A. flos-aquae* and *C. raciborskii* FL-E, while it increased by 3° in the case of *C. raciborskii* IN and decreased by a similar amount for *C. raciborskii* FL-D (Figure 4.2).

Toxicity

Our secondary analysis of growth data from Saker and Griffiths (2000) suggests that toxic and non-toxic *C. raciborskii* strains respond differently to temperature (Figure 4.5A). Additionally, in our measurements of *M. aeruginosa*, we find that the non-toxic strain exhibited a different response than the toxic strains (Figure 4.5B). In both studies, the normalized growth rates (specific growth rate divided by the maximum specific growth rate) of toxic strains appear to be higher below the optimum temperature than in non-toxic strains. Non-toxic strains appear to exhibit higher normalized growth rates than toxic ones above the optimum temperature.

DISCUSSION

Cyanobacteria are believed to have higher optimum temperatures for growth than other groups of phytoplankton (Robarts & Zohary 1987), though a recent study has shown that chlorophytes may also possess similarly high optima (Lürling et al. 2013). The predominance of cyanobacteria when lakes are at their warmest is therefore likely due to a combination of high temperature optima and traits that are beneficial under stratified conditions, such as buoyancy regulation (Huisman et al. 2004; Paerl & Huisman 2009). As a number of cyanobacterial species are capable of buoyancy regulation including the three species considered in this study (Padisák 1997; Reynolds et al. 1987), differences in temperature response and nutrient competitive abilities may be more important in determining the outcomes of competition between them. Differences in temperature response have been shown to successfully predict the outcomes of competition in cyanobacteria in experiments (Chu et al. 2007) as well as in the field, especially in combination with N:P supply ratio and the species' nutrient response (Fujimoto et al. 1997).

The optimum temperatures of the 3 species tested in this study were high and within the range reported for cyanobacteria previously: between 28° and 37°C, with the *C. raciborskii*

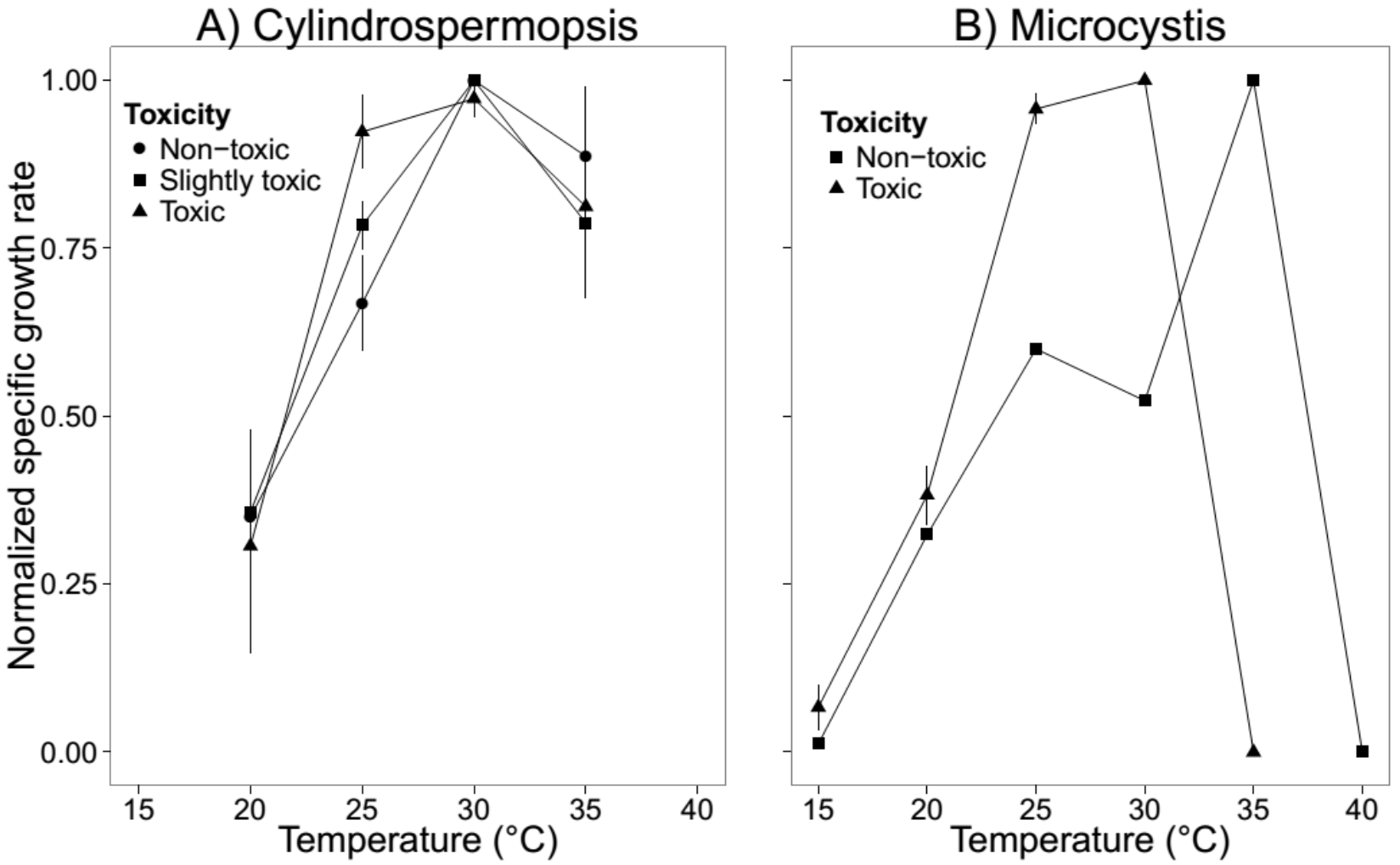

Figure 4.5 Mean normalized growth rates of toxic and non-toxic cyanobacterial strains at different temperatures. Raw growth rates were divided by the maximum growth rate of the same strain, and then the normalized growth rates were averaged within toxic, non-toxic and slightly toxic strains. (A) Normalized growth rates of three toxic strains, two slightly toxic strains, and two non-toxic strains of *C. raciborskii*, data from Saker and Griffiths (2000). (B) Normalized growth rates of three toxic *M. aeruginosa* strains and one non-toxic strain, from this study.

strains ranging from 30° to 32.5° in N-replete medium. Briand et al. (2004) estimated optima between 29° and 31° for other strains of this species using a different model, but as they measured growth rates under lower light conditions, this difference may not reflect true biological differences. *Anabaena flos-aquae* exhibited the highest optimum temperature of 36°, within the 27° to 39° range of estimates for this species (Novak & Brune 1985; Uehlinger 1981). The three toxic *Microcystis aeruginosa* strains exhibited optima around 28°, while the non-toxic strain Bear AC-02 possessed an optimum of 34°. These measurements bookend the range of estimates from earlier studies, which are between 30° and 32° (Imai et al. 2009; Nalewajko & Murphy 2001). Some of these optima are higher than the temperatures these species are likely to experience in their natural environments, a pattern that has been observed in earlier studies of phytoplankton and other taxa (Barker 1935; Karentz & Smayda 1984; Kingsolver 2009; Thomas et al. 2012). These are likely to be adaptive responses to environmental temperature variation, given the physiological constraints that these phytoplankton experience (i.e. an exponential increase in maximum growth rate with temperature and skewness of thermal tolerance curves). An eco-evolutionary model of phytoplankton growth in the ocean found that the best strategy under typical patterns of temperature variation was to have an optimum several degrees above the mean temperature (Thomas et al. 2012). Additionally, variability in the temperature environment may select for higher optima, as growth rates decrease dramatically above the optimum temperature (Martin & Huey 2008). Our findings support the high temperature preference for cyanobacteria over other groups of algae (Reynolds 2006), which implies that rising lake temperatures will promote cyanobacterial dominance (Kosten et al. 2012; Paerl & Huisman 2009).

Growth responses to temperature of multiple strains of *C. raciborskii* have been

measured previously, with maximum growth rates ranging from 0.3 to 1.3 day^{-1} when grown on nitrate (Briand et al. 2004; Mehnert et al. 2010; Saker & Griffiths 2000; Shafik et al. 2001). This places the N. American strains (0.54 to 0.66 day^{-1}, Figure 4.1) at the lower end of the range. One major difference is that all three strains of *C. raciborskii* died at 15° and 40° in our study, while Briand et al. (2004) measured growth in multiple strains at 15° and a few at 40° as well. However, differences in experimental conditions between the studies mean that this does not necessarily reflect strain differences, as Briand et al. (2004) used a lower irradiance level (30 - 50 μmol photons.m^{-2}.s^{-1}) than we did (100 μmol photons.m^{-2}.s^{-1}). We chose the higher irradiance as it is approximately the optimum light intensity at intermediate temperatures, as determined by Briand et al. (2004) and Shafik et al. (2001) at 25° and 27°, close to the optimum temperature for growth. As irradiance level has been shown to alter the response to temperature (Dauta et al. 1990), the inability of the North American *C. raciborskii* strains to survive at the extreme temperatures may be attributable to the different light environments used in the two studies.

Climate change and C. raciborskii

Our data suggest that climate change is likely to favor the invasive *Cylindrospermopsis raciborskii* over the native temperate cyanobacterium *Microcystis aeruginosa* in temperate North America. *M. aeruginosa* strains had higher growth rates at 15°C, but between 20° and 35°, the three *C. raciborskii* strains had higher growth rates on average. The non-toxic *M. aeruginosa* strain Bear AC-02 experienced comparable growth rates at 20° and 25°, but at 30° *C. raciborskii* grew more than 50% faster. The performance of a cyanobacterial species in the 20° - 30° range may be a useful indicator of future success and invasibility in temperate regions, because phytoplankton communities are frequently dominated by cyanobacteria at these temperatures,

and intermediate-sized lakes are expected to spend a greater proportion of the year in this temperature range in future (de Stasio et al. 1996; Magnuson et al. 1997). This invasion may alter lake ecosystems and communities through a variety of pathways – changes in nitrogen supply (as a result of N-fixation), changes in phosphorus concentration (as *C. raciborskii* is an excellent phosphorus competitor), changes in the light environment (due to its shade tolerance), altered zooplankton community abundance and composition (as a result of changes in toxin load and type) (Istvánovics et al. 2000; Padisák 1997). Each of these changes alters the selective environment and may lead to both ecological and evolutionary changes in the local community (Litchman et al. 2010). The outcomes of competition between these species in lakes will depend on other factors as well, including nutrient and light response, and predictions for specific water bodies and regions will need to take these factors into account.

Though *Anabaena flos-aquae* grew as fast as or faster than all the *C. raciborskii* and *M. aeruginosa* strains at every temperature measured, we do not discuss this species in detail except in the context of the cost of nitrogen fixation, as it has been maintained in laboratory cultures since 1967. Therefore, adaptation to laboratory conditions has likely rendered it less representative of the species as laboratory cultures typically select for a copiotrophic lifestyle, leading to important changes in physiology and genome architecture (Swan et al. 2013). However, its temperature response does inform our understanding of the constraints on adaptation to high temperatures under highly favorable growth conditions. Furthermore, physiological trade-offs involving growth under N-limiting conditions will be preserved, although specific parameters will likely change. If the temperature response has not changed significantly since its isolation, our results would lead us to predict that warming will facilitate the invasion of subtropical *A. flos-aquae* strains in temperate lakes.

Adaptation to environmental conditions

The growth curves of the three *C. raciborskii* strains display differences that are consistent with adaptation to different temperature regimes. Temperatures below 15°-17° have been shown to inhibit growth in German lakes (Wiedner et al. 2007), indicating that low temperatures may pose a selective pressure in temperate regions. Consistent with this, the northern strain IN grew faster at 20° than either of the southern strains, while the southern strains grew faster at 35° (Figure 4.1, $p<0.05$). Strain IN also has the lowest estimated optimum temperature of the three under N-replete conditions (Figure 4.2). N-limitation, however, produced different results (Figures 4.3, 4.4). At 20°, strains IN and FL-D grew at similar rates, at 25° and 30° strain FL-D grew fastest, while at 35° strain IN grew nearly twice as fast as the FL strains. As a result, and contrary to our expectations, the estimated optimum temperature is highest for strain IN in N-free medium (Figure 4.2). This unexpected result may be the result of a lack of selection by temperature on enzymes specifically produced under N-limited conditions, such as N-fixation and stress-protection machinery. If the environments from which these strains were isolated did not experience N-limited conditions, we would not expect these enzymes to be evolutionarily optimized for performance at high temperature. Alternatively, differences in the nitrogen environment between the places of origin could explain this pattern as well.

Effects of N-deficiency

N-deficiency showed inconsistent effects on the growth of nitrogen-fixers. It did not change the range of temperatures in which growth was positive, though more finely-spaced measurements, from 15°-20° and 35°-40° may have shown differences. It did alter optimum temperatures for growth, although not in a predictable manner (Figure 4.2 and Table 4.1). Growth rates in N-free medium were lower in most cases, but there was considerable variation

across strains and temperatures (Figures 4.3, 4.4). *A. flos-aquae* did not appear to be strongly affected at temperatures below 30°. On the other hand, strains of *C. raciborskii* had their growth rates reduced by N-deficiency at most temperatures where growth was positive, with the exceptions of strain IN at 35° and to a lesser extent, strain FL-E at 20° and 25°. In the other strains tested, N-deficiency appeared to have its strongest effect on growth rate at 35°.

Toxicity

In our measurements as well as those of Saker & Griffiths (2000) (Figures 4.5 A, B), we see that toxic strains achieve a higher proportion of their maximum growth rate at lower temperatures and also experience a sharper decline in growth rate above 30°. These data are insufficient for a statistical comparison and so need to be interpreted with caution, but are suggestive of a relationship between strain toxicity and temperature response. In the case of *C. raciborskii*, slightly toxic strains (those that secrete quantities of toxin orders of magnitude below other toxic strains) show responses that are intermediate between toxic and non-toxic strains, which is further evidence for this relationship (Figure 4.5 A). Furthermore, of the fours strains of *M. aeruginosa* we tested, the single non-toxic strain (Bear AC-02) exhibited the highest growth rates at 20°, 25°, 30° and 35°, the highest maximum growth rate and highest optimum temperature. In the *C. raciborskii* measurements of Saker & Griffiths (2000), the highest maximum growth rates were measured in slightly toxic and non-toxic strains. This suggests that there may be a trade-off between toxicity and maximum growth rate or high temperature tolerance. We acknowledge that this is limited evidence but hope that future studies will address this question, as this has important implications for the occurrence of HABs in the future. If toxic strains dominate at lower temperatures and lose their advantage over non-toxic strains around 30°, it is possible that climate change could lead to an increase in cyanobacterial bloom

frequency but reduce their toxicity, contrary to expectations from studies that did not elevate temperatures beyond the optimum (Davis et al. 2009).

In conclusion, our study indicates that warming of temperate lakes is likely to favor *C. raciborskii* over the native *M. aeruginosa*, due to the *C. raciborskii*'s higher growth rates at warmer temperatures. *C. raciborskii* may be evolutionary plastic in its temperature response, having possibly adapted to cooler Indiana temperatures. N-deprivation has generally negative though highly variable effects on its growth, making it difficult to predict how nutrients and temperature will interact to affect its performance. Finally, warmer temperatures may stimulate the growth of non-toxic cyanobacterial strains even more than toxic ones, complicating predictions of increased HAB frequency with climate change. However, more studies will be needed to establish this and to improve our ability to forecast future phytoplankton community composition and HAB frequency in temperate lakes.

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CHAPTER 5

INTERACTIVE EFFECTS OF TEMPERATURE AND NUTRIENT CONCENTRATION ON THE GROWTH RATES OF PHYTOPLANKTON

ABSTRACT

Temperature and nutrients are among the most important determinants of productivity globally, but we currently know little about how they interact to determine growth in autotrophs. This lack of understanding limits our ability to predict population growth rates, species ranges, competitive outcomes, community dynamics and productivity in natural environments. Here we propose and test a model that characterizes this interaction in the following manner: i) growth rate is a balance between birth and death rates, ii) both birth and death are exponential functions of temperature, leading to the characteristic left-skewed shape of the thermal reaction norm for certain parameter combinations, and iii) birth rates are also saturating functions of nutrient concentration, but death rates are independent of it. This model makes the novel prediction that optimum temperature for growth is a saturating function of nutrient concentration. New experiments with a phytoplanktonic species, *Thalassiosira pseudonana*, and re-analysis of published experimental data confirm this prediction. These findings imply that studies may underestimate species' vulnerability to environmental warming by failing to account for nutrient dynamics. Our model provides a theoretical foundation with which to explore how interacting abiotic factors influence population and community dynamics.

INTRODUCTION

Modeling population dynamics in complex environments is a fundamental ecological challenge with important practical applications. Population growth is influenced by both abiotic factors and biotic interactions, of which the former include nutrient availability, temperature,

light, and water availability among autotrophs. These factors limit primary production at a global scale (Behrenfeld et al. 2005; Elser et al. 2007; Enquist et al. 1999; Falkowski et al. 1998; LeBauer & Treseder 2008; Lutz et al. 2007; Tyrrell 1999). Of these, temperature and nutrient availability are highly important in aquatic ecosystems, and both are changing in natural environments (Barnett et al. 2005; Greig et al. 2012; Lyman et al. 2010; Vitousek et al. 1997). As organisms have a minimum nutrient requirement, nutrient availability places an upper limit on primary productivity as a result of inefficiencies in recycling rates; growth also declines under low-nutrient conditions. Metabolic constraints also place limits on the absolute rate of growth and can limit production in environments where nutrients are plentiful, such as seasonal temperate and polar environments. Both these factors have highly nonlinear effects on the growth of individuals and populations (Droop 1973; Kingsolver 2009; Monod 1949). These nonlinearities are well-described by models focusing on individual factors, allowing us to predict how single variables affect population dynamics in simple laboratory environments. However, modeling population and community dynamics in complex natural environments will require us to understand how these and other major drivers interact to affect growth. This is particularly urgent because environmental change is changing multiple abiotic factors simultaneously and altering patterns of covariation between abiotic factors, weakening our ability to forecast future responses based on current conditions (Williams et al. 2007).

Temperature exerts a large effect on the growth of organisms and populations, particularly in the case of ectotherms. Across species, increases in temperature lead to exponential increases in important biological rates, including metabolic, birth, death and growth rates (Enquist et al. 1999; Eppley 1972; Gillooly et al. 2001; Gillooly et al. 2002). Within ectothermic species, the change in growth or activity rates with temperature is unimodal and left-

skewed, a shape that is characterized by thermal reaction norms (also known as thermal fitness curves, thermal tolerance curves or thermal performance curves). This skewness has important biological implications for species performance in natural environments (Kingsolver 2009; Martin & Huey 2008). Most importantly, small increases in temperature above the optimum lead to large declines in fitness, implying that even small amounts of environmental warming could threaten populations adapted to current temperature regimes. Studies exploring the effects of predicted temperature changes this century have found that a number of species will be negatively affected by these changes, particularly in the tropics (Deutsch et al. 2008; Martin & Huey 2008; Sunday et al. 2012; Thomas et al. 2012). Therefore, understanding how species respond to temperature is an important step towards predicting species persistence and community composition in a warming environment.

Nutrients strongly influence growth rates (Monod 1949), and the ability of species to persist under low nutrient concentrations is strongly predictive of competitive outcomes in constant environments (Tilman et al. 1982; Tilman 1977; Tilman 1982). In bacteria, growth was found to be a saturating function of limiting resource availability early in the twentieth century (Monod 1949), and later work showed that this shape also described growth of phytoplankton and plants (Eppley et al. 1969; Tilman & Cowan 1989). When modeling competition between species, the Droop model, which explicitly accounts for changes in cellular nutrient stores, has been shown to be a better predictor of competitive outcomes (Droop 1973; Ducobu et al. 1998; Sommer 1991). However, the parameters of the Droop model are far more complex to measure experimentally. Recent work has also shown that the Monod equation describes growth of single populations in environments with rapidly changing nutrient concentrations with a high degree of

accuracy (Bren et al. 2013). Therefore, it a useful formulation with which to begin explorations of how nutrients and other factors interact to affect population growth.

Though previous studies have considered the effects of temperature and nutrients on phytoplankton (e.g. Geider et al. 1997, 1998; Raven & Geider 1988), most have considered their interactive effects on growth rate below their optimum temperature. As many organisms experience temperatures that exceed their optimum at least occasionally (Huey & Bennett 1990; Huey et al. 2009; Thomas et al. 2012), understanding this interaction across a broader temperature gradient will improve our ability to model growth in natural environments.

MODEL

Effect of temperature on growth rate:

The thermal reaction norm has been described using a variety of descriptive and partially mechanistic equations (Briand et al. 2004; Corkrey et al. 2012; Dell et al. 2011; Mordecai et al. 2012; Norberg 2004; Schoolfield et al. 1981). However, many of these suffer from a lack of interpretability of the parameters, making it difficult to understand how to build on these models to incorporate interactions with other factors. Here we use a formulation based on the exponential relationships between temperature and birth rate as well as mortality rate to characterize growth rates as a function of temperature under saturating nutrient levels (Eppley 1972; McCoy & Gillooly 2008; Savage et al. 2004):

$$\mu(T) = b_1 e^{b_2 T} - (d_0 + d_1 e^{d_2 T}) \quad (1)$$

where specific growth rate μ depends on temperature, T, as well as parameters b_1, b_2, d_0, d_1, and d_2. b_1 may be interpreted as the birth rate at a temperature of 0°, b_2 the exponential change in birth rate with increasing temperature. d_0 is the background mortality rate, while d_1 and d_2

describe the exponential increase in mortality rate with temperature relatively to the baseline at 0°. Therefore, the first half of the equation captures the effect of temperature on birth rates, and the second half captures its effect on mortality rates. For certain parameter combinations, the difference between these two exponential curves captures the left-skewed shape that is typical of thermal reaction norms (eg. Fig 5.1A; Kingsolver 2009). However, alternate interpretations of these parameters are possible, and this may also be interpreted as describing the balance between production and respiration within individuals. These alternate interpretations may be useful in descriptions of the effects of temperature on physiological processes other than growth rate (e.g. sprint speed, Hertz et al. 1983).

