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Comparing *Prochlorococcus* temperature niches in the lab and across ocean basins

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Abstract

Niche theory suggests that the realized niche occupied by an organism in the field is a subset of the fundamental niche space of the organism, absent additional biotic and abiotic factors. Though often assumed, this discrepancy is rarely tested for specific organisms, and could act as a source of error in model predictions of biogeographical shifts resulting from temperature change which assume niche theory constraints. Here, we quantify the difference between fundamental and realized temperature niches for four dominant ecotypes of *Prochlorococcus*, including eMED4, eMIT9312, eMIT9313, and eNATL2A, and ask whether the realized temperature niches of each ecotype vary across ocean basins. The realized niches for the four ecotypes are, on average, 3.84°C ± 1.18°C colder (mean ± SD across all ocean basins and ecotypes) and 2.15°C ± 1.89°C wider than the lab-measured fundamental niches. When divided into four ocean regions—North Atlantic, South Atlantic, North Pacific, and South Pacific—we find that the realized temperature niche optimum for a given ecotype compared to the fundamental temperature niche optimum differs across regions by as much as 7.93°C, while the niche width can differ by up to 9.48°C. Colder and wider realized niches may be a result of the metabolic risk associated with living in variable environments when the mean temperature is too close to the optimal temperature for growth or due to physical processes such as dispersal. The strong differences in temperature niches across ocean basins suggest that unresolved genetic diversity within ecotypes, local adaptation, and variable interactive ecological and environmental factors are likely to be important in shaping *Prochlorococcus* realized temperature niches.

Each species of marine microbe in the wild survives in a range of environmental and biotic conditions, which collectively define its realized ecological niche. In contrast, the fundamental niche describes the controlled conditions in which the species can survive in absence of competition, predation, or other interactions (Colwell and Rangel 2009). For microbes, the fundamental niche is typically estimated in the lab by measuring the growth rate of organisms in pure culture, though not necessarily 100% axenic, across a gradient of single or multiple environmental variables, such as temperature (Marãnón et al. 2014), light (Geider et al. 1998), and a range of nutrients (Morel 1987; Burson et al. 2018). The fundamental ecological niches of marine microbes are thought to shape their biogeography and consequently, understanding the processes that shape microbial niches have significant implications for community trait assemblages (Falkowski and Oliver 2007; Thomas et al. 2012; Edwards et al. 2013), biogeographical diversity patterns (Barton et al. 2010; Ibarbalz et al. 2019), and marine ecosystem function (Falkowski et al. 1998). However, niche theory suggests that if the fundamental niche is the measure of physiological tolerance in the absence of biological influence, then the realized niche should be a subset of the fundamental range, controlled by interactions with biotic and abiotic factors (Colwell and Rangel 2009; Soberón and Arroyo-Peña 2017). Understanding the degree of difference between realized and fundamental niches, and the factors that influence the discrepancy and their regional variations, is therefore a question with implications for species biogeography and patterns of diversity, as well as modeling how species ranges shift in response to changing environmental conditions (Irwin et al. 2015; Barton et al. 2016; Bestion et al. 2018; Braschler et al. 2020).

One globally important group of marine phytoplankton is *Prochlorococcus*. *Prochlorococcus* is the most abundant group of

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photosynthetic organisms on the planet and they collectively account for a significant but spatially and temporally variable fraction of primary production in the ocean (Biller et al. 2015). *Prochlorococcus* is most numerous in the oligotrophic subtropical and tropical oceans, and is present in lower densities in higher latitudes and more nutrient-rich systems (Johnson et al. 2006; Flombaum et al. 2013). The predominance of *Prochlorococcus* in warm and nutrient poor systems is due in large part to their small cell size and consequently high affinity for nutrients (Edwards et al. 2012) and light (Moore et al. 1995) combined with adaptations to high temperature (Morán et al. 2010). One projected outcome of anthropogenic warming, increased density stratification, and reduced nutrient supply to the ocean surface over the coming century (Rost et al. 2008; Schaum et al. 2013; Snell-Rood et al. 2015) is that *Prochlorococcus* may become more numerous in oligotrophic regions and perhaps expand poleward (Flombaum et al. 2013).

