Comparison of thermal traits between non-toxic and potentially toxic marine phytoplankton: Implications to their responses to ocean warming

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HIGHLIGHTS

- Our growth experiments revealed that temperature traits were not dependent on the toxicity of phytoplankton, except for optimal temperature and critical thermal maxima.
- Based on our independent analysis of the published datasets of growth responses to temperature, the maximum growth rates, cardinal temperatures, fundamental thermal niche, and skewness were comparable between non-toxic and potentially toxic phytoplankton.
- We found that most marine phytoplankton were thriving within the thermal safety zone in the present climate scenario; however, non-toxic species were more vulnerable to warming than potentially toxic species in a future climate scenario.

ABSTRACT

Understanding the effect of temperature on growth in marine phytoplankton is crucial in predicting the biogeography and phenology of algal blooms in the warming ocean. Here, we investigated the temperature dependence of the growth of non-toxic and potentially toxic marine phytoplankton. Using non-toxic strains (*Prorocentrum* sp. NRR 188, *Prorocentrum micans* CCAP 1136/15, and *Alexandrium tamutum* PARALEX 242) and potentially toxic strains (*Prorocentrum minimum* Poulet, *Prorocentrum lima* CCAP 1136/11, and *Alexandrium minutum* PARALEX 246) of dinoflagellates as test organisms, we measured their growth rates along a wide temperature gradient and estimated their maximum growth rates, thermal traits (e.g. thermal optima (T_{opt}), critical thermal minima (CT_{min}), critical thermal maximum (CT_{max}), fundamental thermal niche (*FTN*), and skewness), thermal sensitivity, and warming vulnerability. To allow a comparison of these traits with an adequate number of observations, we independently analyzed datasets compiled from published laboratory experiments. Our experiments revealed that the temperature traits were independent of the toxicity of phytoplankton, except for T_{opt} and CT_{max}. Also, the results of the analysis of the published datasets showed that maximum growth rates and thermal traits were comparable between non-toxic and potentially toxic phytoplankton. Our findings suggest that non-toxic and potentially toxic phytoplankton have generally comparable temperature traits that they can use to respond to climate change. However, depending on the climate scenario, non-toxic phytoplankton may be more vulnerable to warming than potentially toxic phytoplankton. Further studies are needed to improve our understanding of the response of marine phytoplankton to temperature, which can advance our ability to predict algal blooms in response to ongoing climate change.

Keywords: microalgal ecophysiology, thermal physiology, thermal performance, growth models, growth experiment, toxic microalgae

1. INTRODUCTION

Phytoplankton are ecologically important as primary producers and biological carbon pump regulators (e.g. Behrenfeld et al., 2006; Falkowski, 2012; Falkowski and Oliver, 2007). However, some phytoplankton species may form harmful algal blooms (HABs) that are a global problem due to the production of toxins that pose a risk to public health, the environment, and our economy (Berdalet et al., 2015). Toxic blooms are already a global problem and their current distribution is alarming. Climate change may provide favorable conditions for toxic algae to occur (Hallegraeff, 2010). Toxic blooms and their impacts may likely be exacerbated in the future when their duration, intensity, and frequency may increase in response to changes in the climate (Moore et al., 2008; Tatters et al., 2013). The well-documented effects of toxins on humans and other organisms (Berdalet et al., 2015) and the potential effect of climate change on toxic blooms in the future (Fu et al., 2012) have stimulated studies on the ecophysiology of toxic phytoplankton (e.g. Kellmann et al., 2010; Perini et al., 2014; Ramsey et al., 1998; Stüken et al., 2011). Hence, it is crucial to be able to assess the sensitivity of non-toxic and toxic species to changes in the temperature, which is projected to increase under climate change (IPCC, 2013).

Temperature is one of the most fundamental abiotic factors that influence the growth of phytoplankton (Boyd et al., 2013; de Boer et al., 2004). Increasing temperature enhances growth until it reaches the optimal temperature, while elevated temperature beyond the optimal decreases growth and can be lethal. These thermal responses characterize the typical asymmetry of the growth-temperature curve (also known as the thermal performance curve or the thermal reaction norm), with an asymptotic increase on the colder side, and an abrupt

decline on the warmer side (Ras et al., 2013). Thermal performance curves are often unimodal and negatively skewed in ectotherms (Eppley, 1972; Kingsolver, 2009; Knies and Kingsolver, 2010). The shape of the curves reflects the effect of temperature on the enzymatic rate process and enzyme activation and stability at high temperatures (Knies and Kingsolver, 2010). Growth rates increase gradually with increasing temperature below the thermal optimum (T_{opt}), which is attributed to the exponential increase of the reaction rates with increasing temperature following the Arrhenius kinetics (Arrhenius, 1915). On the other hand, the growth rate decreases with a further increase in temperature above T_{opt}, which is attributed to the denaturation of essential proteins (Hochachka and Somero, 2002). The variability in the trends in growth below or above T_{opt} can be explained by the probability of the activation of rate-limiting enzymes that declines at high and low temperature (Knies and Kingsolver, 2010; Ratkowsky et al., 2005).

Several non-linear models have been used to describe the growth response to temperature (Eppley, 1972; Low-Décarie et al., 2017; Rosso et al., 1993). These models are also used to predict the maximum growth rate (r_{max}) and the thermal traits such as the (i) the cardinal temperatures that correspond to the boundaries of thermal tolerance (i.e. thermal optima (T_{opt}), critical thermal minima (CT_{min}), and critical thermal maximum (CT_{max}), and (ii) the fundamental thermal niche breadth (*FTN*) that correspond to the thermal range on which a species can physiologically tolerate. The temperature range is species-specific that reflects the physiological plasticity of species in response to changes in temperature (de Boer et al., 2004). These thermal traits can be used to infer (i) thermal safety margin (*TSM*) – which measures the difference between a species' thermal tolerance and the temperatures it experiences in the environment (Sunday et al., 2014) – and (ii) warming

vulnerability (V) – that estimates the number of the year prior the local temperatures are expected to exceed CT_{max} in a given location (Bennett et al., 2019). Temperature traits, thermal safety margin, and warming vulnerability provide important information to understand how phytoplankton will respond to ocean warming.

Several studies have examined the effect of temperature on phytoplankton growth rate (e.g., Thomas et al., 2012). However, the differences in the thermal responses between non-toxic and toxic phytoplankton species have not been extensively studied yet. To address this research gap, we conducted growth experiments and analyzed data from multiple studies to determine whether non-toxic and potentially toxic marine phytoplankton exhibit variations in temperature traits.

2. MATERIALS AND METHOD

2.1. Test organisms

Six cultures of dinoflagellate strains were obtained from different culture collections (Table 1). They are ecologically relevant organisms belonging to the phytoplankton genera that make up the majority of the toxic bloom-forming species, i.e. *Prorocentrum* and *Alexandrium* (Abdenadher et al., 2012; Ben-Gharbia et al., 2016; Grzebyk et al., 1997; Quilliam et al., 1996; Vlamis et al., 2015). Three of the strains are listed as “toxic” from their respective culture collections but only one strain was detected for the presence of toxins (e.g. okadaic acid (*OA*) and dinophysistoxins (*DTX1 and DTX2*)), henceforth all of these strains were referred as potentially toxic. Another three strains congeneric to the potentially toxic strains were non-toxic. To minimize the effect of the differences in the source’s culture conditions, all strains were maintained in 35 mL batch cultures in artificial seawater (*ASW*) (Berges et al., 2001) enriched with *K* minimum nutrients (Keller et al., 1987).