Effect of nutrients on growth rate:

We used the Monod equation to describe the effect of nutrients on growth rate at each temperature (Monod 1949):

$$\mu(R) = \mu_{max} \frac{R}{R+K} \quad (2)$$

where specific growth rate μ depends on nutrient concentration, R, as well as the maximum growth rate, μ_{max} and a half-saturation constant, K. This equation captures the saturating relationship between nutrient concentration and growth rate (Figure 5.1B).

Effect of temperature-nutrient interactions on growth rate:

To develop a model predicting how these two factors would interact, we assume that birth rates are nutrient-dependent, while death rates are nutrient-independent. We can therefore replace μ_{max} in equation (2) with the temperature-dependent birth term from equation (1), and incorporate the mortality terms as they are. Uniting equations (1) and (2) in this manner forms a function that describes growth rate as a function of both temperature and nutrient concentrations:

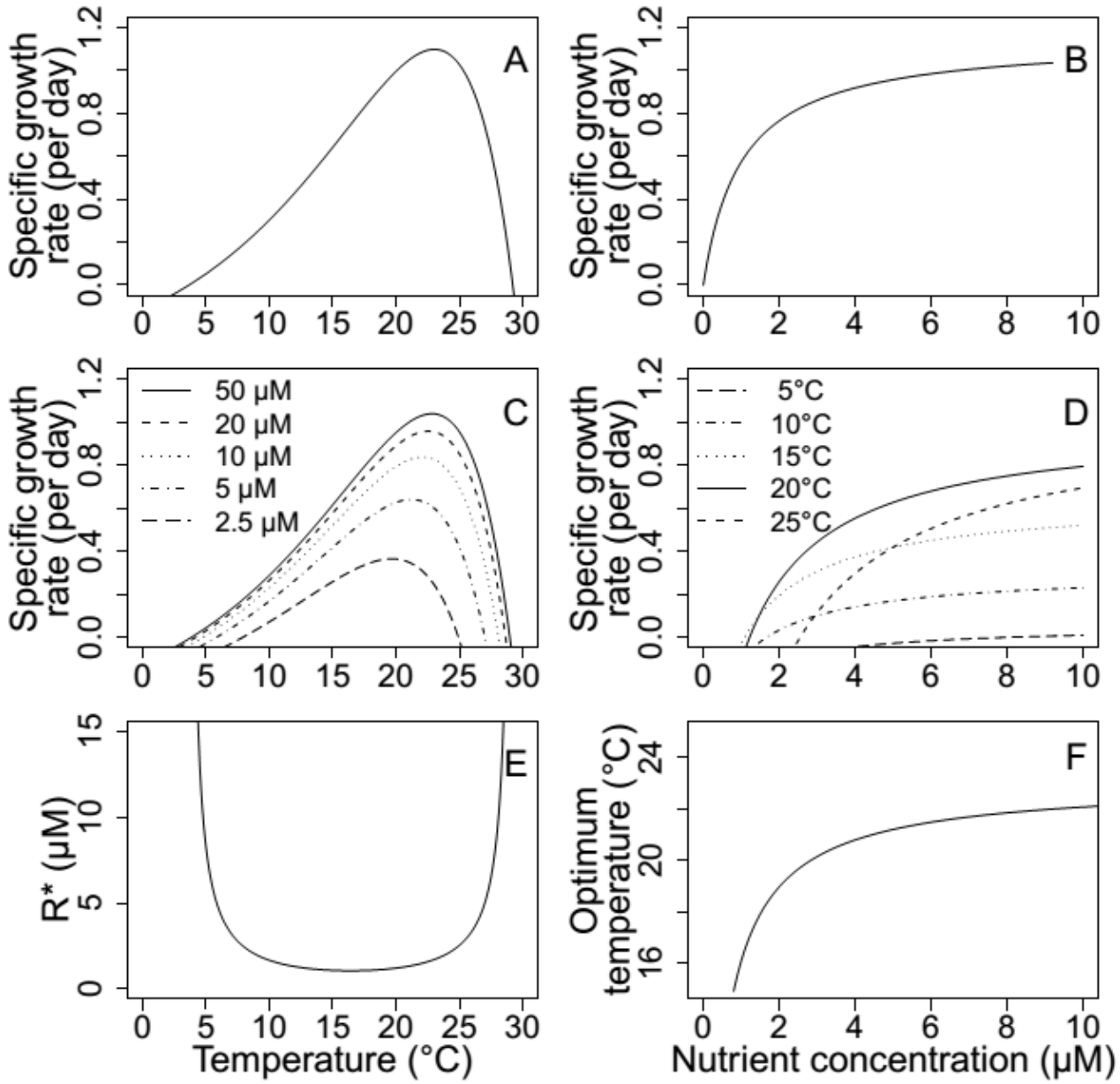


Figure 5.1. The predicted effects of temperature and nutrient interactions. A. Growth rate as a function of temperature, from equation (1). B. Growth rate as a function of nutrient concentration as predicted by the Monod equation. C. Growth rates as a function of temperature at different nutrient concentrations, from equation (3). D. Growth rates as a function of nutrient concentration at different temperatures, from equation (3). E. The minimum nutrient requirement for persistence (R*) in equation (3) is lowest at intermediate temperatures. F. Optimum temperature for growth in equation (3) is a saturating function of nutrient concentration.

$$\mu(T,R) = b_1 e^{b_2 T} \frac{R}{R+K} - (d_0 + d_1 e^{d_2 T}) \quad (3)$$

Mortality terms retain their meaning, but b_1 now refers to the birth rate at a temperature of 0° only under nutrient-replete conditions, due to the nutrient-dependence of birth rate. At nutrient concentrations well above K, this model approximates equation (1); thermal reaction norm shape changes at lower nutrient concentrations.

This model makes novel predictions for important temperature and nutrient traits. Most notably, the optimum temperature for growth, maximum temperature of persistence (the temperature above which population growth rate is negative), and temperature niche width (the range of temperatures over which growth rate is positive) are all predicted to be saturating functions of nutrient concentration (Figures 5.1C, F). Additionally, K is predicted to be lowest in the temperature band where growth rate is highest (Figure 5.1D). R*, the nutrient concentration at which net population growth rate is zero (Tilman 1982), is also lowest at intermediate temperatures (Figure 5.1E).

We performed experiments with a marine diatom, *Thalassiosira pseudonana*, and also re-analyzed previously published data on temperature-nutrient interactions in a freshwater diatom (Tilman et al. 1981) to test the prediction that optimum temperature is a saturating function of nutrient concentration. We did not examine R* and K in our experiments, but we discuss the findings of Tilman et al. (1981) in this regard.

METHODS

Experiment

We measured growth rates in a 5x5 factorial experiment to capture the effect of temperature-phosphorus interactions in *Thalassiosira pseudonana* strain CCMP 1335, obtained

from the Provasoli-Guillard National Center for Marine Algae and Microbiota. The five temperatures (20°, 25°, 27.5°, 30°, 32.5°C) were chosen to span the range that previous experiments had suggested included the optimum temperature and the lethal temperature, and 5 phosphorus concentrations (1, 2.5, 5, 15, and 36.2μM) ranged from concentrations common in natural environments to those commonly used in laboratory experiments.

1. Culture conditions

We grew non-axenic cultures of *T. pseudonana* in autoclaved 125 mL conical flasks containing approximately 50 ml modified ESAW medium (Berges et al. 2001). All glassware and equipment that came in contact with the medium was acid-washed to remove any residue that might cause contamination. Cultures were maintained in a growth chamber at 20°C under cool white fluorescent lights (EcoLux 20W). All growth chambers used during acclimation period and the experiment were set to a 14:10 light/dark cycle, with a light intensity of approximately 100 $\mu E.m^{-2}.s^{-1}$. Before the experiment, all cultures were allowed to acclimate for 2-3 weeks in growth chambers maintained at a light level of 100 $\mu E.m^{-2}.s^{-1}$ and at the experimental temperatures and nutrient concentrations. Cultures were shaken every day by hand and diluted approximately every two days to keep them in exponential growth phase.

2. Growth assays

Experimental assays were carried out in 50 mL conical flasks containing 30 mL of culture. Every 24 hours for 5 days, 2 mL of culture was removed from each flask and the flasks were immediately returned to their growth chambers. These 2 mL subsamples were transferred to individual wells in microwell plates, and we then measured chlorophyll-a fluorescence (excitation wavelength: 436 nm, emission wavelength: 680 nm) using a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA). Microplates were agitated by the

microplate reader before measurements were taken to ensure that the culture was homogeneous. As part of the measurement procedure, each well was divided into a 3x3 grid and 20 fluorescence measurements were made at each point, with the mean of all 180 measurements being used for further calculations.

3. Calculation of specific growth rate

For each culture, we performed linear regressions of log-fluorescence against day number. A visual examination revealed that log-fluorescence plateaued rapidly in low-phosphorus cultures, indicating that they did not experience exponential growth for the full 5 days due to nutrient limitation. Therefore, we only used data points from the first two days of the experiment for all phosphorus concentrations to estimate growth rates. The slope of the resulting regression is the specific growth rate (day^{-1}) of the culture. All growth rate measurements from our experiments are included in the supporting information.

Extraction of published data

We extracted data from Tilman et al. (1981) on the growth of the diatom *Asterionella formosa* across 5 temperatures and a range of silicate concentrations. Data were digitized using the program g3data (Frantz & Novak 2000). Though this paper contained data on an additional species, *Synedra ulna*, temperatures above the optimum temperature of the species were not measured. Therefore, we did not extract or analyze data for this species. We were unable to find other papers with data that met our criteria.

Statistical analyses

1. Fitting equation (3) to the growth data in both species

We used a maximum likelihood approach to estimate the parameter values for equation (1) at each nutrient concentration using the R package bbmle (Bolker & R Core Team 2012). To

fit the model, we assumed that observational error was normally distributed with a variance of σ^2. In addition, we estimated the optimum temperature for growth and maximum growth rate by numerically maximizing the equation after estimating the parameter values.

2. Describing variation in growth rate and estimating optimum temperature for growth

In order to demonstrate that patterns in growth and optimum temperature were not driven by constraints artificially introduced by equation (3), we used generalized additive models (GAMs) to describe variation in growth rates. We did this in two ways: i) we used GAMs with temperature as a smoother term to fit the thermal reaction norms at each phosphate concentration in our experiment. Using this fits, we then estimated the optimum temperature by numerical maximization. ii) We used GAMs with both temperature and nutrient concentration as smoother terms to describe variation in growth rate in both our experiment and the published growth data. Curvature in the GAM-interpolated contours highlight changes in optimum temperature with nutrient concentration.

All analyses were performed using the R statistical environment v. 3.0.2 (R Core Team 2013).

RESULTS

Thalassiosira pseudonana *temperature-phosphate interactions*

Growth rate of *T. pseudonana* was strongly influenced by both phosphorus concentration and temperature, ranging from 0.75 to 1.55 day^{-1}. Equation (3) provided a strong fit to the data with an R^2 of 0.84 (Figure 5.2). In agreement with model predictions, optimum temperature for growth was a saturating function of nutrient concentration, varying by approximately 3.5°C over the range of phosphate concentrations tested (R^2=0.99, Figure 5.3A). GAM fits to growth rates

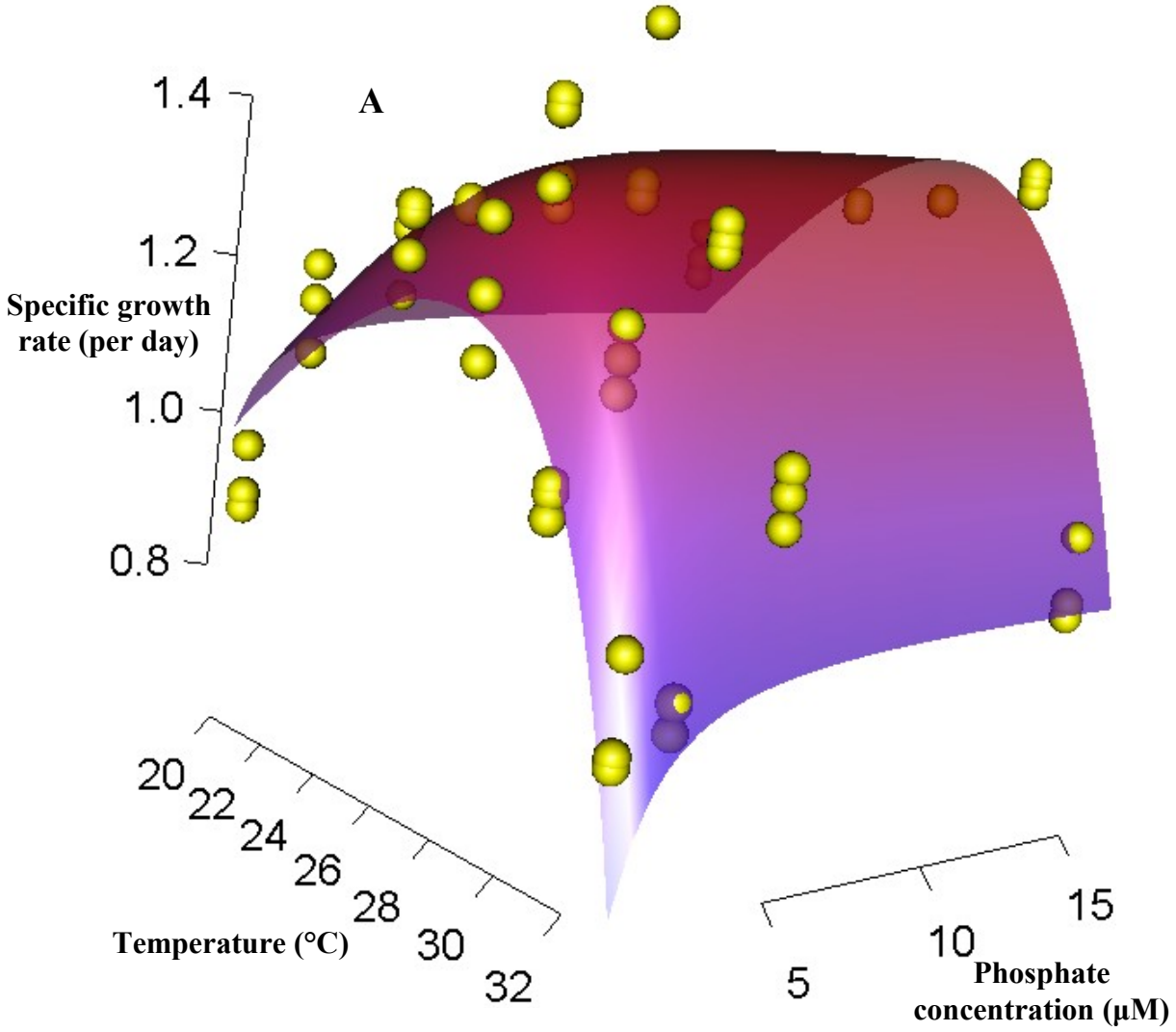
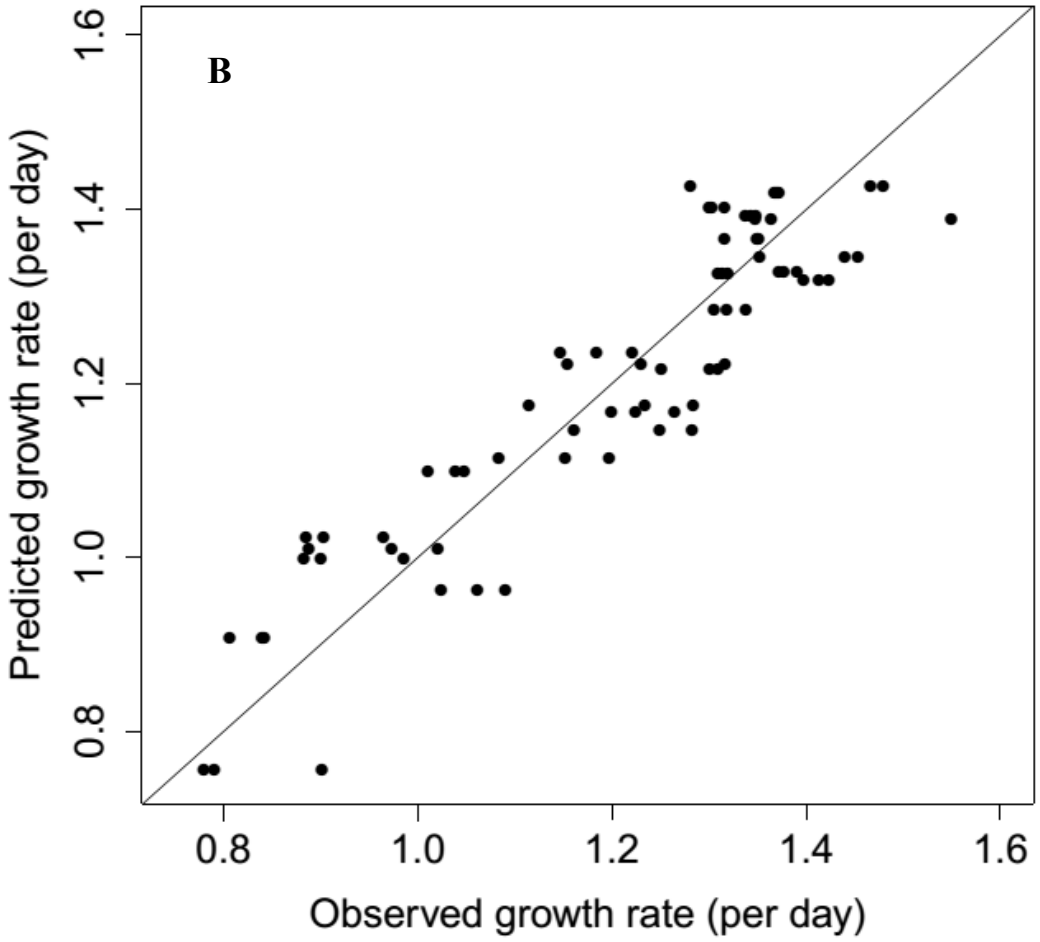


Figure 5.2. Model fits to *T. pseudonana* growth rates at different temperature and phosphate concentrations, compared to measured growth rates. A) Fitted growth rate surface (model R^2=0.84) along with the measured growth rates (yellow spheres). To highlight variation at the lowest phosphate levels, data at the highest phosphate concentration (36.2 µM) are not shown. B) Observed vs. predicted growth rates.

Fig 5.2 (cont'd)



across both temperature and phosphorus gradients also highlighted the decline in optimum temperature at low phosphate concentrations (Figure 5.3B).

The nutrient half-saturation constant for growth also varied strongly across temperatures and was lowest at the temperatures where growth rate was highest, in agreement with our model predictions (Figure 5.4). However, estimates were well below the lowest measured phosphorus concentration, so we do not have high confidence in these estimates.

Asterionella formosa *temperature-silicate interaction*

As silicon concentrations varied between temperature treatments in Tilman et al. (1981), we did not analyze variation in thermal reaction norms at individual nutrient concentrations.