Prochlorococcus is comprised of genetically different ecotypes (or clades) that have different traits, leading to divergent biogeographical patterns. An ecotype is a group of strains with similar phylogenies that exhibit comparable habitat preferences. A strain is similar to a species in that it is genetically unique; however, a strain is not genetically distinct enough to be considered a separate species (i.e., it has <3% genetic divergence from other known strains) (Malmstrom et al. 2013; Biller et al. 2015). *Prochlorococcus* clades, which are delineated by rRNA internal transcribed spacer (ITS) sequence diversity, separate into low light (LL) and high light (HL) adapted groups (Rocap et al. 2003). Currently, each group is further divided into at least six distinct ecotypes. This study focuses on the clades that have culture representatives, HLI, HLII, LLI, and LLIV, which is required to define a fundamental temperature niche. These ecotypes are also referred to in the literature as eMED4, eMIT9312, eNATL2A, and eMIT9313, respectively, which is the nomenclature we use here (Fig. 1). Previous studies have shown how thermal preference, light harvesting, and nutrient acquisition trait differences between these ecotypes of *Prochlorococcus* lead to distinct biogeographical patterns (West and Scanlan 1999; Johnson et al. 2006; Zwirglmaier et al. 2008). Temperature is of particular interest because it is a driving factor in determining phytoplankton biogeography, including for *Prochlorococcus* (Smayda 1958; Moore et al. 1995; Partensky et al. 1999).

Here, we ask how the lab-measured fundamental and field-observed realized temperature niches differ for *Prochlorococcus* ecotypes, and why? We also ask whether and how much realized niches of *Prochlorococcus* ecotypes differ between contrasting ocean basins? We first compile published growth rates across temperature for all strains of *Prochlorococcus*. We then aggregate the data for all the strains into their respective ecotypes in order to define a fundamental temperature niche for all available ecotypes of *Prochlorococcus* (e.g., eMED4, eMIT9312, eMIT9313, and NATL2A). We then define ecotypic realized temperature niches for each of the same ecotypes using field measurements of *Prochlorococcus* abundance from 10 cruises across multiple ocean basins. In addition, we split the data into four different sampling regions (e.g., North and South Atlantic and North and South Pacific) and calculate the realized niche for each ecotype within each region. We discuss how various ecological processes, such as local adaptation, competition, and dispersal may influence the differences between realized and fundamental niches across ocean basins. Understanding the differences between realized and fundamental niches, as well as differences in realized niches across ocean basins, is important for understanding how changing temperatures will impact future biogeographical patterns of important microbial groups like *Prochlorococcus*.

Methods

Laboratory temperature vs. growth data

We conducted a literature search for experiments on *Prochlorococcus* growth rate in response to temperature, with the goal of quantifying the fundamental temperature niche for as many strains of *Prochlorococcus* as possible (*see* Online supplemental Data S1). Our search yielded growth vs. temperature functional response data for 11 strains of *Prochlorococcus*, but for reasons described below some of these strains were excluded from further analysis. We included for further consideration only the data from experiments that were nutrient replete and run under 12:12 or 14:10 h of light/dark cycle with light intensities ≥40 μmol quanta m^{-2} s^{-1}. In other words, we included only experiments where the primary source of variation is temperature instead of other factors such as light or nutrients. We excluded strains that had less than four data points total or had data that did not span across at least four different temperature values. The rationale for this selection criterion is that in order to make a robust temperature vs. growth functional response curve, ample data are required. The resulting database included temperature vs. growth curves for nine strains of *Prochlorococcus*: AS9601, GP2, MED4, MIT9215, MIT9312, MIT9313, MIT9321, MIT9515, and NATL2A (Table 1). Across these nine strains of *Prochlorococcus* we found a total of 143 growth rate measurements from seven publications.

To match the taxonomic resolution from the field measurements of *Prochlorococcus* abundance, we aggregated all available growth rate data for all the strains included in each ecotype (Fig. 1). We aggregated the growth rate data by ecotype instead of strain because qPCR analysis of field samples quantifies relative abundance of each ecotype and cannot resolve taxonomy to the strain level (Zinser et al. 2006).