Cultures were regularly transferred to a fresh *K* medium to maintain exponential growth. The cultures were not axenic. To minimize contamination, all *ASW* and *K* media were autoclaved, and all transfers were performed in a class II biosafety cabinet. The batch cultures were maintained at a constant temperature of 15°C and under a 12:12 hour light-dark cycle at a mean light intensity (± standard error) of 221 ± 12 µmol m^{-2} s^{-1}, measured using a light meter (Li-Cor Li-250A). They were allowed to grow at this condition for at least four transfers before experimental procedures.

2.2. Growth experiments

Plate- and tube-based experiments (Fig. 1; Supplementary Table S1**)** were designed to examine the growth of non-toxic and toxic marine phytoplankton across a wide range of temperatures.

2.2.1. Plate-based experiments

In the plate-based experiments, the temperature gradient was maintained using thermoblocks that were housed in separate growth chambers (Conviron Adaptis CMP6010) with similar growth conditions, except for the air temperature which needed to be different to achieve the desired thermal gradient. Each of the thermoblocks was custom-made metal blocks that were temperature-regulated with flow-through fluid. The temperature gradient of the thermoblock was regulated by the flow of fluid to an external cooling or heating device connected via insulated flexible PVC hoses. At one end of the block, a water bath chiller was used as a cooling device to circulate antifreeze fluid. Whereas, a water bath was used as a heating device to circulate distilled water at the other end of the block. Temperature set points for external cooling and heating devices are adjusted to attain the desired

temperature gradient and stepwise variation in each thermoblock (Supplementary Table S1).

To determine the thermal growth response in each experimental organism, three replicates of 0.2 mL of each of the cultures were inoculated into 1.8 mL *K* medium in each well of the first three rows of the 24-well microplates. Wells in the last row were inoculated with *K* medium to serve as blank. Algal cells in the microplates were incubated in the above-mentioned plate-based thermoblocks for nine days. The microplates were covered with lids with pores that were sheathed with polyvinylidene chloride gas-permeable membranes to ensure gas exchange during the incubation period and were removed aseptically every growth measurement.

Growth rates were quantified from the changes in cell density that were estimated from the optical density (*OD*) measured daily (between 14:00 to 16:00) for nine days using a FLUOstar Omega spectrophotometer (BMG Labtech, Germany) with the following endpoint protocol settings: excitation of 660 nm that corresponds to the long wavelength absorption peak of chlorophyll *a*, horizontal bidirectional reading (start top left), and shaking with a frequency of 400 rpm for 60 seconds before plate reading to homogenize the sample.

OD values were blank corrected and were pre-processed to detect outliers before regression analyses. A total of 324 triplicated observations (36 assay temperatures x 9 days) for every experimental organism were obtained and were quality controlled. The data were trimmed to capture growth within the exponential phase. These pre-processed data were used subsequently in the regression analyses to estimate the growth rates.

2.2.2. Tube-based experiments

Tube-based experiments were performed inside a growth chamber with conditions described in Supplementary Table S1. The thermal gradient in these experiments ranged from 5°C to 30°C at 5°C stepwise variations. Each assay temperature was maintained inside a glass water-jacketed bath using circulating distilled water. The temperature of the circulated distilled water was regulated by external recirculating water baths connected via flexible PVC hoses.

Triplicates of 4 mL of each of the cultures were inoculated into 36 mL *K* medium contained in 50 mL glass test tubes. The tubes were capped with autoclaved foam stoppers to allow gas exchange during the incubation period. Algal cells in the test tubes were incubated in the above-mentioned temperature-regulated water-jacketed bath. Two tube-based experiments were performed. In the first experiment, the cells were incubated for 16 days without stepwise acclimatization. While in the second experiment, the strains were allowed to acclimatize to a new thermal condition for 14 days before the incubation to another 14 days of incubation.

Growth of the cultures was determined using *in vivo* fluorescence as a proxy for phytoplankton biomass, which was measured daily (between 14:00 to 16:00) using a Turner Designs Trilogy Fluorometer. Before the fluorescence measurement, each culture in a test tube was homogenized using a vortex mixer. The test tube was subsequently placed in the fluorometer and a fluorescence reading was obtained. The estimated fluorescence in all samples was corrected with the fluorescence in a blank sample (i.e. 0.04). The corrected estimates of fluorescence were used to compute the growth rates as described in the section below.

2.3. Determination of growth rates and thermal traits

The natural log of *OD* or the fluorescence estimates were fitted against time in a linear model to estimate the growth rate. Only the positive growth rates were included in the subsequent analysis. The growth rates were fitted against temperature in a unimodal response curve using the different non-linear functions (i.e. equ04 – equ15 in the R package *temperatureresponse* (Low-Décarie *et al.* 2017) and Cardinal Temperature Model with Inflexion (*CTMI*; equ16) (Rosso et al., 1993)) presented in Supplementary Table S2.

A modified Levenberg–Marquardt algorithm was used for the robust fitting of non-linear equations to data (Low-Décarie *et al.* 2017). The starting values were estimated from the dataset when the equation parameter values represent features of the dataset, otherwise, the starting values for the parameters were derived the fitted parameters from the source publication of the equation or were set to ensure a downward parabola-like shape. Equations were ranked on each dataset using the Bayesian information criterion (BIC). Similar results of the ranking of equations were observed when other measures of model quality were used such as the Akaike information criterion (AIC) and the AIC corrected for finite sample sizes (AICc).

These non-linear models were used to estimate the following thermal traits: (1) the maximum growth rate (r_{max}, d^{-1}; the highest growth rate within the temperature range), (2) the cardinal temperatures such as the thermal optimum (T_{opt},°C); temperature that corresponds to r_{max}), critical thermal minimum (CT_{min},°C; the lowest temperature at which no positive growth), and critical thermal maximum (CT_{max},°C; the highest temperature at which no positive growth), and (3) the fundamental thermal niche breadth (*FTN*,°C; the width of the temperature range). The skewness of the curve was also calculated as the difference between activation and deactivation rates, which were derived from the mean value of the derivative across

sub- (CT_{min} to T_{opt}) and supra- (T_{opt} to CT_{max}) optimal temperatures, respectively. The skewness was used as a measure of the asymmetry of the thermal growth curve. A positive skew indicates activation is steeper than deactivation, whereas a negative skew indicates that deactivation is steeper than activation.

To obtain an adequate number of observations, this study also analyzed the datasets of published experimental results on marine phytoplankton growth rates across temperatures (Litchman and Klausmeier, 2014; Thomas et al., 2016, 2012). This dataset contains growth responses to temperature in 545 phytoplankton strains/isolates from the major phytoplankton groups, and 74 of the isolates represent 25 potentially toxic species. The strains in this dataset were isolated from 76 deg N to 76 deg S, which gives us a broad geographic coverage (Fig. 2). The species in the dataset that were listed in the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Moestrup et al., 2009) were categorized as potentially toxic, otherwise they are categorized as non-toxic. Out of 545 phytoplankton strains/isolates in the dataset, 74 of which represent 25 potentially toxic species, and about 20% belong to the same taxonomic class as the experimental organisms in this study.

To simplify the results, trait estimates were averaged across models weighted by BIC median rank. All the mean estimates derived from our experiments and published experimental data were pooled and curated to exclude unrealistic estimates of thermal traits with the following inclusion criteria (1) r_{max} within the 0.01 to 3.00 d^{-1} range, and (2) cardinal temperatures within the -7 to 40 °C range.