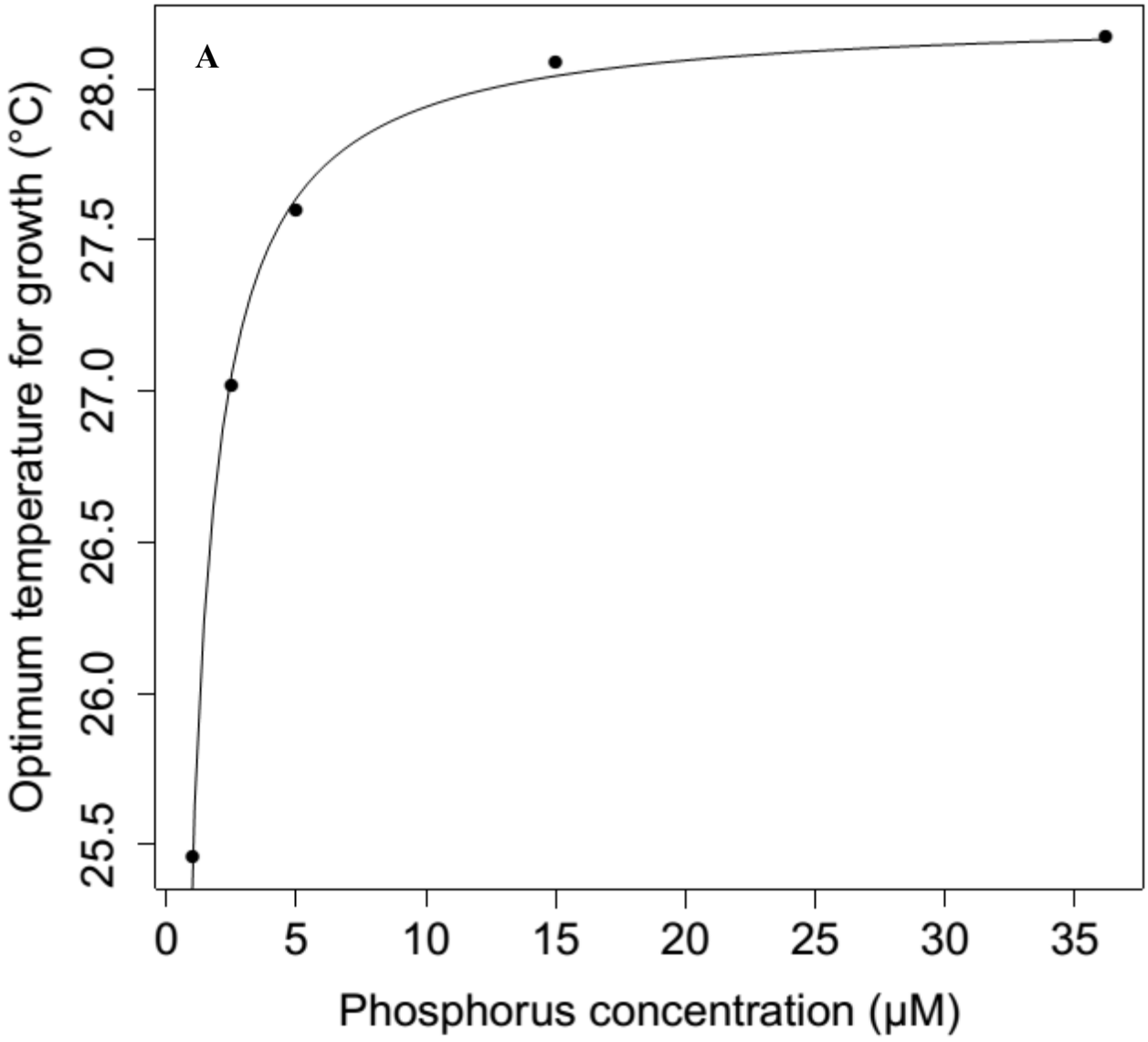
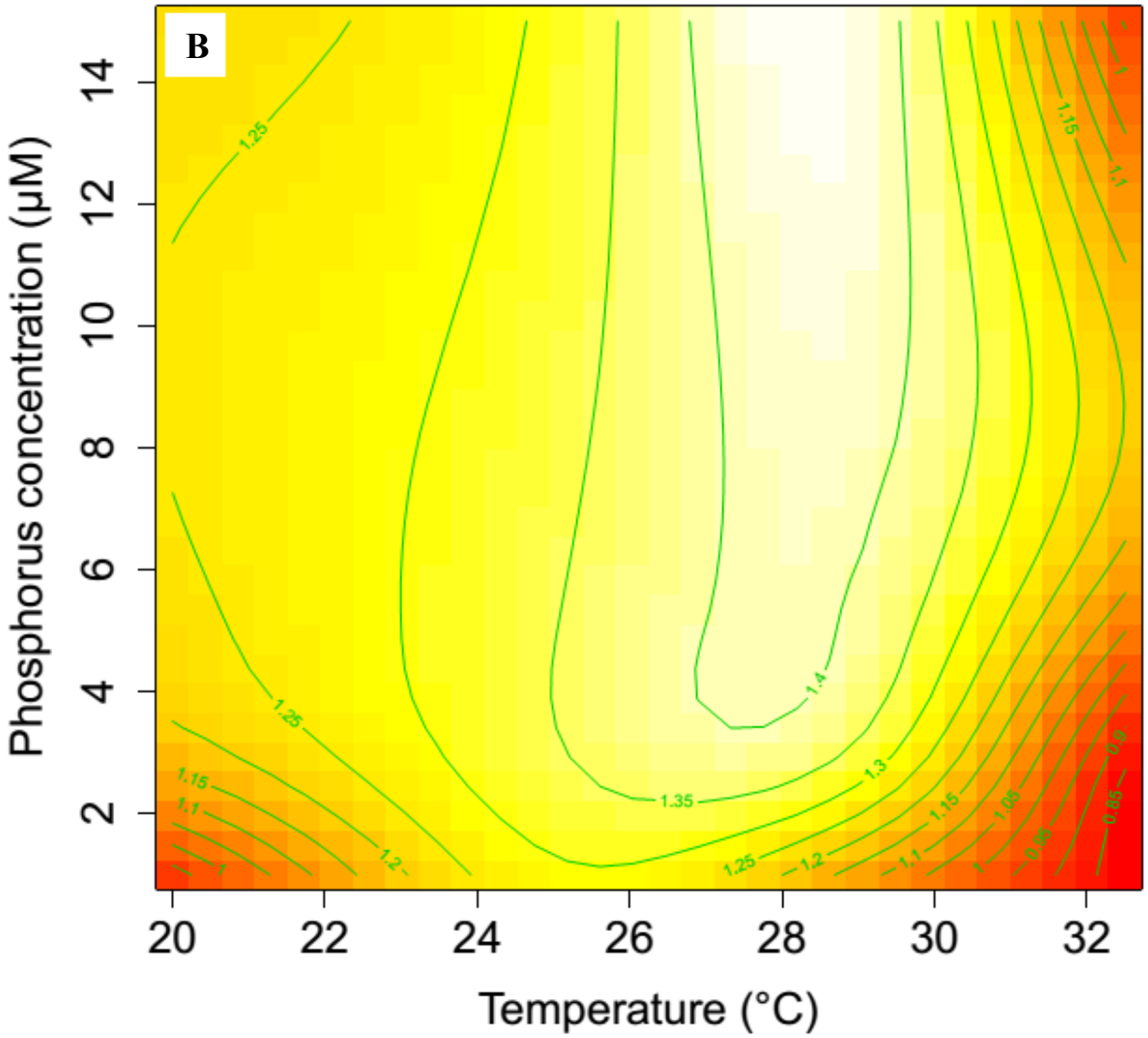


Figure 5.3. Optimum temperature for growth of *T. pseudonana* is a saturating function of phosphorus concentration, as predicted by equation (3). A) Optimum temperature estimated at each phosphorus concentration using GAM fits with temperature as a smoother term. The curve represents a saturating function fit to the points (R^2=0.99). B) Growth rates across temperature and phosphorus gradients, interpolated using a GAM with both temperature and phosphorus as smoother terms. Curvature in the contours showing the highest growth rates indicates the decline in optimum temperature with phosphorus concentration. To highlight variation at the lowest phosphate levels, data above concentrations of 15 µM are not shown.

Fig. 5.3 (cont'd)



Instead, we fit equation (3) to the data, with the model providing an R^2 of 0.80 (Figure 5.5).

GAM fits to growth rates across both temperature and silicate gradients indicate that optimum temperature declines at low silicate concentrations, in agreement with the model predictions (Figure 5.6). Tilman et al. (1981) also found that R* increases at both high and low temperatures, as our model predicts (Figure 5.1E). Contrary to our predictions, however, the same study showed that nutrient half-saturation constant for growth was an exponential function of temperature over the range of conditions tested.

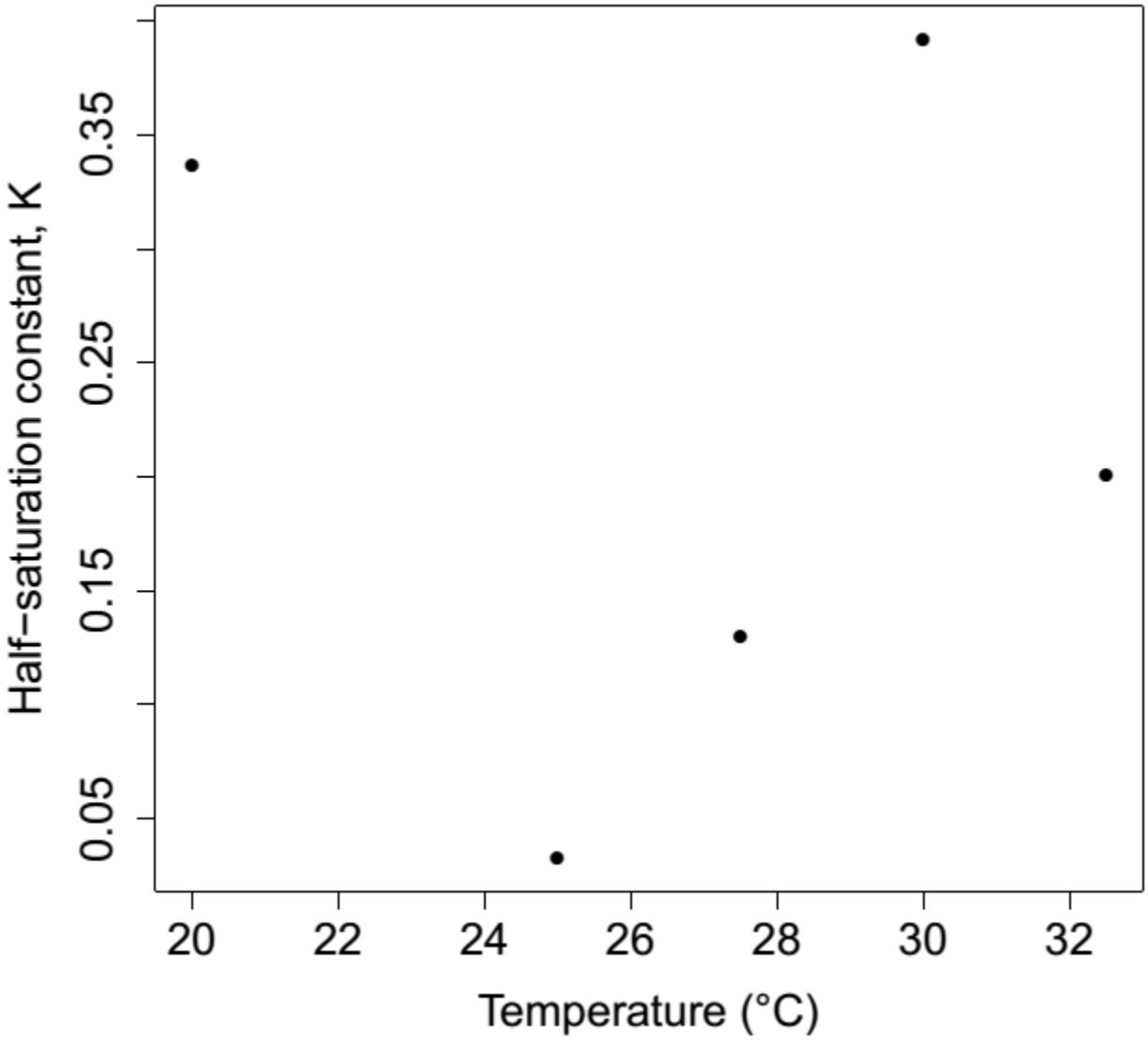


Figure 5.4. Phosphorus half-saturation constant for growth may be lower near the optimum temperature, as predicted by equation (3). However, we have low confidence in these estimates as all are estimated to be well below the range of measured phosphorus concentrations.

DISCUSSION

Though our study focuses on interactions between temperature and nutrients, we have provided a general model framework that allows us to integrate the effects of multiple environmental factors on growth. This enables us to synthesize our understanding of how growth is affected by single parameters into an understanding of how complex environmental variation

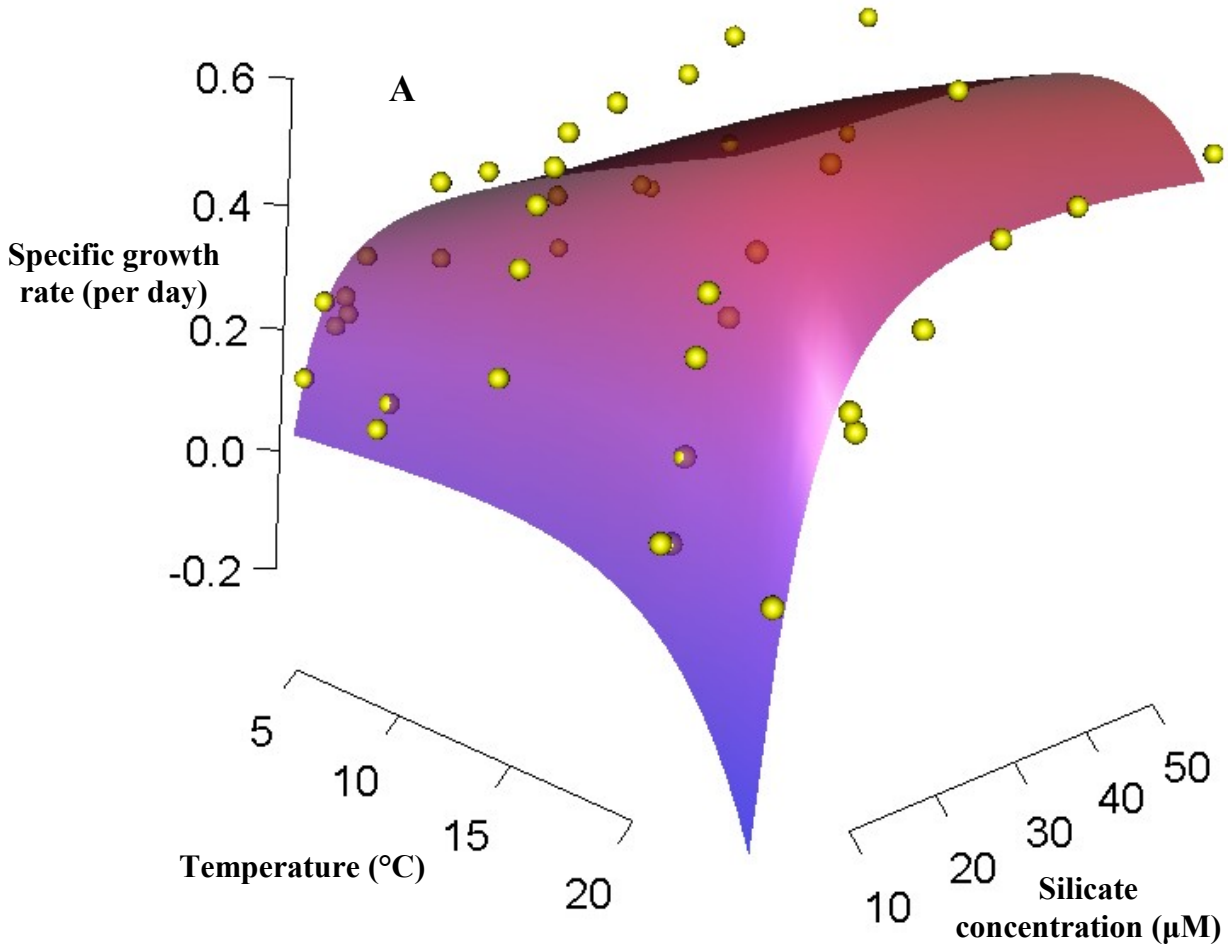
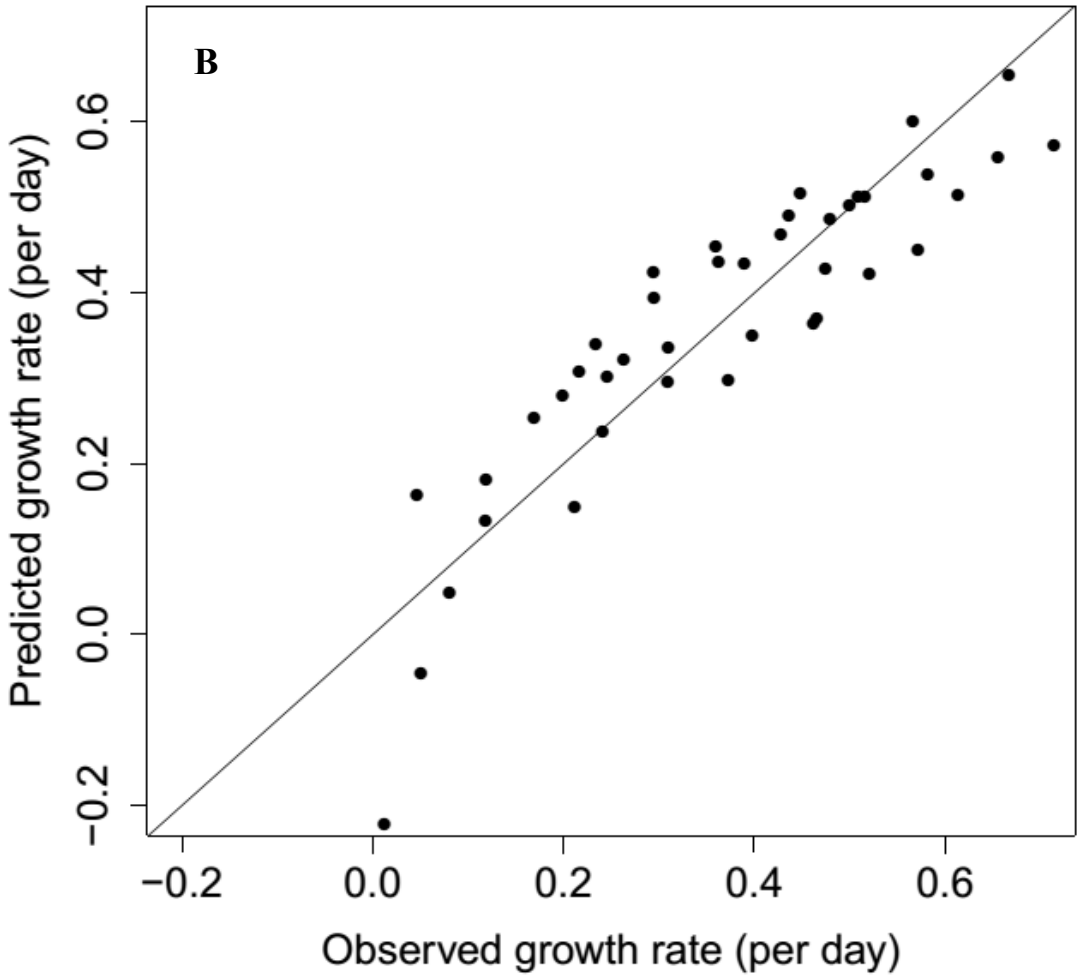


Figure 5.5. Model fits to *A. formosa* growth rates at different temperature and silicate concentrations, compared to measured growth rates. Data are from Tilman et al. (1981). A) Fitted growth rate surface (model R^2=0.80) along with the measured growth rates (yellow spheres). B) Observed vs. predicted growth rates. The highest growth rates may be slightly underestimated.

Fig. 5.5 (cont'd)



affects growth in natural environments. For the environmental factors we studied, this model provides novel, testable, and biologically important predictions.

We find strong support for our primary prediction: that optimum temperature for growth is a saturating function of nutrient concentration (Figures 5.3, 5.6). Moreover, as optimum temperature increased by close to 4°C over the range of concentrations tested, this variation is likely to be biologically relevant. Natural environments experience total phosphorus

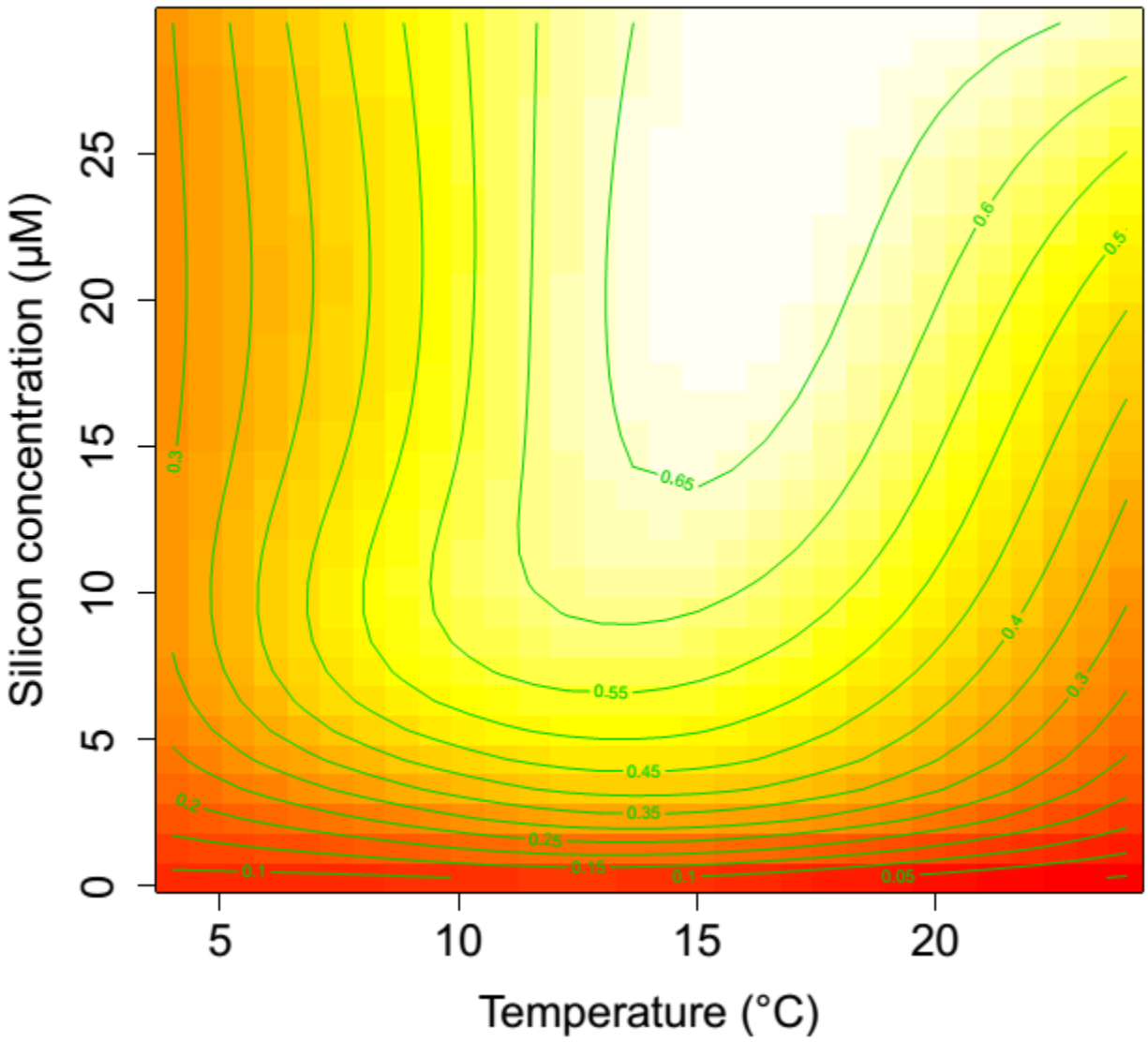


Figure 5.6. Optimum temperature for growth of *A. formosa* is a saturating function of silicon concentration, as predicted by equation (3). Data are from Tilman et al. (1981). As silicate The figure shows predicted growth rates across temperature and silicate gradients, interpolated from the data using a GAM with both temperature and phosphorus as smoother terms. Curvature in the contours along the nutrient gradient indicates the decline in optimum temperature with nutrient concentration. To highlight variation at the lowest silicate levels, data above concentrations of 30 µM are not shown.

concentrations even lower than those used in our experiments (Downing et al. 2001; Tyrrell 1999), suggesting that a further decrease in the environmentally-relevant optimum temperature is possible.