Field observations of Prochlorococcus ecotype abundance

In order to quantify the realized temperature niche for *Prochlorococcus* ecotypes, we aggregated field observations of *Prochlorococcus* ecotype abundance (via ecotype-specific qPCR) and co-located temperature data. Sampled ecotypes included eMED4, eMIT9312, eMIT9313, and eNATL2A and data

Table 1. Strains used in the analysis described by their corresponding ecotype, isolation location, literature reference, and fundamental temperature niche parameter values. T_{opt} is the optimum temperature for growth estimated as the maximum of the fitted left-skewed curve (Thomas et al. 2012). The confidence interval (CI) for T_{opt} is calculated as the interval of the 2.5th–97.5th percentile from a bootstrapped distribution of 80% of available lab data for each strain. *W* is the niche width calculated as the difference between the 99th and 1st percentile of the estimated curve. The CI for *W* is the confidence interval for the niche width presented as the interval of the 2.5th–97.5th percentile of a bootstrapped distribution of widths. Each viable strain is grouped into their respective ecotypes to define an ecotypic fundamental temperature niche. There are four unique ecotypes (HL-I, HL-II, LL-I, and LL-IV) within either the high light (HL) or low light (LL) groups depending on their light and temperature preferences (*see* Fig. 1 for schematic of strains and ecotypes).

				Temperature niche statistics (°C)			
Strain	**Ecotype/clade**	**Isolation locations**	**References**	T_{opt}	**CI**	***W***	**CI**
AS9601	HL-II (eMIT9312)	Gulf stream	Johnson et al. (2006), Zinser et al. (2007)	26.93	[18.0–28.1]	14.68	[0.49–25.2]
GP2	HL-II (eMIT9312)	Sargasso Sea	Stawiarski et al. (2016)	21.35	[21.2–21.6]	11.86	[9.90–12.3]
MED4	HL-I (eMED4)	Pacific, Mediterranean Sea	Moore et al. (1995), Johnson et al. (2006), Kulk et al. (2012), Martiny et al. (2016)	21.93	[21.7–22.4]	17.02	[16.1–19.2]
MIT9215	HL-II (eMIT9312)	Pacific, Sargasso Sea, Mediterranean	Johnson et al. (2006), Martiny et al. (2016)	26.10	[15.0–32.1]	17.59	[1.86–32.2]
MIT9312	HL-II (eMIT9312)	Pacific, East China Sea, Sargasso Sea, Arabian Sea	Johnson et al. (2006), Martiny et al. (2016)	24.55	[15.0–25.7]	14.68	[1.03–20.4]
MIT9313	LL-IV (eMIT9313)	Gulf stream, North Pacific	Kulk et al. (2012), Johnson et al. (2006)	24.99	[24.8–26.3]	10.02	[9.21–15.7]
MIT9321	HL-II (eMIT9312)	Sargasso Sea	Zinser et al. (2007)	26.35	[25.2–27.6]	17.36	[11.2–22.4]
MIT9515	HL-I (eMED4)	Pacific, Sargasso Sea	Johnson et al. (2006), Martiny et al. (2016)	22.48	[22.2–23.5]	19.39	[18.5–23.7]
NATL2A	LL-I (eNATL2A)	Gulf stream	Johnson et al. (2006)	22.67	[22.2–24.0]	14.67	[13.3–20.2]

Fig 1. Schematic showing the division of ecotypes and strains. The ecotype naming convention is the far left column, which can also be identified by the clade naming convention (middle column), and each of the ecotypes/clades are split into individual strains (far right column). For a more comprehensive tree see Malmstrom et al. (2013) and Biller et al. (2015).

originated from 10 separate sampling efforts in the Pacific and Atlantic Oceans (Johnson et al. 2006; Zinser et al. 2007; Malmstrom et al. 2010; Chandler et al. 2016; and this study). All of these sampling efforts are transects except for the Malmstrom study (Malmstrom et al. 2010), which was a multi-year sampling effort at BATS and HOT. Chandler et al. (2016) describe the Pacific Ocean samples from the 2007 WP2 and 2012 POWOW 1 cruises collected at the surface mixed layer. In this study, we also analyzed surface samples from the POWOW 2 and 3 cruises, and samples taken at depths below the surface mixed layer for the WP2 and all three POWOW cruises (Figs. 2 and 3 cruises; *see* Chandler et al. (2016) and Larkin et al. (2016) for cruise details). The new samples processed and analyzed for this study were collected during POWOW 2 and 3 cruises in the North Pacific from ship-board Niskin bottles, filtered and flash frozen, thawed, prepared, and assayed by ecotype-specific qPCR using standards for absolute quantification as previously described (Chandler et al. 2016).