2.4. Determination of thermal safety and vulnerability

Longitude and latitude coordinates were approximated based on the isolation location of the strains. These coordinates were used to determine the sea surface temperature (*SST*) of the coldest and warmest months from 2000 to 2014, which were downloaded from *Bio-ORACLE* (Assis et al., 2018). The *SST* was used to represent the ambient temperature extremes that the strains experience in their local habitats (H_{min} and H_{max} in °C, respectively). The difference between a strain's critical thermal limits (CT_{min} and CT_{max}) and the temperature extremes it experiences represent its sensitivity to cold and warm temperatures (S_{min} and S_{max} in °C, respectively) (Bennett et al., 2019). The thermal sensitivity was used to infer the species' thermal safety margin (*TSM*). A positive *TSM* ($CT_{min} < H_{min}$, hence $S_{min} < 0$; $CT_{max} > H_{max}$, hence $S_{max} > 0$) suggests that a species has physiological thermal safety, whereas a negative *TSM* ($CT_{min} > H_{min}$, hence $S_{min} > 0$; $CT_{max} < H_{max}$, hence $S_{max} < 0$) indicates that a species has to avoid the extreme temperatures or else it is at risk of thermal danger (Sunday et al., 2014). Warming vulnerability (*V*, year) describes the number of years prior the local temperatures are expected to exceed CT_{max} in a given location (Bennett et al., 2019). This was calculated by dividing the species' sensitivity to warm temperature (S_{max}) by the warming rate (*WR*, °C per year) it experiences in a given location. *WR* was derived from the slope of SST of the warmest month between the contemporary and future climate scenarios (i.e. SST predicted in 2050 and 2010 based on *RCP 2.6* and *RCP 8.5*, which were also downloaded from *Bio-ORACLE* (Assis et al., 2018)). Thermal sensitivity, exposure, and vulnerability in the studied *Prorocentrum minimum* strains were not determined because their isolation locations were unknown.

2.5. Data processing and analyses

All data processing and analyses were conducted in R version 4.2.1 (R Core Team, 2022) and implemented in RStudio version (RStudio Team, 2022). Descriptive statistics i.e. minimum, maximum, mean, and standard error (SE) were determined for each trait, and the mean ± SE is reported throughout. Linear mixed models (LMM) were used to analyze the variation using the *lmer* function in lme4 package in R (Bates et al., 2015). The variation in the response variables (i.e. maximum growth rates, thermal traits, thermal sensitivity, and warming vulnerability) between non-toxic and potentially toxic species was analyzed. For the models in our experiments, we take into account the random effects of strain identity and the source of the experimental data (i.e. *lmer(response ~ toxicity + (1|strain) + (1|experiment)*). For the models in the analysis of the published laboratory experiments, we only take into account the random effect of the source of the experimental data (i.e. *lmer(response ~ toxicity + (1|experiment))*). The later model structure was used to compare thermal sensitivity and warming vulnerability between non-toxic and potentially toxic phytoplankton. All the LMMs were compared to a null model using the likelihood ratio (*LR*) test to determine the significance of a single factor by comparing the fit for models with and without the factor (Table S3 – S5).

3. RESULTS

3.1. Thermal performance curves

Our experiments revealed the sensitivity of growth rates of non-toxic and potentially toxic phytoplankton strains to temperature (Fig. 3). Generally, the growth rate increased gradually with temperature until it reached its peak at the optimal

temperature, and it decreased substantially with a further increase in temperature. The shapes of the thermal performance curves were generally negatively skewed.

3.1.1. Non-toxic strains

Prorocentrum sp. NRR 188 had a maximum growth rate of 0.18 ± 0.01 d^{-1} at 19.53 ± 0.59 °C. This strain had a thermal niche breadth of 25.54 ± 1.00 °C and could grow from 5.10°C to 30.65°C. The strain had a skewness of the curve of -0.20 ± 0.04.

The maximum growth rate of *P. micans* CCAP 1136/15 was 0.16 ± 0.01 d^{-1} at 16.45 ± 0.77 °C. This strain had a thermal niche breadth of 25.30 ± 0.80 °C and could grow between 4.27 °C and 29.57 °C. The skewness of the curve in this strain was estimated to be zero (0.002 ± 0.001).

A. tamutum RCC3034 had a maximum growth rate of 0.20 ± 0.01 d^{-1}. It could grow optimally at 19.62 ± 0.46 °C. It had a thermal niche breadth of 25.23 ± 0.74 °C and could grow between 4.41 °C and 29.63 °C. The strain had a skewness of the curve of -0.36 ± 0.09.

3.1.2 Potentially toxic strains

The maximum growth rate of *P. minimum* RCC291 was 0.28 ± 0.03 d^{-1} at 23.16 ± 0.47 °C. This strain had a thermal niche breadth of 26.23 ± 0.45 °C and could grow between 4.52 °C and 30.75 °C. The skewness of the curve in this strain was -1.03 ± 0.33.

P. lima CCAP 1136/11 had a maximum growth rate of 0.11 ± 0.02 d^{-1}. The optimal temperature for growth in this strain was 19.68 ± 0.79 °C. This strain could

grow from 4.73 °C to 30.44 °C. It had a thermal niche breadth of 25.71 ± 0.64 °C. The strain had a skewness of the curve of -0.06 ± 0.02.

A. minutum RCC2649 had a maximum growth rate of 0.24 ± 0.004 d^{-1}. It could grow optimally at 21.94 ± 0.46 °C. It had a thermal niche breadth of 26.36 ± 0.52 °C and could grow between 4.44 °C and 30.80 °C. The strain had a skewness of the curve of -0.63 ± 0.17.

3.2. Variation of maximum growth rates and thermal traits

Based on our experiments, we observed that non-toxic and potentially toxic strains did not differ in maximum growth rate and thermal traits, except in T_{opt} ($\chi^2_{(1, N=54)}$ = 4.30, p = 0.038) and CT_{max} ($\chi^2_{(1, N=54)}$ = 4.02, p = 0.045). Similarly, our analysis of the published laboratory experiments revealed no significant differences in these temperature traits between non-toxic and potentially toxic phytoplankton (Fig. 4).

3.2.1. Maximum growth rate

The maximum growth rate in non-toxic phytoplankton (0.98 ± 0.03 d^{-1}) was twice higher than the estimate in potentially toxic phytoplankton (0.53 ± 0.07 d^{-1}), but the difference was not significant. This trait did not show dependence on toxicity ($\chi^2_{(1, N=264)}$ = 3.28, p = 0.07). Most potentially toxic strains in our experiments had r_{max} close to the median, except for *P. lima* of which the estimate was within the first quartile of the distribution (Fig. 4A). Estimates of r_{max} in all non-toxic strains in our experiments were near the lower limit of the distribution.

3.2.2. Thermal optimum

Thermal optimum in non-toxic phytoplankton (19.42 ± 0.47 ºC) was similar to the estimate in potentially toxic phytoplankton (21.92 ± 1.49 ºC) and no dependence of T_{opt} on toxicity was observed ($\chi^2_{(1, N=264)} = 1.37$, p = 0.24). T_{opt} in non-toxic strains in our experiments was lower than the median, whilst all of the potentially toxic strains, except for *P. lima*, were higher than the median value (Fig. 4B).