This has important implications for efforts to link physiological measurements with organismal performance in natural environments and to model the effects of environmental change on communities. Studies linking species traits to environmental variation to identify patterns of adaptation (e.g. Thomas et al. 2012) may find such patterns confounded due to trait measurements under conditions that do not reflect natural environments. Our model also predicts that the maximum temperature at which species can persist (net population growth rate=0) declines with nutrient concentration, while the minimum temperature of persistence increases. Though we could not test these predictions in our experiment, these also suggest that susceptibility to extreme temperatures may be underestimated for organisms in nutrient-poor environments. In phytoplankton, the predominant use of nutrient-rich growth medium in physiological studies may bias inferences about performance in natural environments. Though both high- and low-temperature tolerance may decrease under nutrient-poor conditions, high temperatures are likely to be a greater threat due to environmental warming and processes that drive negative correlations between temperature and nutrient concentration in natural environments (IPCC Fourth Assessment Report 2007). In aquatic environments, this occurs in part because warming leads to increased stratification, which suppresses nutrient supply via upwelling.

Our model also predicts that the nutrient half-saturation constant K and R* are lowest at intermediate temperatures (Figures 5.1D, E). Tilman et al. (1981) found strong evidence for this pattern in the case of R*. In the case of K, we find some support for this pattern in our experiments (Figure 5.4), but measurements at lower nutrient concentrations are required to rigorously test this prediction. Earlier experiments have not found support for this. Tilman et al. (1981) found an exponential increase in K with temperature in *A. formosa* and no trend in a

different species, *Synedra ulna.* Mechling & Kilham (1982) found some evidence for an increase in K with temperature, but no measurements were made above the optimum. A possible explanation for this variation in experimental findings is that species may alter their physiological response to nutrients with temperature in ways that our model does not capture. For example, cell size, a trait that is strongly related to nutrient response, is also known to change with temperature (Edwards et al. 2012; Marañón et al. 2012; Montagnes & Franklin 2001). Accounting for this plasticity might alter our predictions for the temperature-dependence of nutrient traits. Future studies on multiple species will be needed to examine this prediction and improve our understanding of this interaction.

As we noted earlier, equation (3) may also be interpreted at an organismal level for ectotherms, implying that low resource or food supplies may decrease high-temperature tolerance. This prediction has support in experiments performed on fish by Brett (1971). The optimum temperature for biomass production was a saturating function of food supply, with the greatest decrease in production occurring at the highest temperatures.

Our study highlights how subtle interactions between environmental factors can alter patterns of growth, complicating attempts to model population dynamics. Predicting how populations and communities will respond to environmental change will require a clearer understanding of these interactions. We have shown that models grounded in physiology can generate novel predictions for how environmental variation affects species, improving our ability to predict how environmental change will affect species and communities.

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APPENDICES

APPENDIX 1

APPENDIX 1

1 Materials and Methods

We provide additional details on methods, statistical analyses, models, and data sources in this supplement. A graphical overview of the various data sets and analyses composing this project can be seen in Figure A1.10. §1.1 describes the selection criteria used to identify the growth rate data used in this study. §1.2 details the statistical procedures used to fit and describe the thermal tolerance curves. §1.3 outlines the bootstrapping approach used to appropriately account for uncertainty in estimates of thermal traits. §1.4 covers the statistical analysis used to characterize temperature regimes. §1.5 describes statistical methods used to demonstrate trait-environment relationships while accounting for trait uncertainty. §1.6 explains our analysis of trait variation as a function of taxonomy. §1.7 describes the structure and analysis of our eco-evolutionary model. §1.8 provides extra details on the data and methods employed in our species distribution models. §1.9 lists the various pieces of computational software we employed.

1.1 Curve selection criteria

We assembled a data set containing growth rate measurements of marine and estuarine phytoplankton at different temperatures that have been published over the past century. All measurements were digitized using g3data (Frantz & Novak 2000). Strains were treated as independent, due to the existence of considerable intraspecific variation and uncertainty in species boundaries for some taxa.

Several criteria were used to determine the inclusion of species/strain data in our analyses.

1) To facilitate comparisons across studies we only included data for growth rates measured in units that could be converted to specific growth rate.

2) Because we were primarily concerned with estimating the temperature at which strains/species achieve their maximum growth rates, we rejected curves where the largest measured growth rate occurred at the lowest or highest temperature considered.
3) Curves with fewer than four measured growth rates were excluded, as were curves showing strong bimodality, which we attributed to imprecise experimental measurements.
4) Where curves were measured under different experimental conditions (salinity, nutrient limitation, light levels, day length), we preferentially selected curves meeting the following conditions:
    a. Salinity between 30 and 40 parts per thousand.
    b. Light levels greater than or equal to 100 microeinsteins.$m^{-2}.s^{-1}$.
    c. Not experimentally limited by nutrients
    d. Day lengths of greater than or equal to 10 hours.

    When no curves for a particular isolate satisfied these experimental constraints, we settled for using data from the curve(s) that were closest to the desired light and salinity levels.
5) We considered only marine and estuarine strains not isolated from inland waters.

After applying these criteria, we had data for a total of 252 separate curves, divided among 194 isolates/strains belonging to approximately 130 species, from 111 unique isolation locations ranging in latitude from 76°N to 75°S (Figure A1.1, Table A1.5).

1.2 Statistical analysis of thermal tolerance curves

Temperature dependent specific growth rates can be described by the following equation:

$$f(T) = ae^{bT}\left[1 - \left(\frac{T-z}{w/2}\right)^2\right] \quad \text{(S.1)}$$

Here specific growth rate *f* is an explicit function of temperature, *T*. The shape of the thermal tolerance curve is controlled by two important species traits, *z* and *w*. The range of temperatures over which growth rate is positive, or the thermal niche width, is given by *w*. Species trait *z* determines the location of the maximum of the quadratic portion of this function. In the case where parameter $b = 0$, this value is identical to the temperature at which a species achieves its maximum growth rate. However, when *b* is non-zero, the maximum value of S.1 falls above (or potentially below, $b < 0$) the value of *z*, and can be found through numerical optimization.

Norberg (2004) fixed parameters *a* and *b* according to the values of the Eppley curve (Eppley 1972), an exponential relationship thought to provide the community-level upper bound (95% quantile) on phytoplankton growth as a function of temperature. However, in fitting growth curves to data for individual strains, we recognized that species may not strictly follow this community level constraint, potentially due to the effects of other constraints such as light or nutrient limitation. For this reason, we allowed *a* and *b* to be free parameters, fit simultaneously with *z* and *w*.

To describe the growth data for each isolate, we used a maximum likelihood approach, such that the mean growth rate at a given temperature followed equation (S.1),

$$\mu = f(T) + N(0, \sigma^2) \quad \text{(S.2)}$$

Here observational error was described by a normal distribution with a mean of zero and variance of σ^2.

1.3 Determining physiological parameter uncertainty

While confidence intervals for the point estimates of the parameters of (S.1) were easy to obtain, it was not straightforward to determine uncertainty for implicit properties such as the temperature at which growth rate is maximized (or, the 'optimum temperature'). Yet, this was the property that we were mainly interested in, leading us to adopt a parametric bootstrapping approach.

We used a Monte Carlo approach such that for each thermal tolerance curve having *n* data points, we simulated *n* new data points, drawn from a normal distribution such that:

1) The mean of the distribution corresponds to the value of (S.1) at each of the original experimental temperatures, given the coefficients previously estimated for the original curve.
2) The standard deviation of the distribution, σ, was obtained by adjusting the original maximum likelihood estimate, $\hat{\sigma}$, to account for uncertainty in its estimation (Gelman & Hill 2007):

$$\hat{\sigma}^2 = \sigma^2\sqrt{(n-1)/X} \qquad \text{(S.3)}$$

where *n* is the number of points, and *X* is a random number drawn from the χ^2 distribution having (*n* - 1) degrees of freedom.

Equation (S.1) was then fit to the simulated data using maximum likelihood estimation, and the new parameter values, as well as the numerically estimated optimum temperature, were retained. Repeating this process a total of 10,000 times (for each isolate), yielded bootstrapped distributions of all parameter estimates. From these distributions we calculated the 95%

confidence intervals as the range between the 2.5th and 97.5th quantiles. These estimates of uncertainty were vital for subsequent analyses, as they allowed us to appropriately account for the inherent differences in uncertainty between different isolates expected to arise any time that data are synthesized across many individual studies.

1.4 Environmental data analysis

To provide the environmental data necessary for investigating trait-environment relationships, we turned to historical sea surface temperature (SST) estimates available through NOAA as ¼ degree, AVHRR/AMSR+AVHRR daily optimum interpolation SST (Reynolds et al. 2007). These data covered the period between 1981 and 2010. Because of the fine spatial resolution of these data, we were able to closely match the location of each isolate to the nearest location having SST data, minimizing error due to spatial variation. When more than one ¼ degree location was equidistant from an isolation location, a specific grid location was selected randomly from the various options. Our initial set of 111 distinct isolation locations were matched in this manner to a set of 106 distinct SST locations. We then assembled time series of sea surface temperature at each of these locations from Sept. 1st, 1981 to Jan. 18th, 2011.

Temperature regimes were described by fitting the following modified sinusoidal function to the entire 30-year time series at each location, again using a maximum likelihood approach:

$$\mathrm{T(t)} = \phi + r\left[\sin\left(\frac{\pi}{365}(t + \beta)\right)\right] \qquad \text{(S.4)}$$

In this model, $|r|$ describes the range of temperatures achieved, while ϕ provides the maximum ($r < 0$) or minimum ($r > 0$) temperature. Parameters α and β describe the skewness and temporal

shift of the temperature oscillations, respectively. The addition of the α parameter extends a typical sine function to capture asymmetrical seasonality (for example, longer warm periods than cold periods). Note however, that for $\alpha = ½$, equation (S.4) reduces to an ordinary sinusoidal model. These regressions generally fit the data well (n = 106 time series, mean R^2 of 0.81, standard deviation of 0.17). Note that due to the inherent assumption of observation error, standard regression models describing temperature time series (such as S.4) will always underestimate the extreme range of temperatures.

We calculated mean temperatures and temperature ranges directly from time series data for each location, as well as the mean and range of the deterministic model fit to the time series. These results are used subsequently in establishing trait-environment relationships, and (in the case of the deterministic model) to describe realistic temperature fluctuations in our eco-evolutionary model.

1.5 Trait-environment regression randomizations

We wanted to calculate regressions relating latitude and mean temperature to phytoplankton optimum temperatures, while accounting for the varying levels of uncertainty attached to each estimate of optimum temperature. Typically meta-analyses handle this issue by performing a weighted regression, using the inverse of the uncertainty associated with each data point. This approach implicitly assumes that the uncertainty around each point estimate is symmetric, which was clearly not the case for our traits (see for example the bootstrapped confidence intervals around point estimates of optimum temperatures, Figure A1.9). Consequently, we employed a different approach using resampling techniques, which, to be useful, needed to provide: 1) regression coefficients, 2) uncertainty in regression fit, and 3) a means of comparing the fits of a set of alternative models. Note that we wanted to account for

two kinds of uncertainty in this process, both the uncertainty arising from error in estimates of optimum temperature and the uncertainty associated with fitting a regression to data.

Recall that during our earlier parametric bootstrapping calculations we obtained 10,000 estimates of the thermal optimum of each of $n = 194$ isolates, via a process that is independent across isolates. For the ith iteration of this resampling procedure, we did the following:

1) Drew the ith value (out of 10,000) from each of the 194 distributions of estimated thermal optima. This captured error associated with uncertainty in our thermal optima estimates.
2) Fit each of the regression models under consideration to this randomized data
3) Performed standard model comparison of these regressions, saving the Akaike weights associated with each model. Akaike weights capture the relative likelihood of a model, given the data and set of competing models under consideration (Burnham & Anderson 2002).
4) To capture uncertainty associated with each of these models, we conducted residual resampling, associating each predicted value from a given regression with a new residual, and repeating the regression.
5) Saved the estimates of the resulting regression coefficients.

This process was repeated a total of 10,000 times across all of the bootstrapped optimum temperature values. The result was a distribution of parameter estimates for each of the regression fits. From these we calculated confidence intervals on the regression parameters, as well as confidence bands for the relationships as a whole. Finally, we determined the model(s) with the most explanatory power by looking at the average Akaike weight (across the 10,000 replicates) associated with each.

This method was used to examine the relationship between optimum temperature and latitude (having considering linear and quadratic models), resulting in Figure 2.1. A more extensive set of models was examined when considering the relationship between optimum temperature and the mean and range of environmental temperatures. The set of models considered, as well as the corresponding average Akaike weights, are provided in Table A1.4, while the model receiving the greatest weight is presented in Figure 2.2A.

1.6 Taxonomic model comparison

As the genetic sequence data necessary to construct a complete phylogeny for the species in our dataset were not available, we tested for a taxonomic signal (Kerkhoff et al/ 2006; Schwaderer et al. 2011) in optimum temperature and temperature niche width. Strains were classified according to Algaebase (Guiry & Guiry 2012) supplemented with a few entries from ITIS (ITIS 2012). In the case of optimum temperature, we constructed a full linear mixed model containing environmental parameters (mean annual temperature) as fixed effects and all taxonomic levels (from domain to strain) as nested random effects. This was compared against a set of models that omitted each individual taxonomic level using Akaike Information Criterion (AIC), a tool used in information theoretic approaches to model selection that measures goodness of fit of a statistical model (Table A1.1). In the case of niche width, the models contained taxonomic levels, but not environmental covariates, as none explained variation in niche width. A summary of the full models is in Table A1.2. Analyses were repeated using an alternate method in which taxonomic levels were added in sequentially and compared against the previous model. The results did not change our conclusions, though we were unable to test for the effect of Domain on either trait.

1.7 Eco-evolutionary modeling

1.7.1 Model setup.

We used a version of an eco-evolutionary modeling framework called adaptive dynamics (Abrams 2001; Geritz et al. 1998) to determine the thermal strategy or strategies that represent evolutionary equilibria for each temperature regime represented in our dataset. All parameters used in the subsequent model are presented in Table A1.3, along with their units and the numerical values used in our simulations, where appropriate. Our approach consisted of defining the per capita growth rate of phytoplankton strain *i* as a function of its traits (focusing on z_i) and environment (nutrient level and temperature) as follows:

$$\frac{1}{N_i}\frac{dN_i}{dt} = \mu(z_i, T)\frac{R}{R+k} - m(T) \quad \text{(S.5)}$$

In this model, strains exhibit a temperature dependent growth rate $\mu(z_i, T)$, equation (S.7), subject to the availability of resource *R* and half-saturation constant *k*. Given that very little is known about the interactive effects of temperature and resource dynamics, we treated resource dynamics as simply as possible (algebraically):

$$R(t) = R_{in} - \sum_i N_i(t) \quad \text{(S.6)}$$

Growth rates were taken to be both trait and temperature dependent:

$$\mu(z_i, T(t)) = 0.81e^{0.0631T(t)}\left[1 - \left(\frac{T(t)-z_i}{w/2}\right)^2\right] \quad \text{(S.7)}$$

where temperature dynamics follow (S.4). This equation is similar to that of Norberg et al. (2004) and (S.1), but instead of using Eppley's original coefficients (Eppley 1972) we made use of more recently refined parameters (Bissinger et al. 2008). The value of this function (S.7) depends critically on strain traits z_i and w, which are the temperature at which a strain touches the Eppley curve and the strain's thermal niche width, respectively. Three different values of niche width w were investigated, approximately spanning the range of niche widths that we could estimate from literature data with confidence (6.1 to 33.9 °C). Trait z_i can be interpreted as the temperature at which a strain's thermal tolerance curve lies tangent to the Eppley curve. To obtain predictions of thermal optima comparable to those in the rest of the paper, we used numerical methods to determine the temperature at which (S.7) reaches its maximum. In addition to depending on trait z_i, growth rate depends on the temperature, $T(t)$, which varies over time following (S.4) with specific parameters that differ depending on the particular environment under consideration (see section 'Environmental data analysis'). In the model, all strains experience a temperature dependent mortality rate, $m(T)$ that is 5% of the Eppley curve (i.e. 5% of the maximum growth rate), regardless of their traits.

Finally, we allowed strains to evolve or change their trait value z_i dynamically through time according to (Abrams 2001):

$$\frac{dz_i}{dt} = \epsilon \frac{d}{dz_i}\left[\frac{1}{N_i}\frac{dN_i}{dt}\right] \tag{S.8}$$

In this model, the change in trait over time is driven by the fitness gradient of a strain, or how much the per capita growth rate (fitness) of a strain increases (or decreases) with small changes in its trait value. This rate is scaled by parameter ε, which we take to be small so that traits are approximately constant within a period, effectively forcing the separation of time scales

between ecological and evolutionary dynamics (Lande 1976). Equation (S.8) is analogous to the breeder's equation drawn from quantitative genetics (Abrams 2001).

1.7.2 Predictions of optimal trait values - adaptive dynamics simulations.

Together, (S.5-S.8) provide a system of differential and algebraic equations that can be analyzed numerically and used to predict the evolutionary stable strategies (ESSs), or equilibrium trait value(s), of phytoplankton species in a given environment. These predictions allowed us to test whether our understanding of the interaction between physiological constraints and environmental variation, and ecology and evolution, is enough to predict trait-environment relationships observed in the real world. Eco-evolutionary models have rarely been used as a source of predictions for comparing with data, with notable exceptions (Childs et al. 2011; Metcalf et al. 2008; Stegen et al. 2012).

We performed eco-evolutionary simulations for each of the 106 distinct historical temperature regimes earlier matched to isolates. The temperature forcing (S.4) for these simulations are parameterized using the results from the earlier section 'Environmental data analysis'. For each location, we started with four species having initial trait values z_i uniformly distributed across that site's range of temperatures. We then used Wolfram Mathematica version 8 to numerically solve this system of eight differential equations forward in time, until they reached their dynamic attractor. During this process, the trait and population dynamics of each strain were monitored, such that:

1) If the trait values of any two strains converged on each other to within $1x10^{-4}$, their biomasses were summed, and they were merged into a single pair of equations, and

2) If the biomass of an individual strain integrated over one time period fell below $1x10^{-12}$, it was considered to have suffered extinction and its pair of equations was removed from the system.

Once this system reached its dynamic attractor, we took the average value of z_i over a single period for each of the remaining strains and determined the temperature at which the corresponding thermal tolerance curve achieved its maximum growth rate. This provided us with estimates of the ESS optimum temperatures and diversity of coexisting strains, matched to that particular environment.

We then repeated this process across each set of environmental conditions, for three different settings of niche widths (see Figure A1.3 and parameters in Table A1.3). For environments where the ESS consisted of a single strain, we found that its optimum temperature was consistently higher than the mean annual temperature by a factor determined by the strain's niche width. These strains all shared the property that their z (the temperature at which their thermal tolerance curves were tangent to the Eppley curve) exactly matched the mean annual temperature they experienced. In other words, their most adaptive strategy is to have values of z such they grew as fast as theoretically possible at the mean annual temperature. However, because their thermal tolerance curves are bounded by an increasing function, the value of z is not the same as the temperature at which each strain achieves its own fastest growth rate, usually defined as its optimum temperature (see Figure A1.2). The difference between the temperatures at which these two points occur is determined by the niche width, explaining the offset we observe.

This particular method of eco-evolutionary modeling is only one of several methods that could be applied to this problem. We selected it because under some situations, such as with

fluctuating environments, it can be more computationally tractable than more traditional 'Adaptive Dynamics' methods. Additionally, in future work it can easily be extended to account for trait change on ecological time scales (Abrams 2001, 2005) by increasing ε, enabling us to potentially study evolutionary responses to climate change within the same framework. It is worth noting that the maximum number of coexisting strains that can be identified using this approach is constrained by the total number of initial strains (four in our case), so it remains possible that temperature variation could support higher levels of diversity than we determined in this study. However, this seems unlikely as the most diverse result obtained consisted of only three strains.