In total, there were 5285 observations for each ecotype with depths ranging from 0 to 200 m. We partitioned this dataset into four ocean basins (Fig. 2; North Pacific, South Pacific, North Atlantic, South Atlantic). Using the computational methods outlined below, we calculated a realized temperature niche across all depths for each ecotype within each ocean basin to compare to the fundamental temperature niche of each ecotype. Our intention here was to contrast realized niches across ocean regions that are relatively disconnected by dispersal. Dispersal timescales are long between ocean basins and across the Equator, up to 100 years, while within gyres

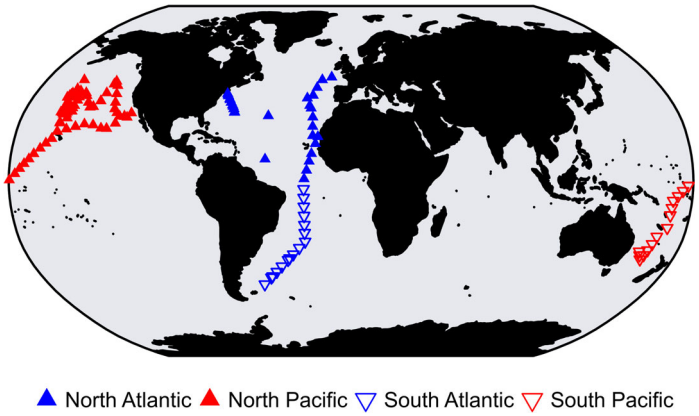

Fig 2. *Prochlorococcus* ecotype data collection locations grouped into ocean basins. Blue colors represent data in the Atlantic Ocean, and red represent data in the Pacific Ocean. Filled-in arrows pointing up represent data collected in the northern hemisphere, and open arrows pointing down represent data collected in the southern hemisphere. The same symbols are used in Fig. 6. The data collection efforts are listed here: (a) POWOW 1, North Pacific (Chandler et al. 2016); (b) POWOW 2, North Pacific (this study); (c) POWOW 3, North Pacific (this study); (d) WP2, North to South Pacific (Chandler et al. 2016); (e) AMT 13, North to South Atlantic (Johnson et al. 2006); (f) EN 351, North Atlantic (Zinser et al. 2007); (g) EN 375, North Atlantic (Zinser et al. 2007); (h) MP01, North Atlantic (Zinser et al. 2007); (i) BATS, North Atlantic (Malmstrom et al. 2010; Chandler et al. 2016); and (j) HOT, North Pacific (Malmstrom et al. 2010; Chandler et al. 2016).

timescales of dispersal and mixing are relatively fast, approximately 0–10 years (Martiny et al. 2009; Jönsson and Watson 2016).

Temperature niche characterization

Here, we describe our approach for estimating the realized and fundamental niches from field and lab data, respectively. We desired methodologies for characterizing temperature niches that (a) allowed for the limited data, particularly for the lab-measured growth rates; (b) robustly described the width and central tendency of the underlying distributions; (c) enabled comparison across the fundamental and realized temperature niches with minimal methodological biases; and (d) are easily understood and reproduceable across a variety of systems. While the fundamental temperature niche is typically defined as a left-skewed curve (Thomas et al. 2016), the realized temperature niches are not skewed in the same manner, therefore fitting both distributions with the same curve would be undesirable. As a result, we calculated the fundamental temperature niche optimal temperature and niche width using the left skewed temperature niche form described by Thomas et al. (2012), and calculated the realized temperature niche median and niche width using non-parametric percentile estimates.

The fundamental temperature niche is often defined as a left-skewed curve with the following equation (Norberg 2004; Thomas et al. 2012):

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

where $\mu(T)$ (day^{-1}) is the growth rate as a function of temperature and T is temperature (°C). The exponential term (ae^{bT}) describes how growth rate exponentially increases with temperature, with a and b as constants typically estimated from data (Eppley 1972; Thomas et al. 2012; Kremer et al. 2017). The latter portion of the equation characterizes the left-skewed nature of the fundamental niches where w is the range of temperatures where the curve is >0 (°C), and z is the temperature where the quadratic portion of the equation is tangent to the exponential portion of the equation (°C). We fit Eq. 1 to the growth rate data for each strain, and each ecotype using non-linear least-squares regression from the *nls* function in the R package "stats." We used published estimates of the constant b (b = 0.063; Thomas et al. 2012) but fit a using the collected growth rate data for *Prochlorococcus*. The estimates for each of the free parameters (a, z, w) and b are then used to recreate the curve across a range of temperatures from − 2°C to 40°C, where the fundamental temperature niche is the range of temperature where growth rate is >0 2012). In cases where a strain did not have measured zero growth values at temperature extremes (*see* Fig. 3), the curve was extended to estimate