3.2.3. Critical thermal limits

Non-toxic phytoplankton had lower critical thermal minimum and maximum (4.41 ± 0.35 ºC and 26.28 ± 0.59 ºC, respectively) than the estimated values in potentially toxic phytoplankton (5.32 ± 0.97 ºC and 29.77 ± 1.56 ºC, respectively), but no significant variation in the traits was found. Both critical thermal limits were not dependent on the toxicity of phytoplankton (CT_{min} : $\chi^2_{(1, N=264)} = 0.96$, p = 0.33; CT_{max} : $\chi^2_{(1, N=264)} = 1.32$, p = 0.25). The critical thermal limits in all strains in our experiments were within the 25% - 75% percentile (Fig. 4C and 4D).

3.2.4. Fundamental thermal niche

The fundamental thermal niche in non-toxic phytoplankton (21.87 ± 0.50 ºC) was comparable to the estimated value in potentially toxic phytoplankton (24.46 ± 1.33 ºC). *FTN* did not exhibit dependence on the toxicity of the phytoplankton ($\chi^2_{(1, N=264)} = 0.34$, p = 0.56). All strains in our experiments had *FTN* near the median, except for *P. micans* which had *FTN* above the 75% percentile (Fig. 4E).

3.2.5. Skewness

Skewness of the thermal performance curves in non-toxic and potentially toxic phytoplankton was negative (-0.22 ± 0.05 and -0.24 ± 0.11, respectively) and

did not show dependence on toxicity ($\chi^2_{(1, N=197)} = 0.12$, $p = 0.73$). The skewness of the TPC in all strains in our experiments was within the second and third quartile (Fig. 4F).

3.3. Thermal safety and vulnerability

The majority of the phytoplankton had higher critical thermal maxima (CT_{max}) than the maximum *SST* projected in 2050 and 2010 at different climate scenarios (RCP 2.6 and RCP 8.5) (Fig. 5). About 80% of the marine phytoplankton (78% of the non-toxic strains and 100% of the potentially toxic strains) had CT_{max} higher than the environmental temperature projected in 2050 at RCP 2.6, with the mean difference of 7.25 ± 0.31 °C (Fig. 5A). The remaining 20% of the marine phytoplankton (all were non-toxic) had mean CT_{max} that was 8.56 ± 0.98 °C lower than the projected local environmental temperature. Similar observations were found in the projections in 2050 at RCP 8.5 (Fig. 5B) and 2100 at RCP 2.6 (Fig. 5C). However, a noticeable difference in the statistics was observed for the projections in 2100 at RCP 8.5 (Fig. 5D). Approximately, 71% of the marine phytoplankton (69% of the non-toxic strains and 90% of the potentially toxic strains) had CT_{max} higher than the environmental temperature projected in 2100 at RCP 8.5, with the mean difference of 5.69 ± 0.31 °C. The remaining 29% of the marine phytoplankton (31% of the non-toxic strains and 10% of the potentially toxic strains) had a mean CT_{max} that was 7.99 ± 0.87 °C lower than the projected local environmental temperature in 2100 at RCP 8.5.

The majority of the phytoplankton strains had lower CT_{min} and higher CT_{max} than the local minimum and maximum *SST*, respectively. As a result, they had sensitivity to cold (S_{min}) and sensitivity to warm (S_{max}) temperatures below and above zero, respectively, occupying the thermal safety zone. About 58.33% of the strains

had thermal safety, whereas the remaining 41.67% were at risk of cooling (23.41%), warming (15.08%), or both (3.17%).

S_{min} and S_{max} in non-toxic phytoplankton (-4.27 ± 0.40 °C and 5.21 ± 0.52 °C, respectively) were comparable to the estimate in potentially toxic phytoplankton (-5.54 ± 0.63 °C and 11.15 ± 0.78 °C, respectively) (Fig. 6A and 6B). These traits did not exhibit dependence on the toxicity of phytoplankton (S_{min} : $\chi^2_{(1, N=276)} = 2.17$, p = 0.14; S_{max} : $\chi^2_{(1, N=276)} = 0.27$, p = 0.60).

Fig. 6C and 6D present the vulnerability to warming of non-toxic and potentially toxic phytoplankton in *RCP 2.6* and *RCP* 8.5 climate scenarios. Vulnerability to warming at *RCP* 2.6 climate scenario was dependent to toxicity ($V_{2.6}$: $\chi^2_{(1, N=227)} = 7.59$, p = 0.0059). The mean estimate of $V_{2.6}$ in non-toxic phytoplankton (913 ± 45 years) was lower compared to the value in potentially toxic phytoplankton (1154 ± 106 years). However, no significant difference in $V_{8.5}$ was found between groups ($V_{2.6}$: $\chi^2_{(1, N=229)} = 3.76$, p = 0.052). The local maximum temperature was projected to exceed the CT_{max} of non-toxic phytoplankton after 247 ± 13 years at *RCP* 8.5 climate scenarios, which was similar to the projections in potentially toxic phytoplankton, i.e. 299 ± 20 years.

4. DISCUSSION

4.1 Comparison of thermal traits

Generally, our analysis of the datasets of growth responses to temperature revealed that the maximum growth rates, cardinal temperatures, fundamental thermal niche, and skewness did not show dependence on the toxicity of

phytoplankton. This suggests that non-toxic and potentially toxic phytoplankton have comparable temperature traits that they can exploit in response to ocean warming.

Variations in the thermal traits cannot be explained by the toxicity of phytoplankton. However, these traits can vary among strains and experiments, suggesting that these traits are dependent on physiological plasticity and evolutionary history (Kremer et al., 2017; Thomas et al., 2016, 2012). Interspecific and intraspecific variations in growth rates and thermal traits of marine phytoplankton have been demonstrated in several studies (Boyd et al., 2013; Chen and Laws, 2016; Kremp et al., 2012; Thomas et al., 2016). Species that are heat stress-sensitive have narrow thermal tolerance limits, while those that can survive through acclimation or adaptation have a wider range (Chen, 2015). Most of the species exhibited a negatively skewed pattern of their thermal growth curve suggesting that their growth is more sensitive to warming than cooling, which is an important trait given the projected change in temperature in the next decades. Few species exhibit a less skewed curve (i.e. nearly symmetrical), a trait characterized by a constant growth over an optimal temperature range that decreases at extreme temperatures at similar rates. The symmetrical thermal growth curve suggests that the growth of the species is equally sensitive to decreasing and increasing temperature from the T_{opt}. The differences in the traits among species and strains imply that the phytoplankton community composition may be altered in response to environmental change, such as ocean warming.

4.2. Vulnerability to ocean warming

The findings showed that nearly all the non-toxic and potentially toxic phytoplankton were thriving within the thermal safety zone in the present climate

scenario. Depending on the climate scenario, non-toxic phytoplankton may be more vulnerable to warming than potentially toxic phytoplankton.

The vulnerability of phytoplankton to warming is attributed to the influence of temperature change on the physiological processes and growth, which consequently alter marine ecosystem structure and function (Regaudie-De-Gioux and Duarte, 2012; Thomas et al., 2012; Toseland et al., 2013). Recent studies have demonstrated the effect of elevated temperature on metabolic and growth rates in phytoplankton (de Boer *et al.*, 2004; Regaudie-De-Gioux & Duarte, 2012; Boyd *et al.*, 2013; Toseland *et al.*, 2013). Typically, photosynthesis rises with elevated temperature until it reaches its optimum, and decreases with further warming; while respiration, on the other hand, increases with increasing temperature. This elevation in metabolic rates is likely to expand the growth rate of photoautotrophs in warming conditions (Hochachka and Somero, 2002). Several species exposed to a high temperature display higher photosynthesis and lower respiration rates, but exhibit a reduction in their cell size (Staehr and Birkeland, 2006). Shrinking their size can neutralize the imbalance between these metabolic processes (Peter and Sommer, 2013). Also, nutrient uptake by phytoplankton becomes strongly limiting at elevated temperatures (Sterner and Grover, 1998). Cell size reduction can improve nutrient uptake rates and lessen metabolic costs, which is a good strategy in response to increasing resource demand due to warming (Atkinson et al., 2006). Furthermore, cyst germination in dinoflagellate is controlled by temperature (Anderson et al., 2005), which may be altered by changing climate. It can be increased under warm conditions and can be inhibited at extreme temperatures (Anderson et al., 2005).