1.8 Species distribution modeling.

The thermal tolerance curve of a given species allowed us to calculate its growth rate under any given environmental temperature. A time series of environmental temperatures can thus be converted into a time series of estimated growth rates. We can then calculate the average per-capita growth rate of a species over the duration of the time series, in the absence of other limiting factors. Environments in which this growth rate was positive were considered to be within the geographic limits of the species' fundamental niche, with respect to temperature. Repeating this exercise for time series data across the world oceans for each strain in our study, we estimated the spatial extent of the strain's fundamental temperature niche. We then examined how these species distributions differ given historical and predicted future thermal regimes.

1.8.1 Data sets

For this analysis we drew on two additional sources of sea surface temperature data:

1) Monthly mean historical sea surface temperatures, 1° x 1°, and

2) Projections of future temperature regimes obtained from the NOAA GFDL CM2.1 (Delworth et al. 2006; Griffies et al. 2005). This model was driven with the SRES A2 emissions scenario, characterized by rapid population growth and heterogeneous development. This dataset contained global ocean temperature projections from 2001 to 2100, with a spatial resolution of 1° x 1° and a temporal resolution of 1 month. We focused on the final ten-year period from 2091-2100, at the end of which CO_2 concentrations reach 800 ppm.

These data sets have similar resolutions, but use different spatial grids (fixed versus Gaussian, respectively). To generate predictions that are directly comparable between historical and future temperature regimes we used bounded universal kriging to conduct spatial interpolations, basing the requisite theoretical variograms on Gaussian model fits to the semivariograms of each spatial data set. Because the world is round, kriging methods applied to global data sets based on latitude/longitude are prone to boundary effects. We minimized this problem for our kriging by generating two different interpolations, where the longitudinal boundary fell at 0° and 180°, respectively. We then generated a corrected interpolation by combining these results, while excluding edge values within the local neighborhood size employed in the bounded universal kriging. This interpolation allowed us to generate spatially congruent data sets, based on which we could make valid area, range shift and diversity comparisons.

1.8.2 Range shift estimates.

Using the above methods, we generated spatial predictions of the fundamental temperature niches (i.e. all locations where its growth rate averaged over its thermal environment was positive) or species distributions of all 194 strains in our data set, for both historical and

future environments. These calculations were performed without allowing for strain evolution, such that the traits of contemporary strains were assumed to be the same traits with which they will confront future environments. Comparing these results enabled us to quantify shifts in the fundamental range of these species, including changes in both range size and range position (see Figures A1.4–A1.6).

1.8.3 Diversity change estimates.

When examined in the aggregate, species distribution models allowed us to examine broad-scale patterns in potential diversity. We generated potential diversity predictions for each ocean location by calculating the number of strain ranges it fell within, under both historical and predicted future temperature regimes. Historical diversity estimates were kriged (see above) to make them comparable to estimates of future diversity. The historical diversity at each location was then subtracted from future diversity to generate predictions of diversity change over the 100-year time period (see Figures A1.7 and A1.8).

1.9 Software employed.

All statistical analyses were performed using R (R Core Team 2013), as well as the following packages: bbmle *(49)* (for maximum likelihood regressions and model comparison), reshape (Wickham 2007) (for data manipulation), ggplot2 (Wickham 2009) (for figure creation), ncdf4 (Pierce 2010) (for working with environmental data in NetCDF formats), gstat (Pebesma 2004) (for performing spatial interpolation/kriging), and lme4 (Bates et al. 2013) (for mixed model analyses).

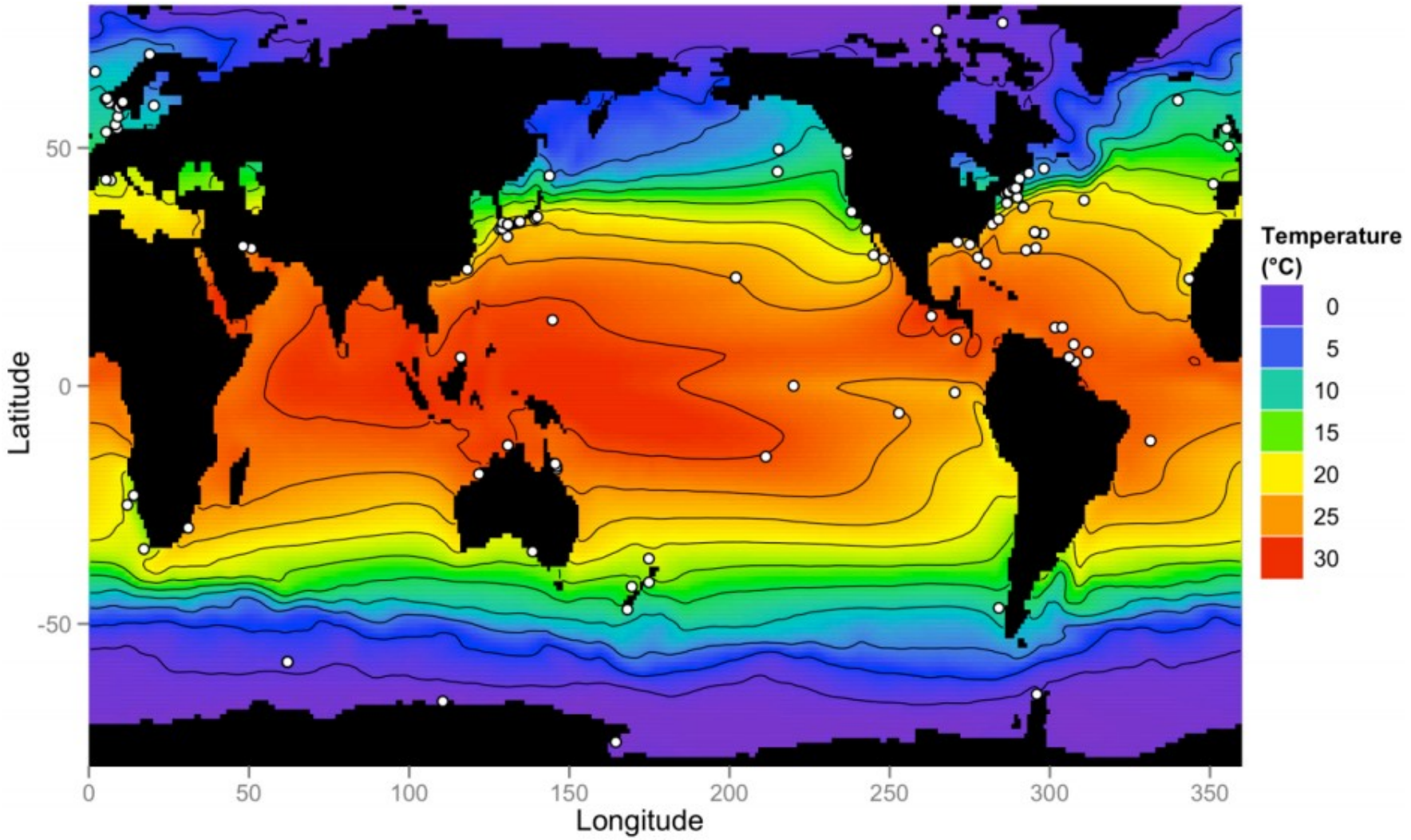


Figure A1.1. Mean annual temperatures across the oceans and the isolation locations of the 194 strains in our dataset, indicated by white dots. While most strains are isolated from coastal regions, we capture almost the entire temperature gradient, including the polar and tropical extremes.

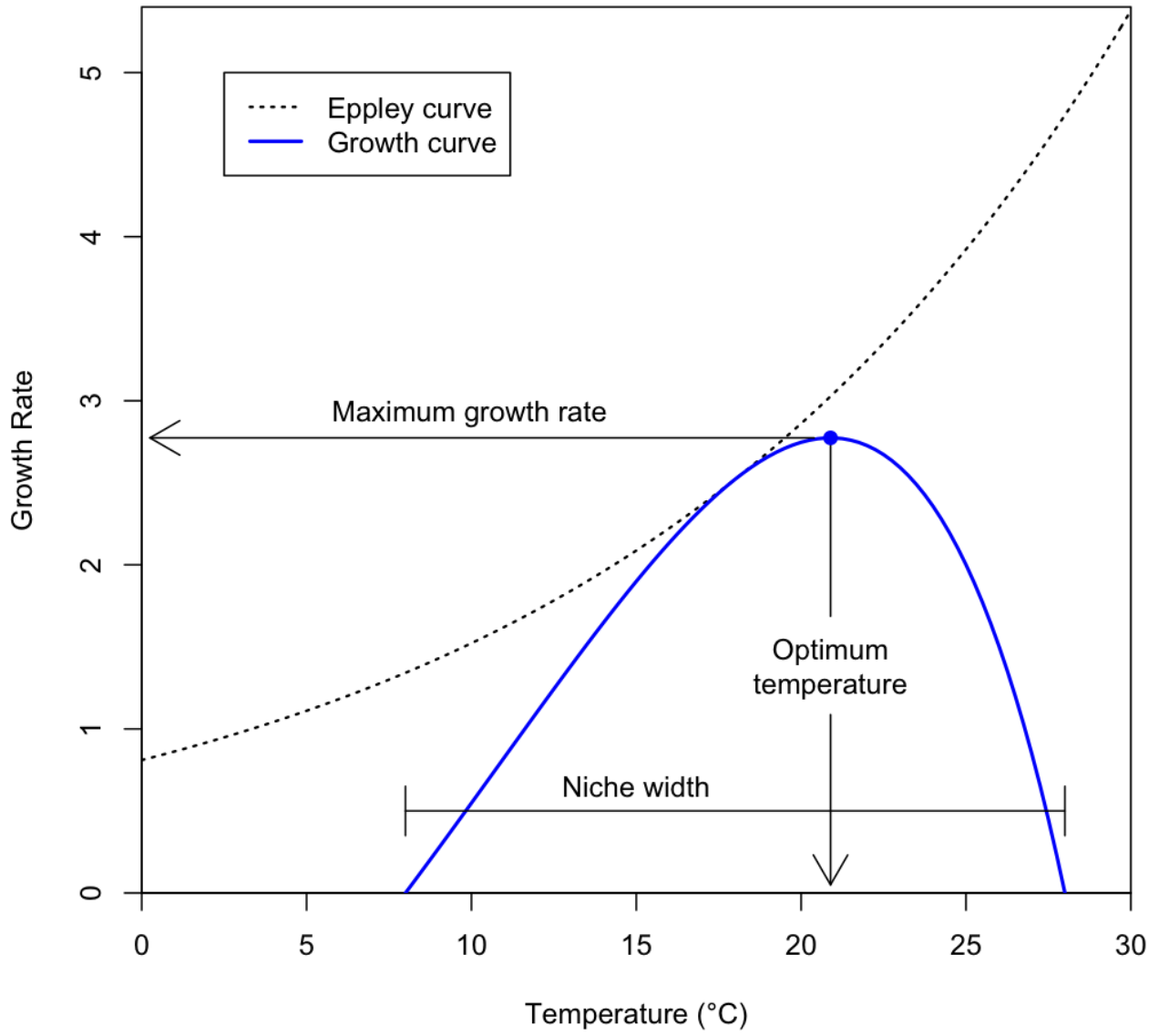


Figure A1.2**.** An example thermal tolerance curve, illustrating the skewness typical of all known ectotherms, including reptiles, amphibians, fish, algae, bacteria, and viruses. Niche width and the optimum temperature (at which the strain reaches its maximum growth rate) are shown. As in our model, this strain is bounded by the Eppley curve.

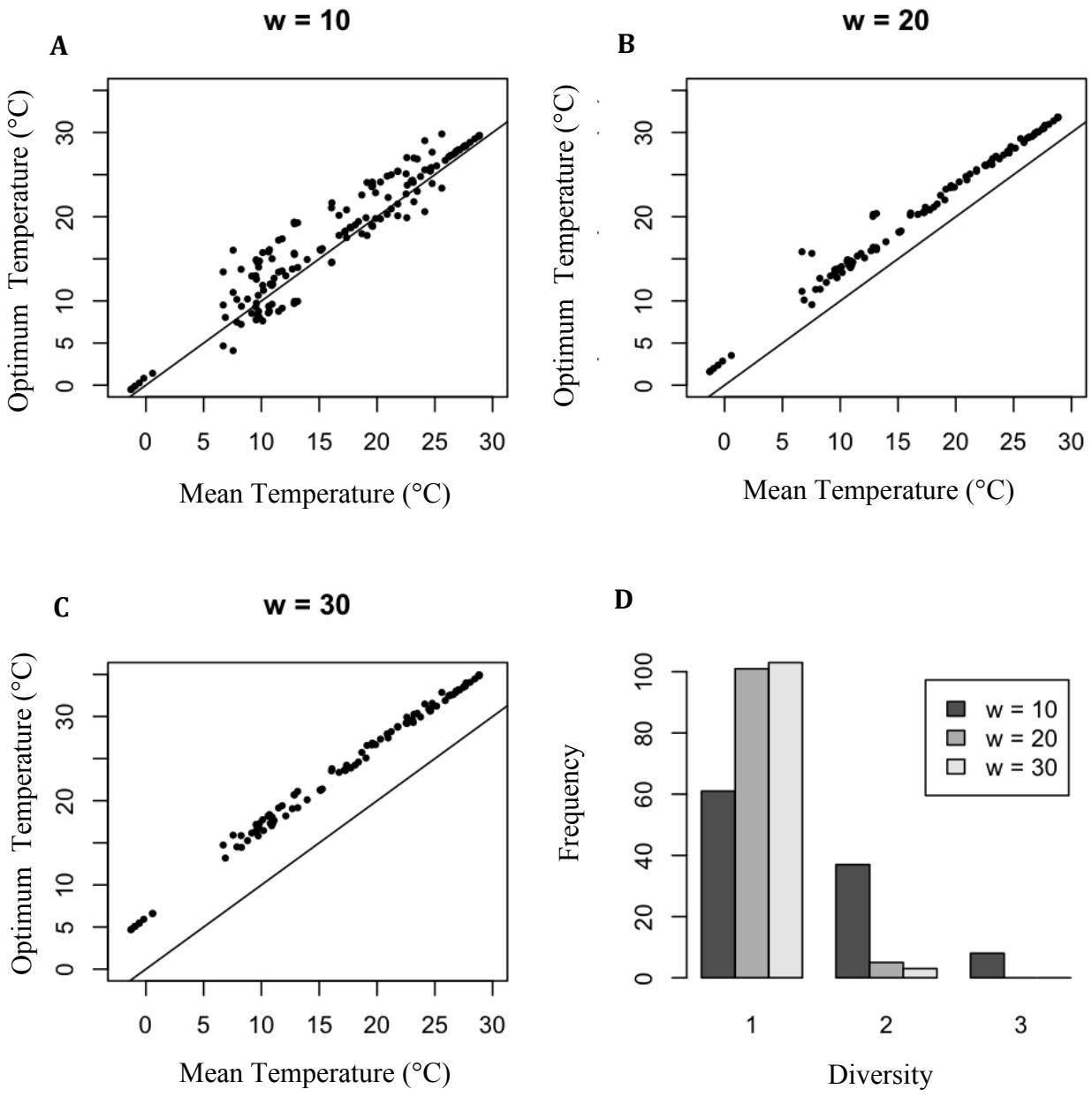


Figure A1.3. Eco-evolutionary model predictions of the evolutionarily stable optimum temperatures for growth given different niche widths (panels A-C; w = 10, 20, and 30°C, respectively). Also shown in D is the number of environments for which the equilibrium community consisted of 1, 2, or 3 coexisting strains, displayed for each of the various niche width assumptions. As niche width increases the frequency of obtaining multiple coexisting species declines.

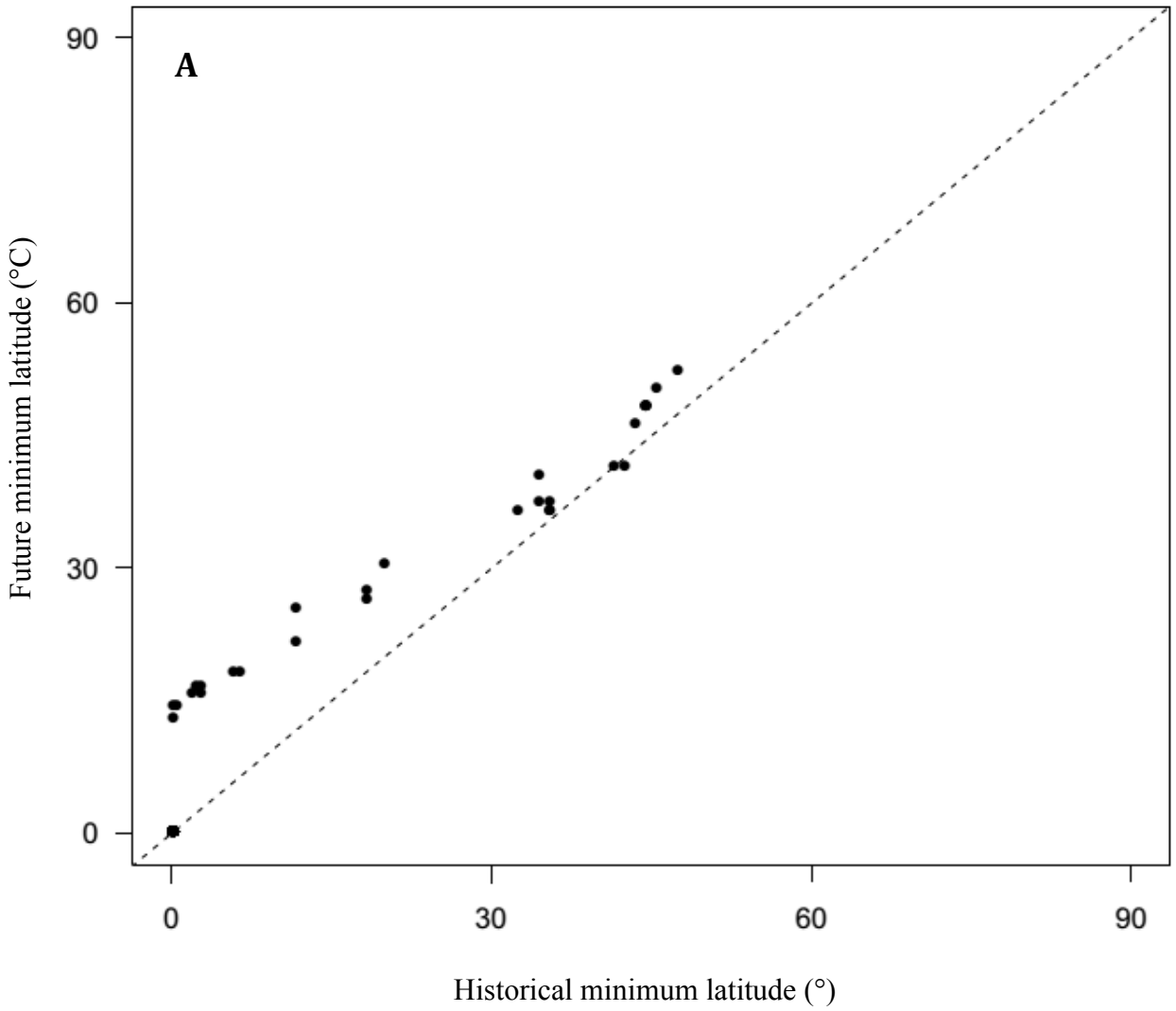
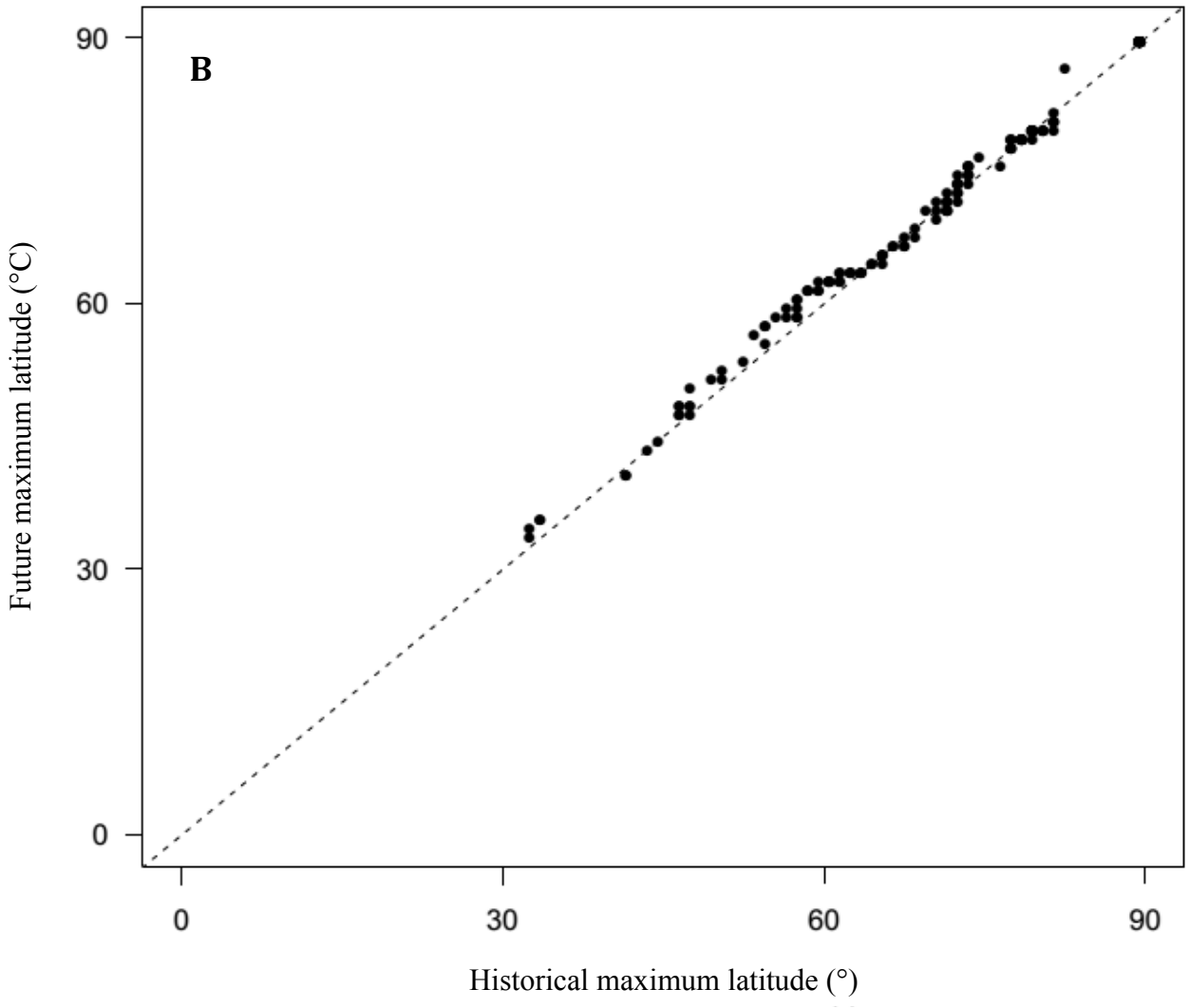
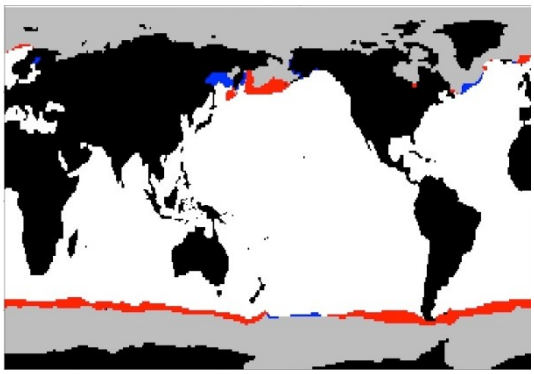


Figure A1.4. Predicted shifts in the A) equatorial and B) polar boundaries of all 194 strains. A) Points above the 1:1 dashed line have experienced a poleward shift in their lowest latitude at which they can grow. Almost all strains experience no change or poleward shifts. B) Points above the 1:1 dashed line have experienced a poleward shift in their highest latitude at which they can grow. Most strains experience a fairly small change in this latitude.