The effect of temperature change on their physiological processes and growth may alter marine ecosystem structure and function. Most marine

phytoplankton are generally living in the present climate scenario within the thermal safety zone. However, the warming temperature may likely exceed the physiological limits of marine phytoplankton species. They must avoid extreme temperatures or else they are at risk of thermal danger. They may either adapt or migrate to new favorable habitats to survive, otherwise, their extinction is inevitable.

4.3. Caveats

We acknowledge the limitation of using only six dinoflagellate strains isolated from limited geographic regions in our experiments. Extrapolation of the thermal response of dinoflagellates as model organisms to the whole phytoplankton is inherently problematic. Because our experiments are limited to dinoflagellates strains (only one strain has been confirmed to be toxic, two are potentially toxic and three are considered non-toxic), it is recommended that future studies should consider conducting laboratory experiments using representatives from the major phytoplankton taxa (i.e. diatoms, haptophytes, and cyanobacteria) with confirmed toxicity. Although the majority of toxic species belong to dinoflagellates, characterization of the thermal response curves in representatives from the other taxa is crucial to advance our knowledge of the taxon-specific differences in the growth thermotolerance between non-toxic and toxic phytoplankton. Analysis of the datasets of published laboratory experiments allows the comparison of thermal growth response between phytoplankton groups with an adequate number of observations with broad geographic coverage.

With our analysis of the datasets of published laboratory experiments, we acknowledge that the multifaceted interference from different protocols implemented across individual studies may also limit the usefulness of the compiled datasets.

However, the experimental results generated in this present study provide the groundwork to evaluate the value of the published datasets in comparing traits between non-toxic and toxic marine phytoplankton. As observed, there is a discrepancy in the findings between the analyses using our results and published experimental results, which may be related to the data quality used in thermal trait analysis. For instance, our experiments reveal that the maximum growth rates in toxic strains are higher than the rates in non-toxic strains of dinoflagellates, which are found to be comparable in the analysis of the pooled datasets. This suggests that the maximum growth rates between non-toxic and toxic phytoplankton are not robust across a range of experimental protocols, which may be attributed to the sensitivity of the trait to light or nutrient conditions (Boyd et al., 2013).

Furthermore, we acknowledge the uncertainties inherent in fitting growth rates with temperature and extracting the traits from the reaction norm. One challenge of modeling the thermal growth response is that there is no single equation that fits all data (Low-Décarie et al., 2017), suggesting that different equations may describe different processes that are still unresolved. Another is the limitation of the statistical uncertainty of the estimation of the thermal physiological limits and thermal niche breadth, as these parameters are frequently extrapolated beyond the data. This limitation constrains our understanding of the responses of non-toxic and toxic phytoplankton to climate extremes. There are also limitations linked with low temperature resolution, incomplete observation of full thermal range, over-representation of non-toxic phytoplankton, and few observations on toxic species that are mostly dinoflagellates.

4.4. Implications and Future Directions

More studies that address the abovementioned limitations are needed to further elucidate the responses of non-toxic and toxic phytoplankton to temperature that is expected to increase with climate change. These studies are important because climate change responses of the non-toxic and potentially toxic phytoplankton have ecological implications. For instance, toxic species may employ thermal acclimation and adaptive strategies to expand their thermal tolerance and toxin production may provide toxic species a selective advantage under future climate scenarios; hence, toxic species may dominate over the non-toxic species in the changing climate. Warming may provide favorable conditions for harmful algae, including toxic ones to occur (Brandenburg et al. 2019). Toxic blooms and their impacts may likely be exacerbated in the future when their duration, intensity, and frequency may increase in response to changes in the climate. The possible impacts of climate change on toxic blooms have important implications on how to manage and control harmful algal blooms (HAB) in the future. Furthermore, we need more studies to improve our predictive understanding of the ecological responses of non-toxic and toxic marine phytoplankton to future climate scenarios. For instance, the thermal performance curves (*TPC*) obtained in our experiments can be used to develop a mechanistic model to establish a causal relationship between species distribution and temperature. This mechanistic model is useful in predicting climate-induced ecological trends such as changes in range, habitat suitability, diversity, and community composition.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

This work was supported by a doctoral fellowship from the University of the Philippines awarded to Brisneve Edullantes.

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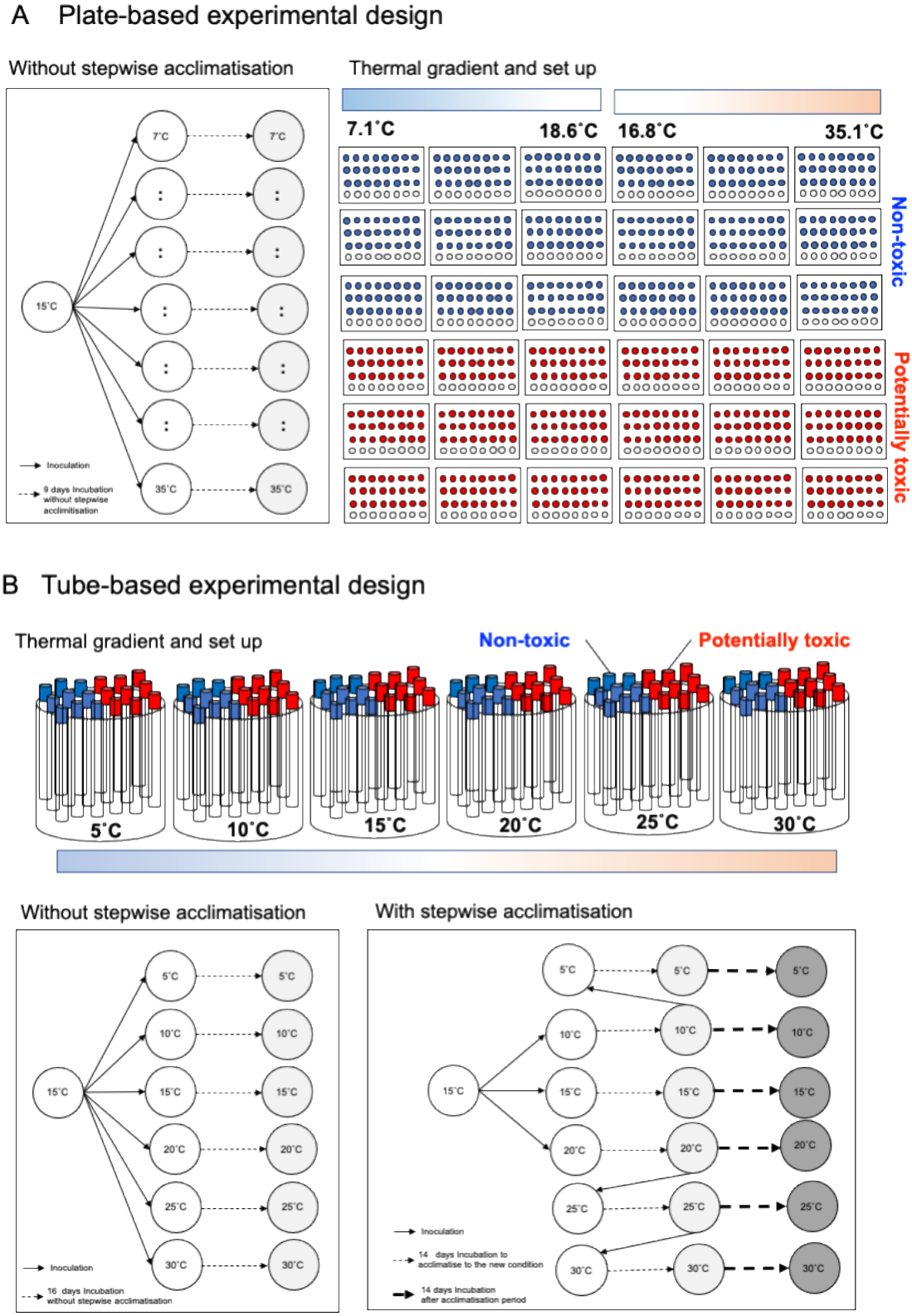

Fig. 1 Schematic representation of the plate- and tube-based experimental designs to examine the effect of temperature on growth in marine phytoplankton.

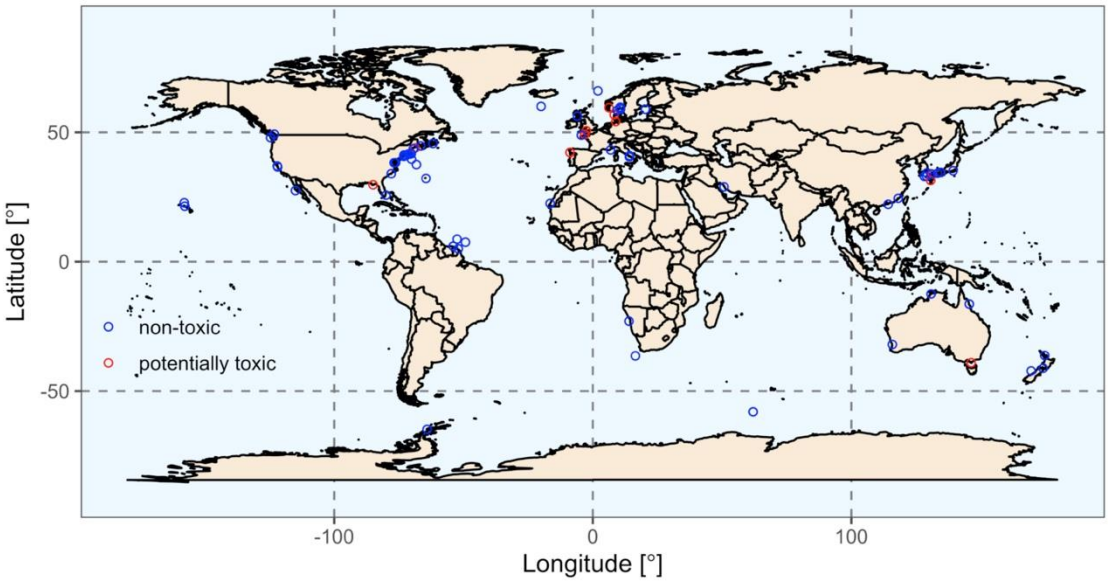

Fig. 2 Isolation locations of non-toxic and potentially toxic phytoplankton that were investigated in our experiments and in published laboratory experiments.

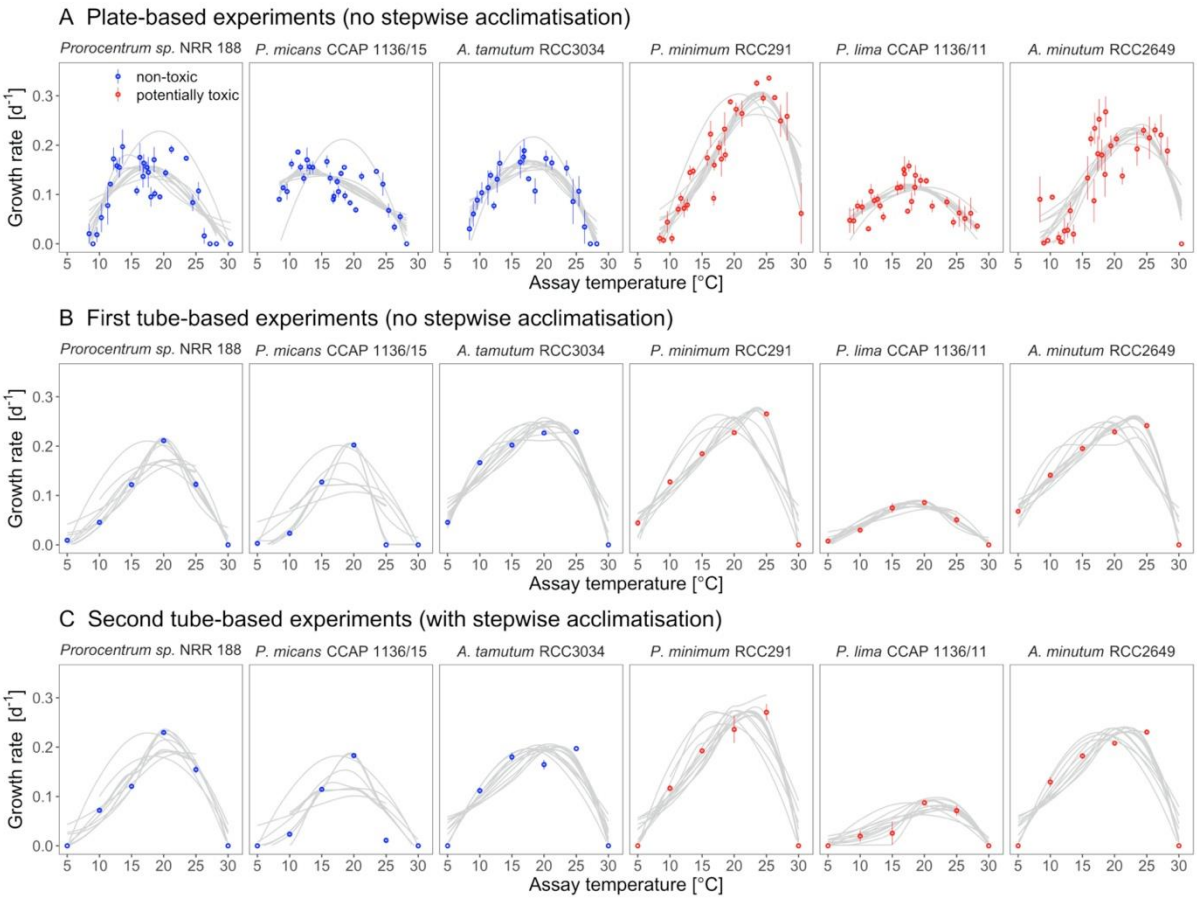

Fig. 3 Growth rates in non-toxic and potentially toxic strains of marine phytoplankton across temperature obtained from plate-based experiments (*PB*) and tube-based experiments without and with stepwise acclimatization (*TB1* and *TB2*, respectively). Each data point shows the mean growth rate with standard error as error bars. The grey solid lines denote the non-linear models fitting growth rate against temperature.