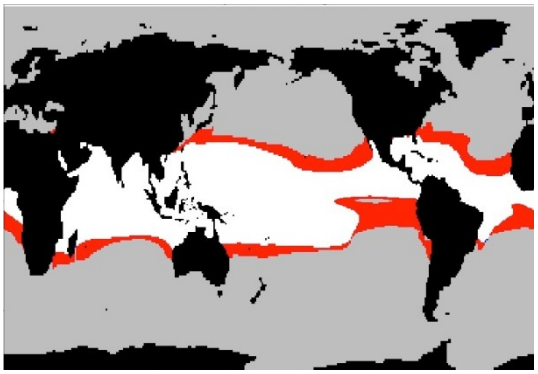
Fig. A1.4 (cont'd)



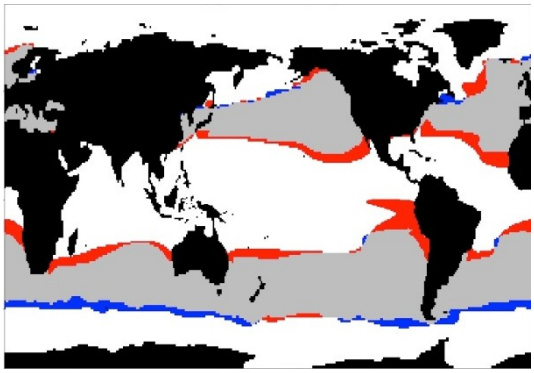
Stellarima microtrias



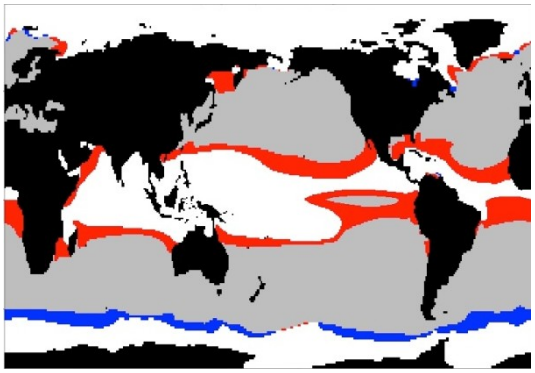
Emiliania huxleyi



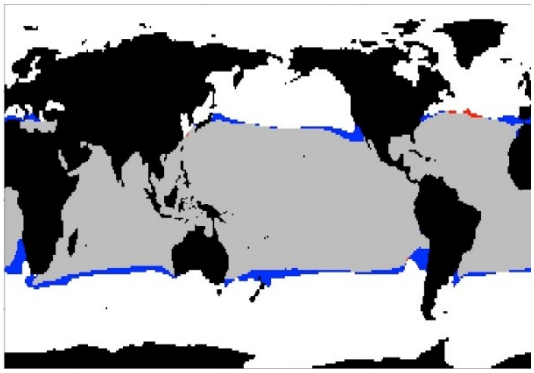
Calcidiscus leptoporus



Coccolithus pelagicus ssp. braarudii



Trichodesmium erythraeum



Chaetoceros lorenzianus

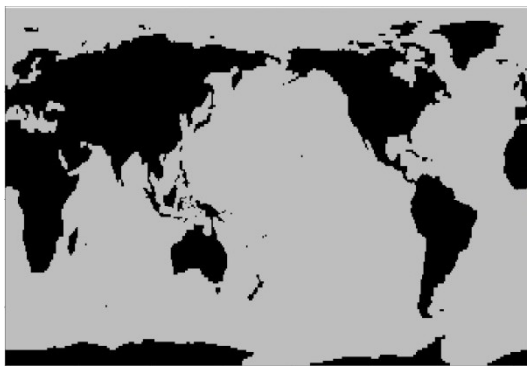


Figure A1.5. Examples of changes in the fundamental niche illustrating the diversity of ways individual strains may be affected. At each location a strain can persist (grey), be absent from the environment (white), or undergo range expansion (blue) or contraction (red) in the future.

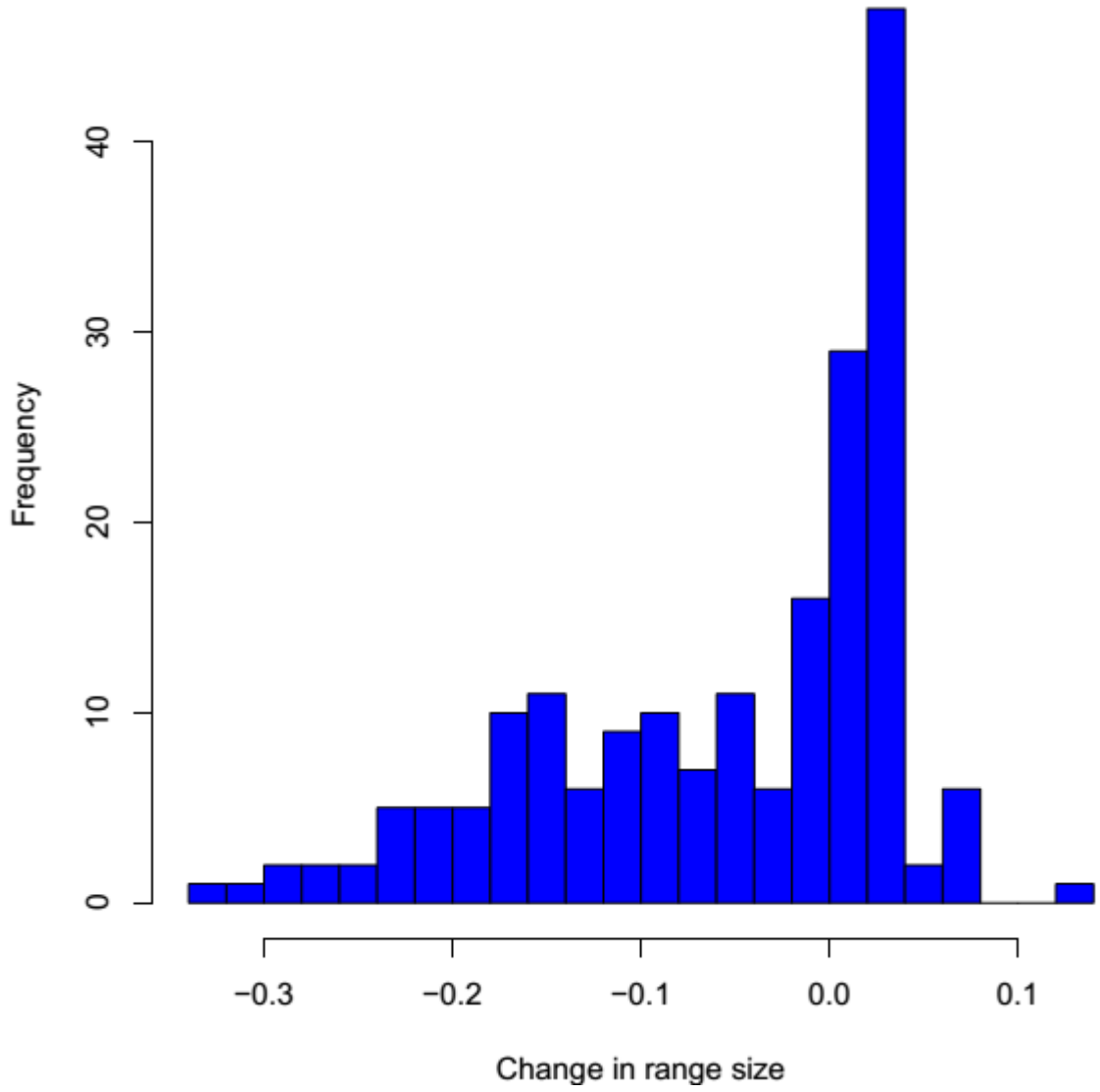


Figure A1.6. Histogram of per cent change in predicted strain range sizes. A number of strains experience a slight increase in their range, but a large number experience small to moderate decreases.

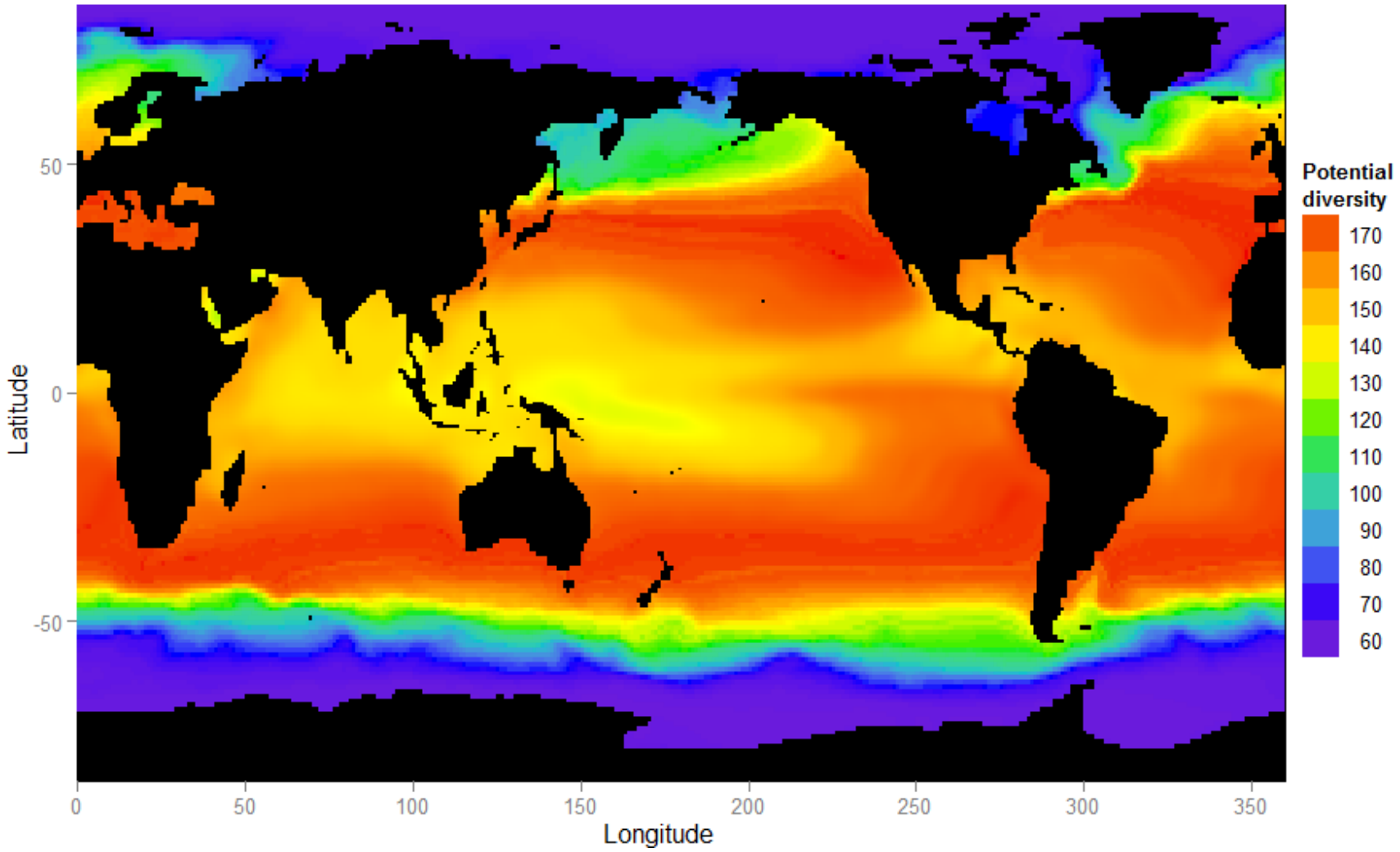


Figure A1.7. Potential diversity under past (1991-2000) temperature regimes. High diversity in temperate waters is a result of sampling bias, as most strains in our dataset were isolated in temperate waters, specifically off the US east coast, the European west coast and Japan.

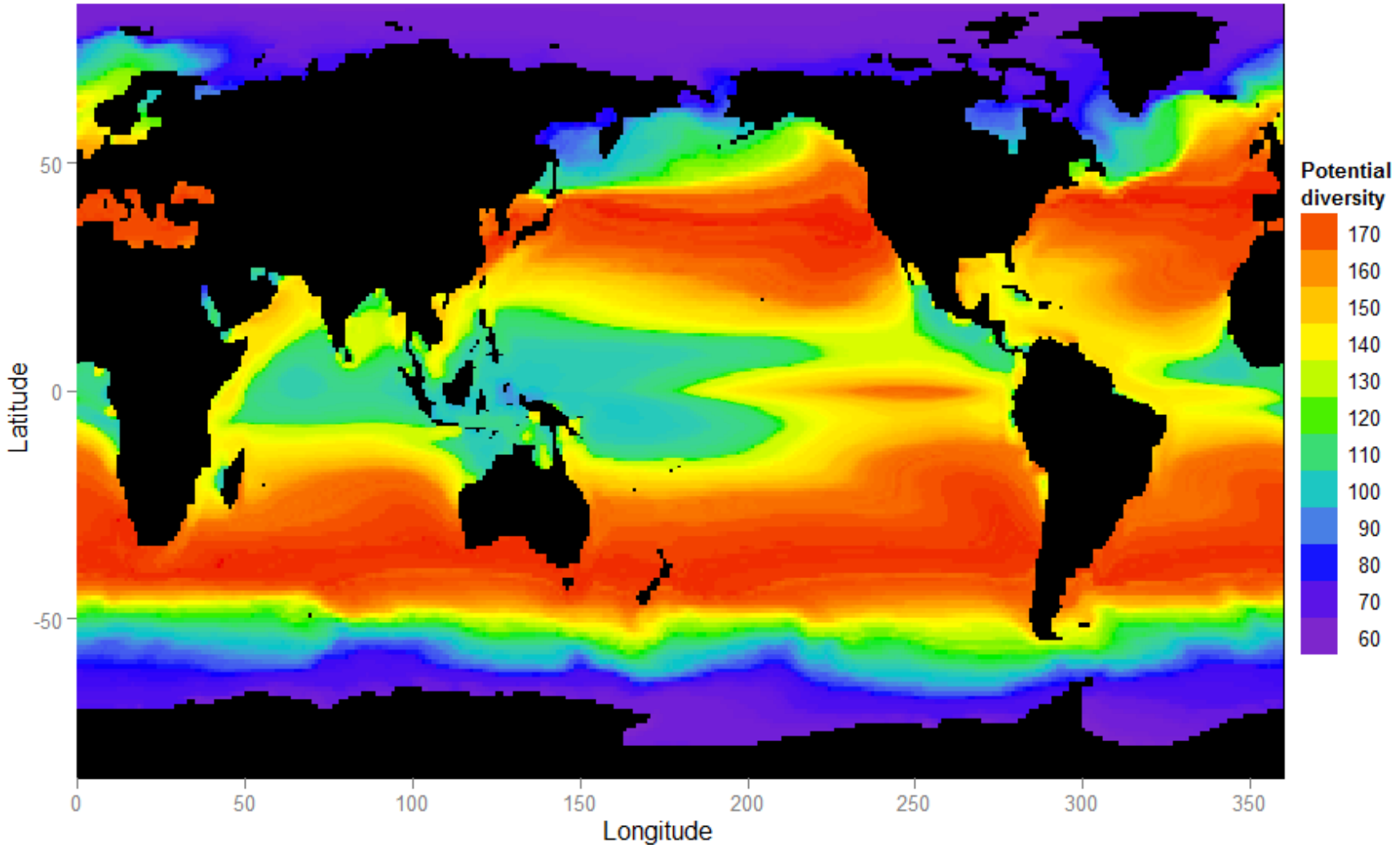


Figure A1.8. Potential diversity under future (2091-2100) temperature regimes. Temperature increase drives a large reduction in the potential diversity of the tropical Indian Ocean, Pacific Ocean and western Atlantic Ocean and an increase in the Antarctic Ocean.

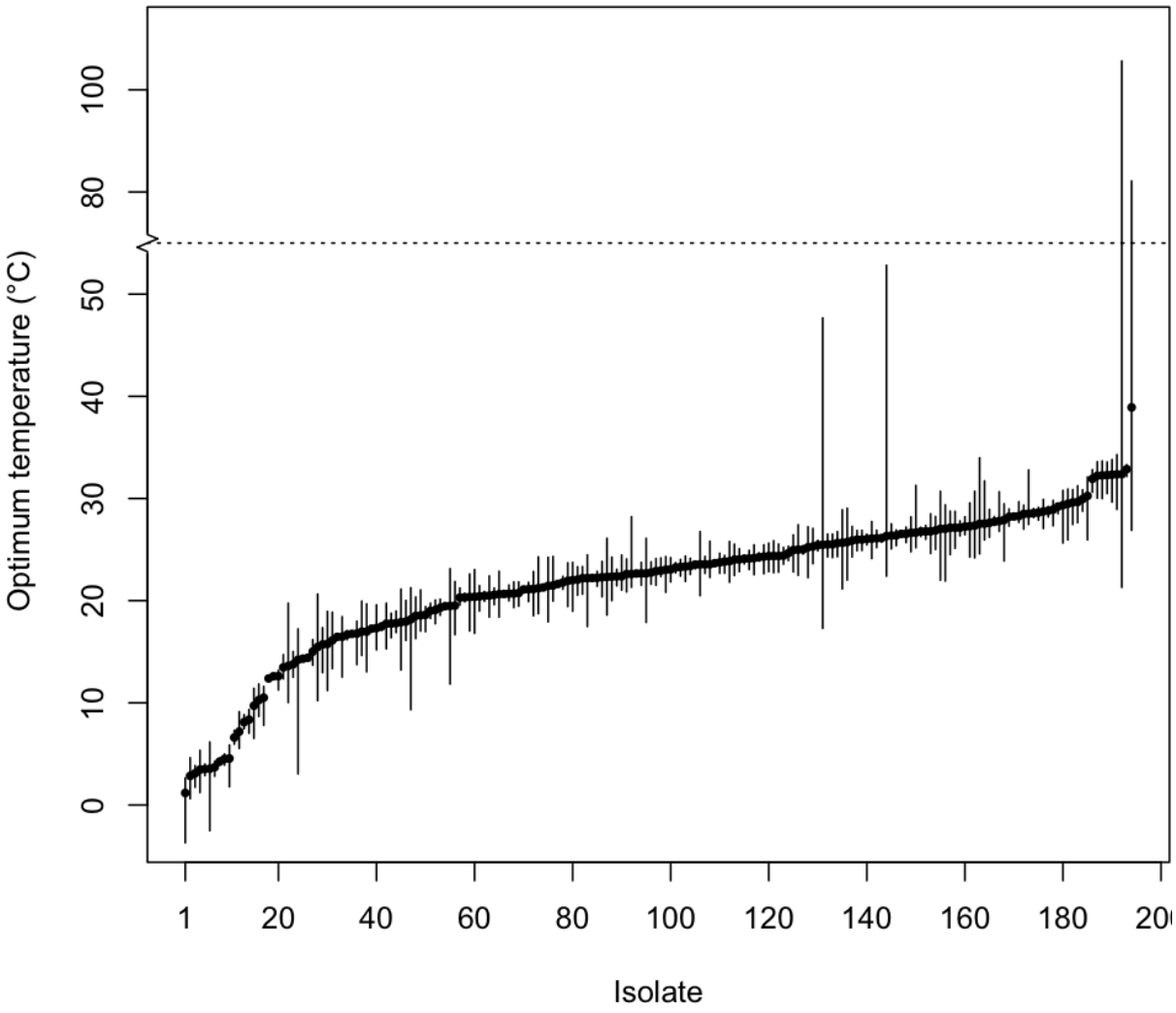


Figure A1.9. Estimates of uncertainty in optimum temperature for all strains, obtained through bootstrapping. Estimated optima are shown as points, while the 95% confidence intervals for the point estimates are shown by the error bars, obtained via our randomization analysis. Strains are ranked in ascending order of optimum temperature.