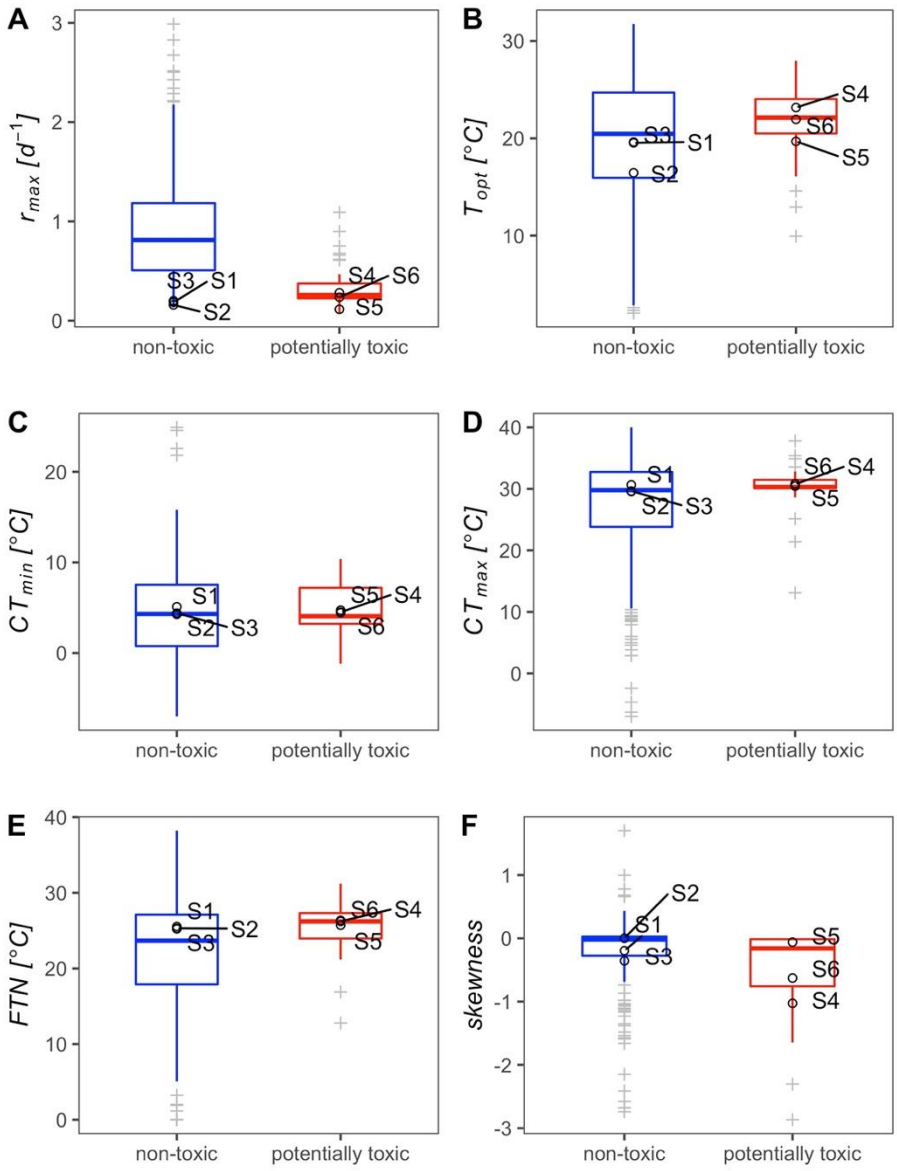

Fig. 4 Variation in maximum growth rates and thermal traits between toxicity in marine phytoplankton. Box plots show the distribution of maximum growth rates (r_{max}), thermal optimum (T_{opt}), critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}), fundamental thermal niche (*FTN*), and skewness in non-toxic (blue) and potentially toxic (red) strains from our experiments and published laboratory experiments. Outliers are indicated as grey crosses. Traits in strains (S1, S2, and S3 refer to non-toxic strains of *Prorocentrum sp.*, *P. micans*, and *A. tamutum*, respectively; while S4, S5, and S6 refer to potentially toxic strains of *P. minimum*, *P. lima*, and *A. minutum*, respectively) used in our experiments are labeled and indicated as black circles.

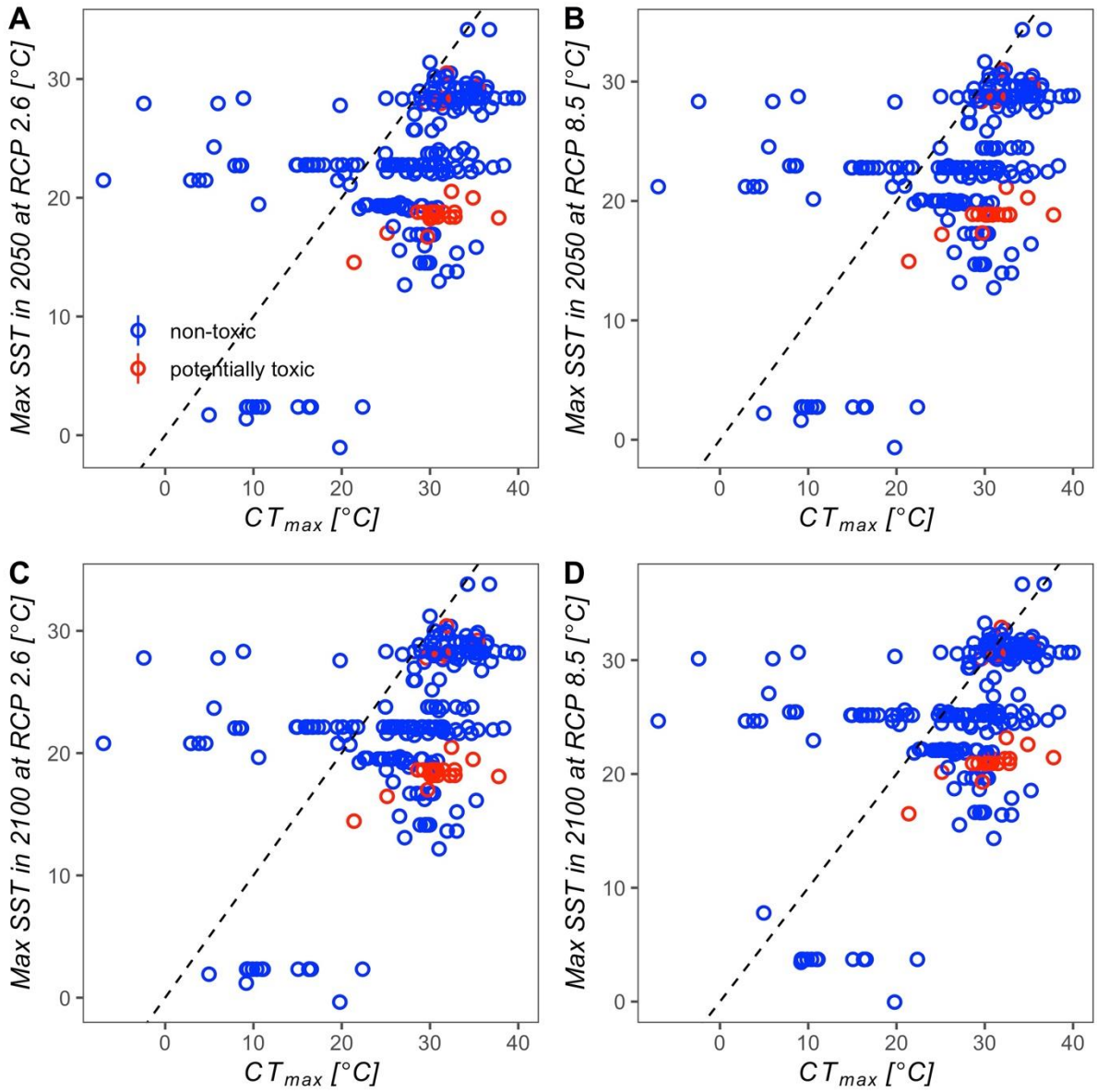

Fig. 5 Scatter plots showing the critical thermal maximum (CT_{max}) of non-toxic (blue) and potentially toxic (red) marine phytoplankton strains in relation to their habitat's maximum sea surface temperate (SST) projected in 2050 and 2100 at different climate scenarios (RCP 2.6 and RCP 8.5). The points above the threshold (broken line) indicate that the projected SST exceeds the CT_{max}.