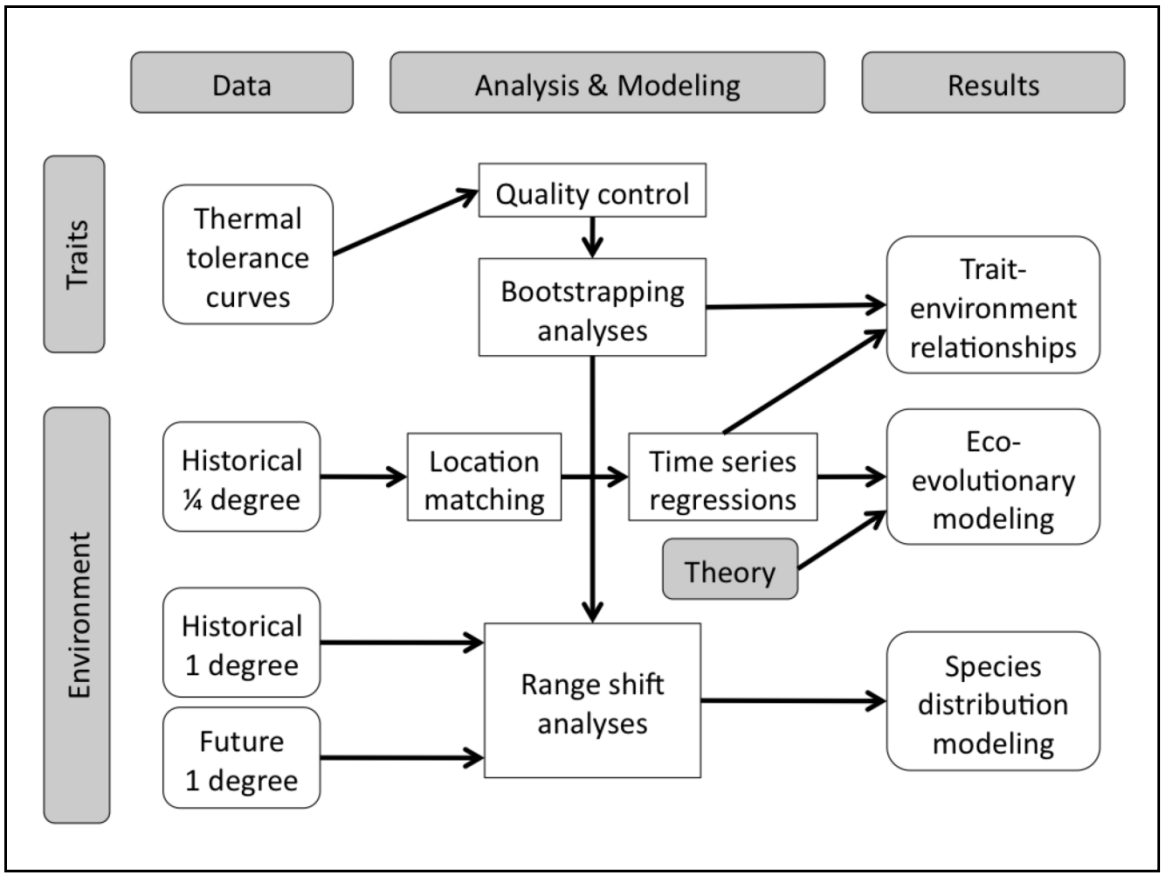


Figure A1.10. A schematic outlining all analyses and modeling performed.

Trait	Model	AIC	dAIC
Optimum temperature for growth Full model: Mean temperature + (Mean temperature)^2 + Domain + Kingdom + Phylum + Class + Order + Family + Genus + Species + Strain	Full model	1091.407	0
	Full model - Domain	1089.407	2
	Full model - Kingdom	1090.960	0.447
	Full model - Phylum	1089.407	2
	Full model - Class	1089.407	2
	Full model - Order	1089.407	2
	Full model - Family	1089.407	2
	Full model - Genus	1092.006	-0.599
	Full model - Species	1098.854	**-7.447**
	Full model - Strain	1089.447	1.96
Temperature niche width Full model: Domain + Kingdom + Phylum + Class + Order + Family + Genus + Species + Strain	Full model	1522.816	0
	Full model - Domain	1521.898	0.918
	Full model - Kingdom	1520.816	2
	Full model - Phylum	1522.256	0.56
	Full model - Class	1520.816	2
	Full model - Order	1520.816	2
	Full model - Family	1520.816	2
	Full model - Genus	1521.336	1.48
	Full model - Species	1546.125	**-23.309**
	Full model - Strain	1520.816	2

Table A1.1. Taxonomic model comparisons. Taxonomic levels are considered significant if their removal leads to an increase in AIC of >2 (i.e. dAIC < -2) relative to the full model, and are indicated here in boldface.

Optimum temperature for growth		Temperature niche width	
Random effects			
Taxonomic level	*Variance explained*	*Taxonomic level*	*Variance explained*
Domain	<0.001	Domain	52.044
Kingdom	0.937	Kingdom	<0.001
Phylum	<0.001	Phylum	14.775
Class	<0.001	Class	<0.001
Order	<0.001	Order	<0.001
Family	<0.001	Family	<0.001
Genus	1.867	Genus	7.142
Species	**4.722**	Species	**55.514**
Strain	0.173	Strain	<0.001
Residual	8.868	Residual	91.489

Fixed effects

Term	*Estimate*	*Std. error*	*t-value*	*Term*	*Estimate*	*Std. error*	*t-value*
Intercept	7.216	1.141	6.326	Intercept	14.114	5.875	2.402
Mean temperature	1.358	0.133	10.238				
(Mean temperature)^2	-0.024	0.004	-5.657				

Table A1.2. A summary of the full models (as written in Table A1.1) used to test for a taxonomic signal. Significant random effects indicated in boldface.

	Symbol	Definition	Value or units
Resource variables	R	Available resource	Dynamic
	k	Half-saturation constant	1
	R_{in}	Total resource level	100
Biomass variables	N	Biomass	Dynamic; measured in resource units
	$m(T)$	Temperature dependent background mortality rate	Specific growth rate; $m=0.5(ae^{bT})$
	μ	Growth rate	Specific growth rate
	a	Eppley curve coefficient	0.81
	b	Eppley curve exponent	0.0631
	z_i	Competitive optima	°C
	w	Niche width	{10, 20, 30}, °C
Temperature variables	T	Temperature	°C
	τ	Period of temperature fluctuation	365 days
	ϕ	Minimum (maximum) temperature, if r is positive (negative)	°C
	r	Range of temperature fluctuation	°C
	α	Skewness of temperature fluctuation	Not applicable
	β	Temporal shift of temperature fluctuation	Days
Evolutionary parameters	ε	Evolution rate	0.01

Table A1.3. Eco-evolutionary model parameters.

Model	Average Akaike weight
Optimum ~ Mean	0.0153
Optimum ~ Range	0.0002
Optimum ~ Mean + Range	0.0313
Optimum ~ Mean + Mean^2	0.6661
Optimum ~ Mean + Mean^2 + Range	0.2871

Table A1.4. Trait-environment models and associated Akaike weights.

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APPENDIX 2

APPENDIX 2

Methods

1. Temperature curve data collection and quality control

We assembled a data set containing >6000 published measurements of phytoplankton growth rates at different temperatures from published literature. All measurements were digitized using g3data. The data are included as supporting information.

Several criteria were used to determine whether the data were to be included in our analyses:

1) We only included data for growth rates measured in units that could be converted to specific growth rates.
2) We rejected curves where the largest measured growth rate occurred at the lowest or highest temperature considered.
3) Curves with fewer than four measured growth rates were excluded, as were curves showing strong bimodality, which we attributed to imprecise experimental measurements.
4) Where curves were measured under different experimental conditions (salinity, nutrient limitation, light levels, day length, pH), we preferentially selected curves meeting the following conditions:
    a. Light levels greater than or equal to 100 microeinsteins.$m^{-2}.s^{-1}$.
    b. Not experimentally limited by nutrients.
    c. Day lengths of greater than or equal to 10 hours.
    d. pH between 5 and 9.
    e. Salinity between 30 and 40 parts per thousand (only in the case of marine species).

    When no curves for a particular isolate satisfied these experimental constraints, we settled for using data from the curve(s) that were closest to the desired levels.

5) We excluded isolates from saline lakes and hot springs, preferring to focus on freshwater, estuarine and marine strains. Estuarine and marine strains are not always possible to separate (typically due to imprecise isolation location information) and were grouped together for analysis.

After applying these criteria, we had data on a total of 442 isolates belonging to approximately 252 species (not all were identified to the species level) from at least 256 unique isolation locations (48 were from unknown locations). 201 were from freshwater environments and 241 were marine. The known isolation locations ranged in latitude from 76°N to 78°S (Figure A2.1).

Additional criteria were used for specific analyses:

1) In analyses of Tmax, only maxima that were estimated to be within 5 degrees of the highest measurement temperature were considered.

2) Similarly, in analyses of Tmin, only minima that were estimated to be within 5 degrees of the lowest measurement temperature were considered.

3) In analyses of niche width and thermal tolerance curve skewness, only curves that satisfied both the previous criteria were considered.

2. Temperature trait estimation

Trait estimation followed Thomas et al. (2012), which we summarize here. Temperature-dependent specific growth rates can be described by the following equation:

$$f(T) = ae^{bT}\left[1 - \left(\frac{T-z}{w/2}\right)^2\right] \quad (1)$$

The shape of g is controlled by two important parameters, z and ω, which determine the location and scale of the thermal tolerance curve, respectively. The range of temperatures over which growth rate is positive, or the temperature niche width, is given by ω. The location of the thermal tolerance curve is governed by parameter z, which determines where the quadratic portion of g achieves its maximum. When $b = 0$, z is identical to the optimum temperature maximizing g. Otherwise, the optimum temperature for growth can be calculated by numerical optimization. Though this optimization can also be used to estimate maximum growth rate, we restricted ourselves to the use of empirically-determined maximum growth rates. Maximum (T_{max}) and minimum persistence temperatures (T_{min}) can be calculated by subtracting $\omega/2$ from parameter z.

Finally, we developed the following metrics to describe the skewness of thermal tolerance curves around their optimum temperature. We transformed g into a probability density function $G(T)$ on the interval [Tmin, Tmax], where g(Tmin) = g(Tmax) = 0, as follows:

$$G(T) = g(T) / \int_{T_{min}}^{T_{max}} g(T)dT \quad (2)$$

Absolute skewness then is given by M_3, the third moment of G around a fixed point, T^*:

$$M_3 = \int_{T_{min}}^{T_{max}} (T - T^*)^3 G(T)dT \quad (3)$$

In particular, we selected $T^* = z$, to quantify skewness around the point of maximum growth rate, rather than considering a central moment (where T^* would instead be the mean of G). The value of M_3 increases with increasing niche width, so we also explored a measure of relative skewness, standardizing deviations from T^* by the niche width of each curve:

$$\widehat{M}_3 = \int_{T_{min}}^{T_{max}} \left[\frac{(T-T^*)^3}{\omega}\right] G(T)dT \quad (4)$$

Ultimately, we found no convincing relationship between relative skewness and environmental or functional group covariates. We did find that absolute skewness differed between environments, but that this was driven by differences in the mean niche width between environments (Figures 3.5A, 3.5B, A2.4). When we controlled for niche width either through our relative skewness metric or by including niche width in the model, we could not detect any signal of environment on skewness. Therefore, the skewness of thermal tolerance curves may be unimportant in nature, arise as a by-product of physiological constraints, or simply have a much weaker effect on fitness than optimum temperature and niche width. Alternatively, we might not be able to determine the role of skewness without more detailed descriptions of the thermal environments these species face.

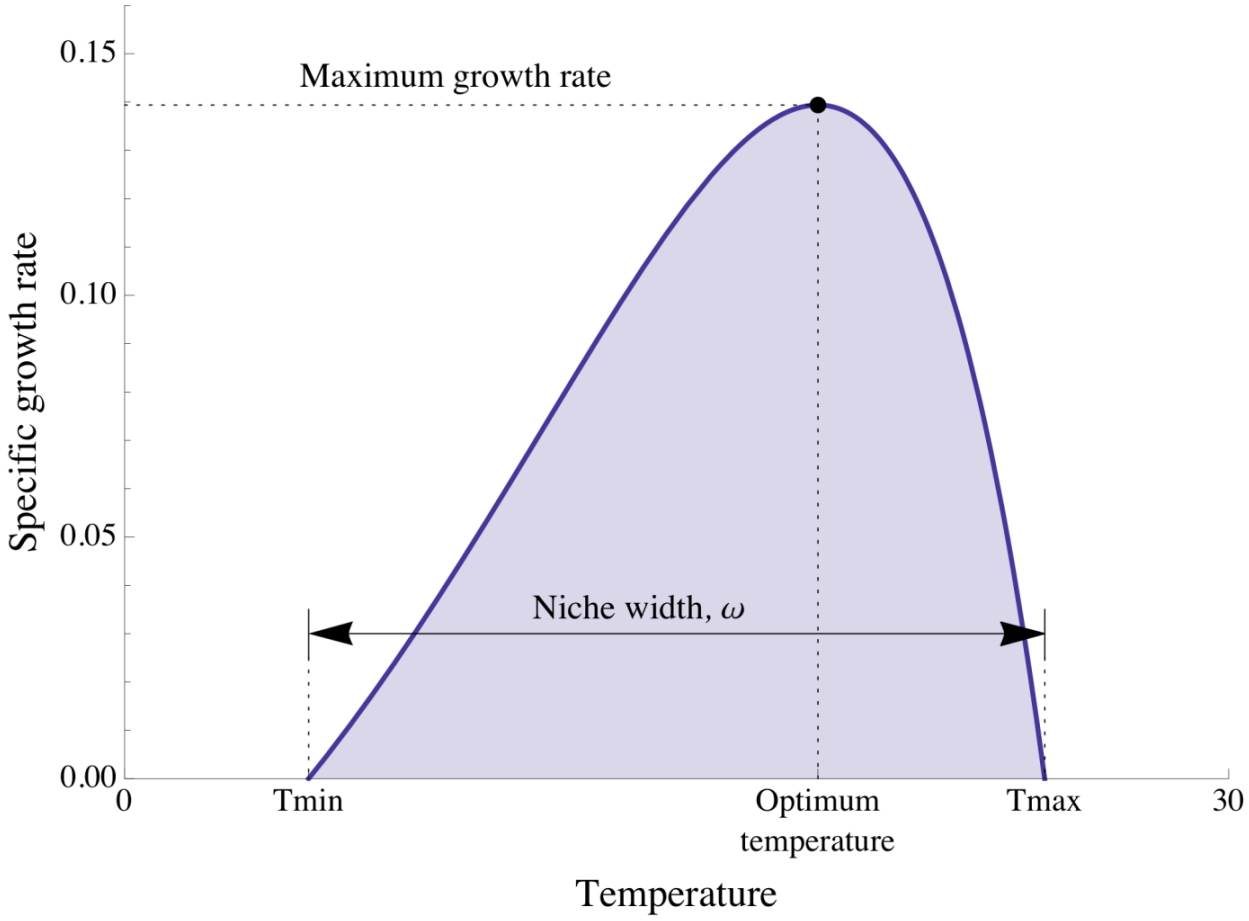

Figure A2.1. Traits characterizing the thermal reaction norm.

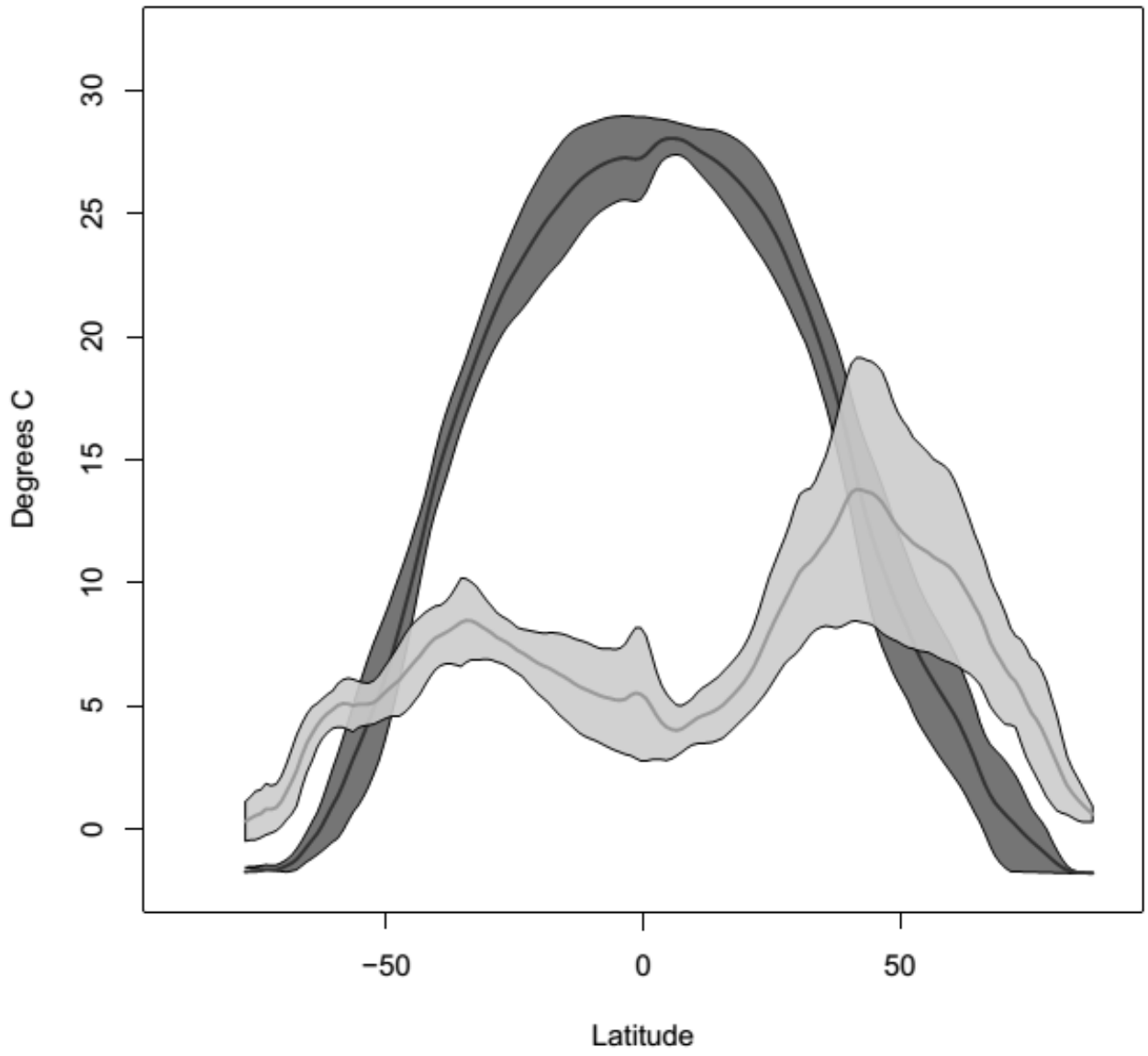

Figure A2.2. Mean temperature (dark grey) and annual temperature range (light grey) in the oceans across latitude, with 95% confidence bands. Note that peak temperatures occur at the equator but that the highest variability is at temperate latitudes. Data from Reynolds et al. (2007).

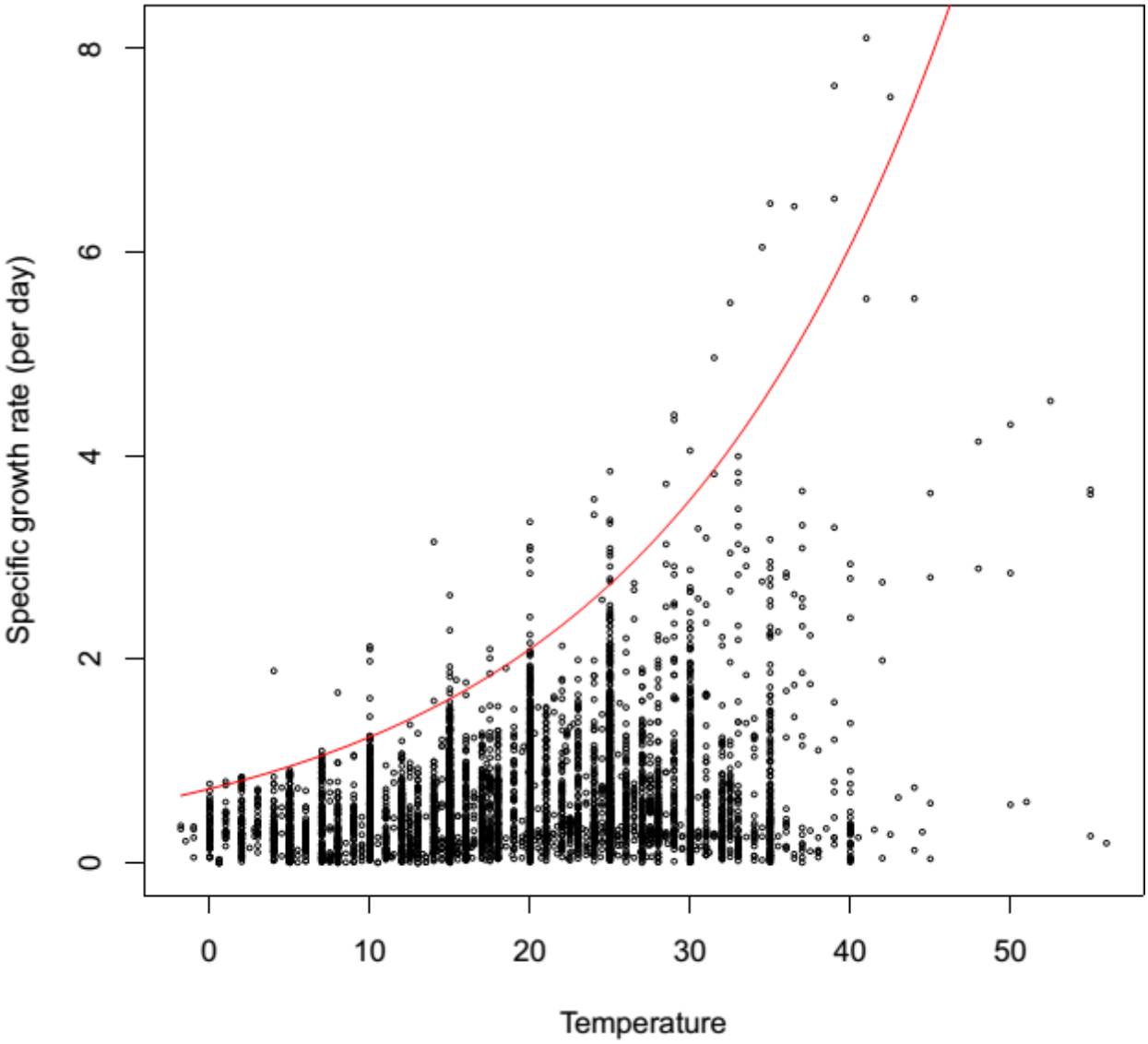

Figure A2.3. The highest attainable growth rate increases exponentially with temperature (Eppley 1972, Bissinger et al. 2008); the red curve represents the 99% quantile, estimated through quantile regression. We re-estimated these parameters using more data than either of the previous studies and found slight differently values: the equation for the above curve is $\mu_{max} = 0.73e^{0.0529T}$

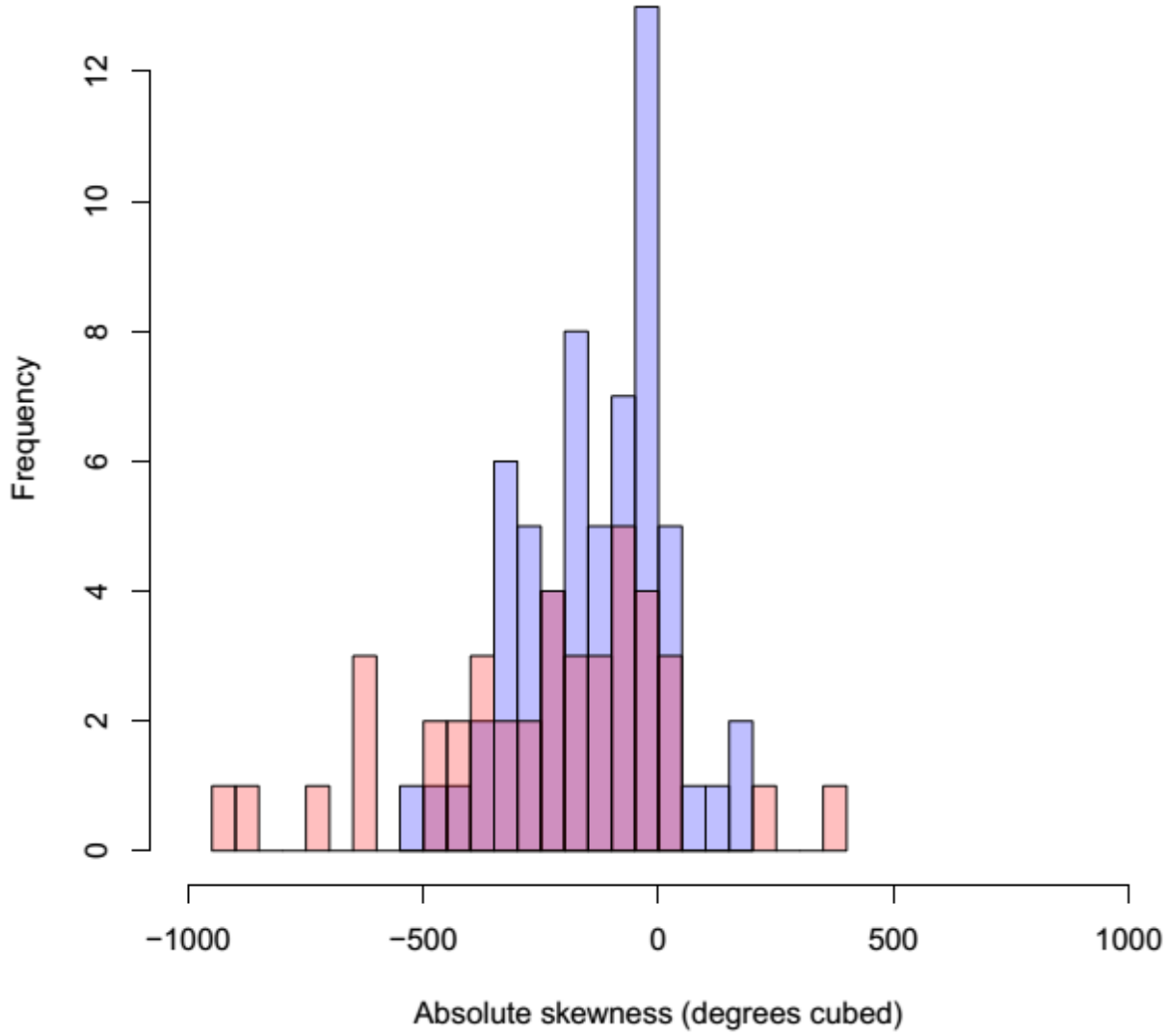

Figure A2.4. Stacked histogram showing differences between absolute skewness of the three major groups between marine and freshwater environments. Blue bars represent marine curves, pink bars represent freshwater curves and purple represents the region of overlap between the two.

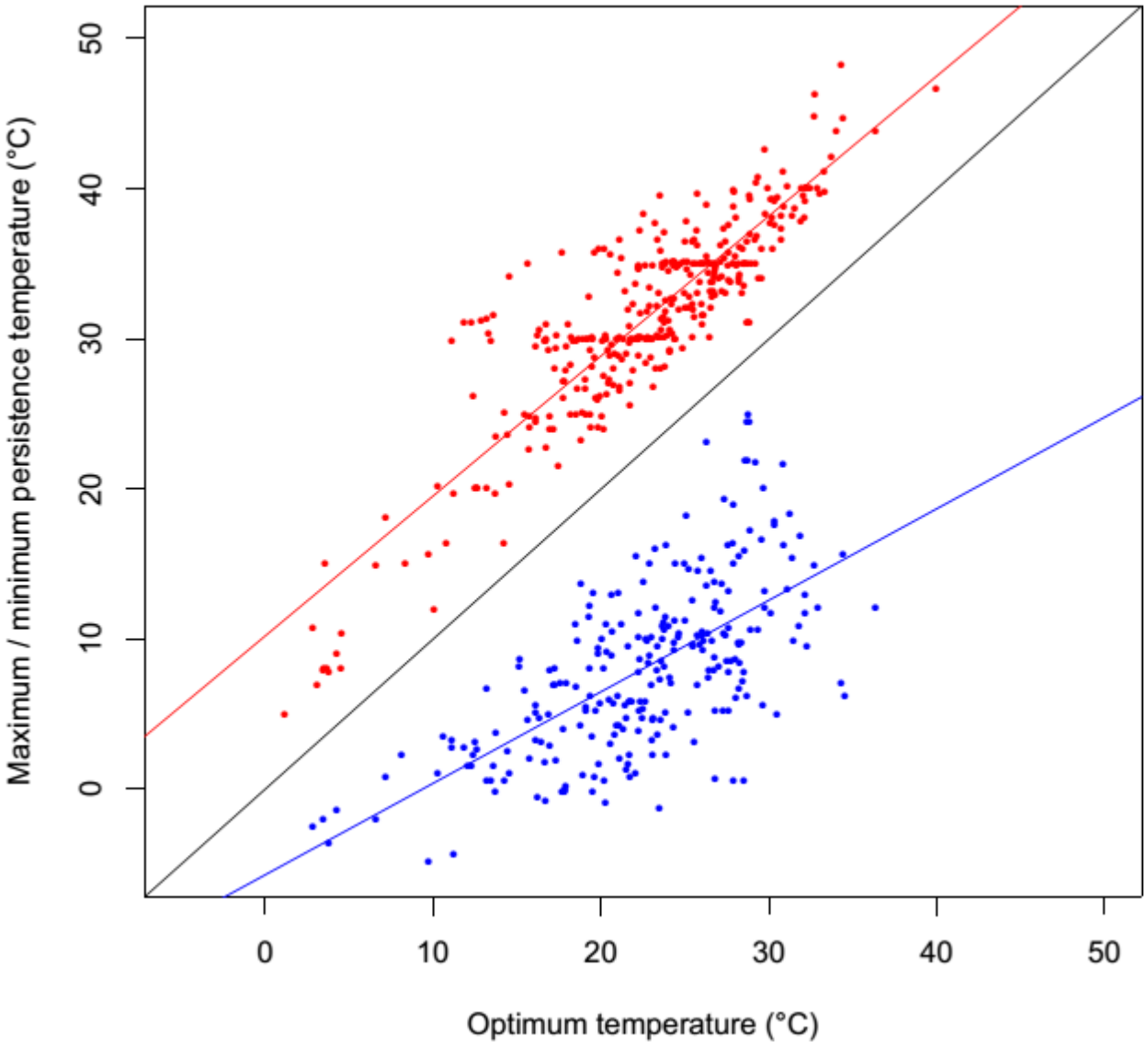

Figure A2.5. Correlations of optimum with Tmax (red points) and with Tmin (blue points). The correlation is stronger with Tmax (r=0.88) than Tmin (r=0.65). Data sets differ for these calculations due to differing quality control criteria (see methods in supporting information).

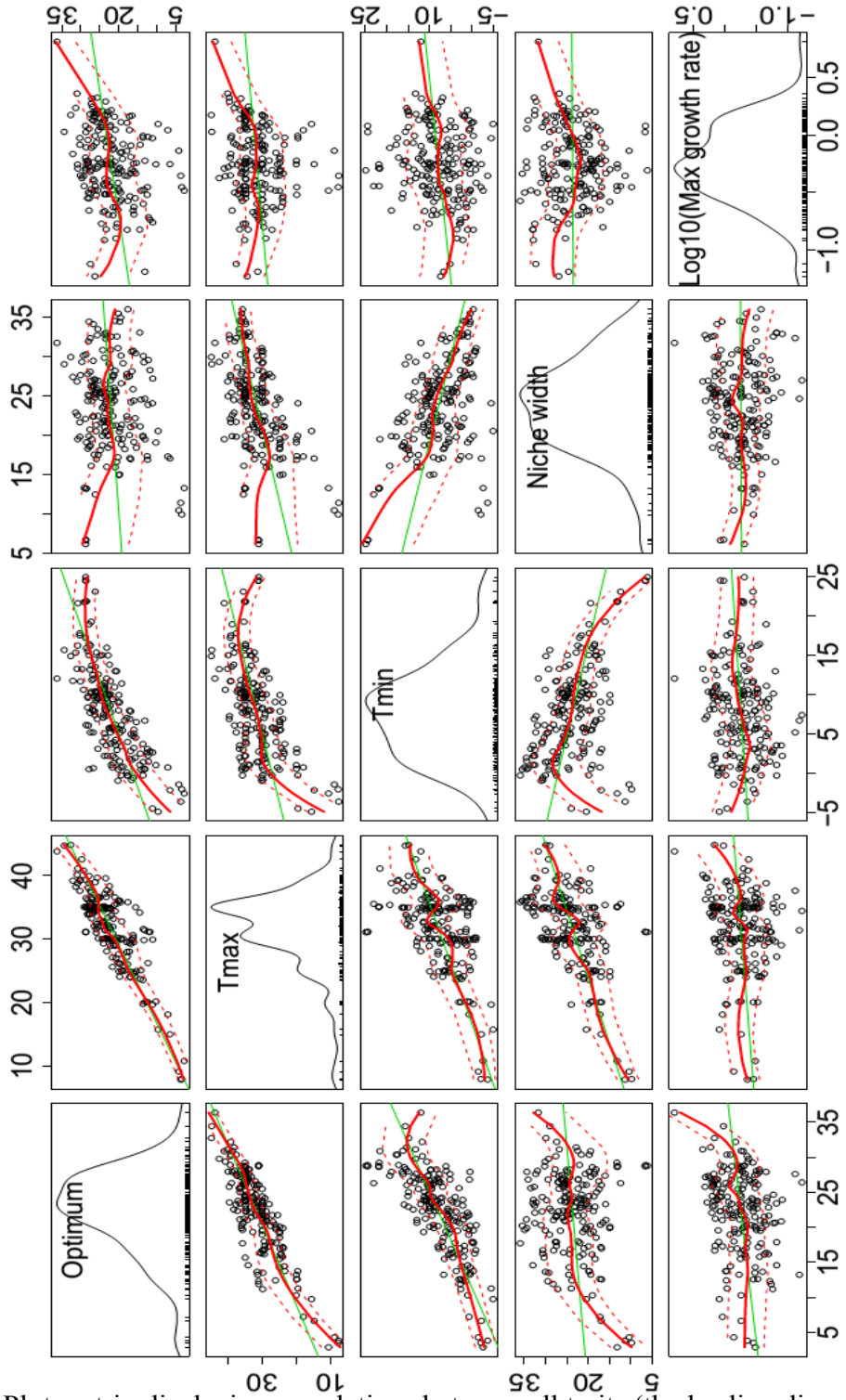

Figure A2.6. Plot matrix displaying correlations between all traits (the leading diagonal displays kernel density plots of the individual traits). As quality control criteria differed between traits, we present the correlations based on a subset that met quality control standards for all traits. Red lines indicate loess fits with confidence bands, while the green line indicates the linear regression between pairs of traits.

Trait	Marginal R^2	Conditional R^2	Model parameters	Bootstrap p value
Optimum	0.50	0.83	quadratic latitude : group	<0.001
			environment	<0.01
Tmax	0.46	0.90	quadratic latitude : group	<0.01
			environment	<0.01
Tmin	0.49	0.82	group : environment	<0.05
			quadratic latitude : group	<0.05
Niche width	0.35	0.77	group : environment	<0.01
Maximum growth rate	0.29	0.61	group	<0.001
			quadratic latitude	<0.05

Table A2.1: Summary of best models for all traits

A

Parameter	Estimate	Lower CI	Upper CI
intercept	26.5467	24.0175	29.0978
quadratic latitude	-0.0015	-0.0024	-0.0007
group (Diatoms)	-0.1609	-3.4952	2.7710
group (Greens)	1.3334	-2.3676	5.0435
environment (freshwater)	3.8090	1.8681	5.8070
quadratic latitude : group (Diatoms)	-0.0027	-0.0039	-0.0016
quadratic latitude : group (Greens)	-0.0014	-0.0025	-0.0002

B

Group	Parameter	Estimate	Lower CI	Upper CI
Dinoflagellates	intercept	26.4628	24.3412	29.1424
	quadratic latitude	-0.0026	-0.0037	-0.0016
Haptophytes	intercept	26.6279	23.3946	29.9625
	quadratic latitude	-0.0035	-0.0050	-0.0023
Coccolithophores	intercept	20.9092	16.5737	25.0050
	quadratic latitude	-0.0006	-0.0016	0.0005
Raphidophytes	intercept	29.0604	23.8873	33.9062
	quadratic latitude	-0.0032	-0.0057	-0.0007

Table A2.2. Optimum temperature model parameters and 95% CI (A) Major groups. (B) Minor groups

Table A2.2 (cont'd)

Desmids	intercept	26.9585	23.5446	30.6439
	quadratic latitude	-0.0010	-0.0023	0.0002
Chrysophytes	intercept	16.4633	11.9309	20.7464
	quadratic latitude	0.0012	-0.0009	0.0035

A

Parameter	Estimate	Lower CI	Upper CI
intercept	33.4557	30.3041	36.9279
quadratic latitude	-0.0008	-0.0018	0.0000
group (Diatoms)	0.1958	-3.2236	3.8296
group (Greens)	5.8543	1.5963	10.4270
environment (freshwater)	3.8311	1.2053	6.3655
quadratic latitude : group (Diatoms)	-0.0030	-0.0040	-0.0019
quadratic latitude : group (Greens)	-0.0026	-0.0040	-0.0011

B

Group	Parameter	Estimate	Lower CI	Upper CI
Dinoflagellates	intercept	32.0365	29.3149	34.5312
	quadratic latitude	-0.0017	-0.0028	-0.0007
Haptophytes	intercept	35.9352	32.5621	39.4186
	quadratic latitude	-0.0048	-0.0064	-0.0032
Coccolithophores	intercept	29.6084	25.3707	33.6613
	quadratic latitude	-0.0007	-0.0016	0.0002

Table A2.3 Maximum persistence temperature model parameters and 95% CIs (A) Major groups. (B) Minor groups.

Table A2.3 (cont'd)

Raphidophytes	intercept	36.3818	30.9516	41.5351
	quadratic latitude	-0.0027	-0.0052	-0.0001
Desmids	intercept	34.7504	33.0835	36.4885
	quadratic latitude	-0.0001	-0.0006	0.0004
Chrysophytes	intercept	21.4145	16.2192	25.6767
	quadratic latitude	0.0031	0.0004	0.0059

A

Parameter	Estimate	Lower CI	Upper CI
intercept	17.3168	14.1401	20.5200
quadratic latitude	-0.0015	-0.0028	-0.0004
group (Diatoms)	-5.6682	-9.8337	-1.5927
group (Greens)	-3.8281	-9.2162	2.1166
environment (freshwater)	-5.1904	-9.3559	-1.2242
quadratic latitude : group (Diatoms)	-0.0027	-0.0045	-0.0007
quadratic latitude : group (Greens)	-0.0009	-0.0025	0.0005
group (Diatoms) : environment (freshwater)	8.1434	2.3836	13.3798
group (Greens) : environment (freshwater)	4.9232	-1.4039	10.2283

B

Group	Parameter	Estimate	Lower CI	Upper CI
Dinoflagellates	intercept	11.5633	9.5285	13.5022
	quadratic latitude	-0.0020	-0.0028	-0.0012
Haptophytes	intercept	11.3954	7.7572	15.0335
	quadratic latitude	-0.0029	-0.0047	-0.0010

Table A2.4. Minimum persistence temperature model parameters and 95% CIs (A) Major groups. (B) Minor groups.

Table A2.4 (cont'd)

Coccolithophores	intercept	8.1271	3.7556	12.7477
	quadratic latitude	-0.0004	-0.0029	0.0018
Raphidophytes	intercept	12.1882	8.3801	16.3253
	quadratic latitude	-0.0022	-0.0041	-0.0006
Desmids	intercept	4.9819	3.1474	6.8701
	quadratic latitude	-0.0002	-0.0008	0.0003
Chrysophytes	intercept	10.5458	1.8248	19.2667
	quadratic latitude	-0.0023	-0.0068	0.0021

A

Parameter	Estimate	Lower CI	Upper CI
intercept	15.8823	11.8629	19.9610
group (Diatoms)	7.6336	3.2771	12.0995
group (Greens)	6.2223	0.5540	11.0534
environment (freshwater)	11.2912	6.2503	16.3909
group (Diatoms) : environment (freshwater)	-7.3054	-14.7441	0.1127
group (Greens) : environment (freshwater)	-8.1436	-14.7857	-1.6023

B

Group	Parameter	Estimate	Lower CI	Upper CI
Dinoflagellates	intercept	21.6338	20.0439	23.3714
Haptophytes	intercept	19.7885	14.8453	24.7316
Coccolithophores	intercept	20.9271	18.4952	23.3607
Raphidophytes	intercept	23.4735	20.8584	26.1418
Desmids	intercept	31.5671	29.6268	33.3960
Chrysophytes	intercept	20.6125	15.9919	25.2330

Table A2.5. Temperature niche width model parameters and 95% CIs (A) Major groups. (B) Minor groups

Parameter	Estimate	Lower CI	Upper CI
Intercept	-0.4025	-0.7417	-0.0930
quadratic latitude	-0.000025	-0.000047	-0.000002
group (Diatoms)	0.4727	0.1227	0.8242
group (Green algae)	0.3196	-0.0014	0.6674
group (Coccolithophores)	0.3076	-0.0724	0.7220
group (Haptophytes)	0.2953	-0.0567	0.6547
group (Cyanobacteria)	0.1994	-0.1328	0.5395
group (Raphidophytes)	0.0536	-0.3207	0.3899
group (Dinoflagellates)	0.0354	-0.2962	0.3605
group (Chrysophytes)	0.0265	-0.3473	0.4024
group (Desmids)	0.0046	-0.3374	0.3654

Table A2.6. Maximum growth rate model parameters and 95% CIs: all groups

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