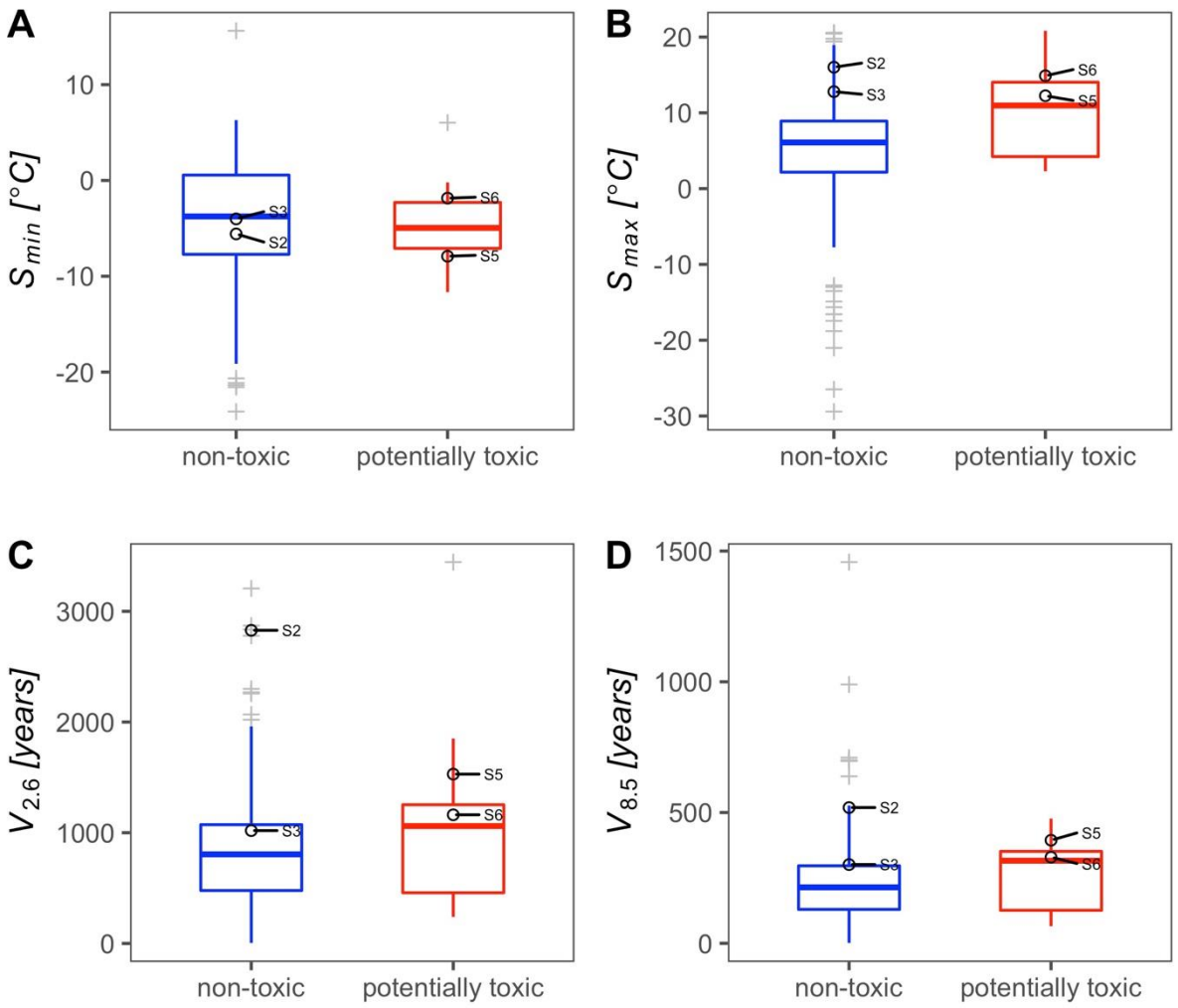

Fig. 6 Variation in thermal sensitivity and vulnerability between toxicity in marine phytoplankton. Box plots show the distribution of thermal sensitivity to cold and warm temperatures (S_{min} and S_{max}, respectively; A and B, respectively) and vulnerability to warming at *RCP* 2.6 and *RCP* 8.5 climate scenarios ($V_{2.6}$ and $V_{8.5}$, respectively; C and B, respectively) in non-toxic (blue) and potentially toxic (red) strains from the combined present and published experimental data. Outliers are indicated as grey crosses. Traits in strains (S2 and S3 refer to non-toxic strains of *P. micans*, and *A. tamutum*, respectively; while S5 and S6 refer to potentially toxic strains of *P. lima*, and *A. minutum*, respectively) used in this present study are labeled and indicated as black circles. Data for *Prorocentrum* sp. (S1) and *P. minimum* (S4) were not available.

Table 1. Information on the identity, origin, culture condition, and toxicity of experimental organisms obtained from different culture collections.

Experimental Organism	Origin	Source's culture condition	Toxicity
Prorocentrum sp. (NRR 188)	Maintained at University of Essex culture collection; Information on isolate's origin is not available.	Medium: f/2 in natural sea water (*NSW*) Temperature: 15 °C Light intensity: 100 µmol m^{-2} s^{-1}	non-toxic
Prorocentrum micans (CCAP 1136/15)	Isolated at Lynn of Lorne, Argyll, Scotland, UK; maintained at Culture Collection of Algae and Protozoa (*CCAP*) at the Scottish Association for Marine Science (*SAMS*)	Medium: L1 in *NSW* Temperature: 15 – 20 °C Light intensity: 30 – 40 µmol m^{-2} s^{-1}	non-toxic
Alexandrium tamutum (PARALEX 242)	Isolated at Kerloc'h, Dinan, English Channel, France; maintained at Roscoff Culture Collection (ID: RCC 3034)	Temperature: 19 °C Light intensity: 100 µmol m^{-2} s^{-1}	non-toxic
Prorocentrum minimum (Poulet)	Maintained at *RCC* (ID: RCC 291); Information on isolate's origin is not available.	Medium: K in *NSW* Temperature: 20 °C Light intensity: 100 µmol m^{-2} s^{-1}	potentially toxic
Prorocentrum lima (CCAP 1136/11)	Isolated from Vigo, Spain; maintained at CCAP at SAMS	Medium: L1 in *NSW* Temperature: 15 – 20 °C Light intensity: 30-40 µmol m^{-2} s^{-1}	potentially toxic
Alexandrium minutum (PARALEX 246)	Isolated from Britanny coast, English Channel, France; maintained at RCC (ID: RCC 2649)	Medium: f/2 in NSW Temperature: 18 °C Light intensity: 100 µmol m^{-2} s^{-1}	potentially toxic

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Brisneve Edullantes reports financial support was provided by University of the Philippines System.