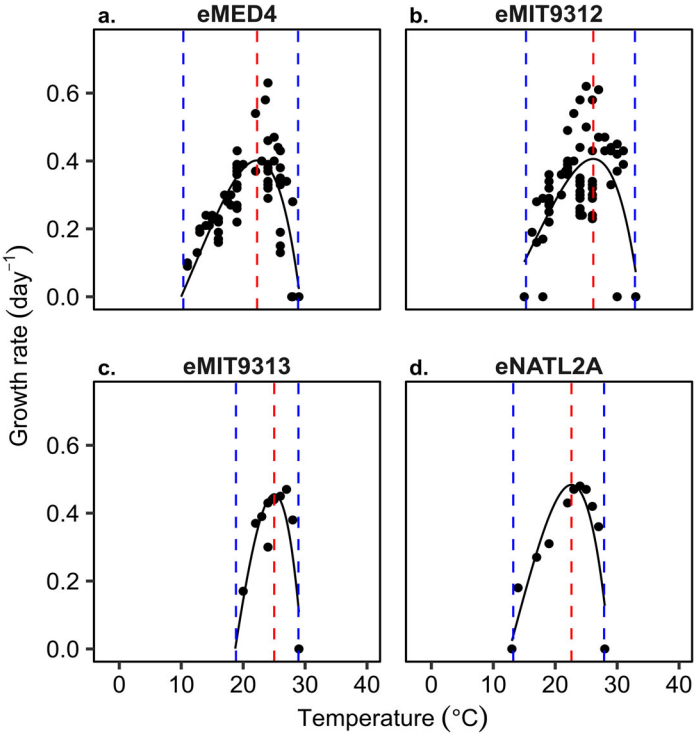

Fig 3. Fundamental temperature niche for (**a**) eMED4, (**b**) eMIT9312, (**c**) eMIT9313, and (**d**) eNATL2A. The filled black circles indicate lab growth rate data from all the strains within each ecotype (described in Fig. 1). The temperature optimum (T_{opt}) is the temperature where the curve is maximum (dashed red line) and the niche width (W) is the difference between the 1st and the 99th percentiles (the percentiles are indicated by dashed blue lines).

where a zero growth value may be. If an ecotype had zero growth values at temperature extremes, the curve was bounded by those temperature extremes.

From these temperature niche estimates, we calculated the following niche metrics: the temperature at which growth is maximum is the optimal temperature (T_{opt}), and the difference between the 1st and the 99th percentile we defined as the niche width (W). The choice of defining the width by the temperature difference between the 99th and 1st percentiles is somewhat arbitrary, but experimentation with other criteria (e.g., 95th minus the 5th percentiles) indicated that our criteria provided a reasonable estimate of niche width (Fig. 3). The 95% confidence intervals for T_{opt} and W for each ecotype were calculated by refitting Eq. 1 to a random subsample of 80% of the available lab data 1000 times, and then recalculating the optimal temperature and niche width for each iteration. From these bootstrapped distributions, we found the 2.5th and 97.5th quantiles of T_{opt} and W to estimate the 95% confidence intervals. Because lab data are relatively sparse, the confidence intervals are not symmetric about the niche metrics.

The realized temperature niches were estimated by calculating the 1st, 50th, and 99th percentiles of the observations, excluding data below the qPCR detection limit which is 0.65 cells mL^{-1}. We defined the 50th percentile of all available observations as $\tilde{T}_{obs}$. Similar to the fundamental temperature niche, the realized temperature niche widths (W_{obs}) were defined as the difference between the 99th and the 1st percentile of the observations for each ecotype. Though these bounding niche width percentiles are somewhat subjective, they provided reasonable estimates of niche width for the field data that are comparable to the lab data (Figs. 3–5). We tested other percentile cut offs, such as the 20th–80th and the 10th–90th for the niche width range but selected the 1st and 99th percentiles as they described the niche width best. The 95% confidence intervals for $\tilde{T}_{obs}$ and W_{obs} for each ecotype were found by randomly selecting 80% of the observations, repeated 1000 times, and recalculating $\tilde{T}_{obs}$ and W_{obs} over each of the 1000 iterations. From these bootstrapped distributions of $\tilde{T}_{obs}$ and W_{obs} we took the 2.5th and 97.5th percentiles to create a 95% confidence interval.

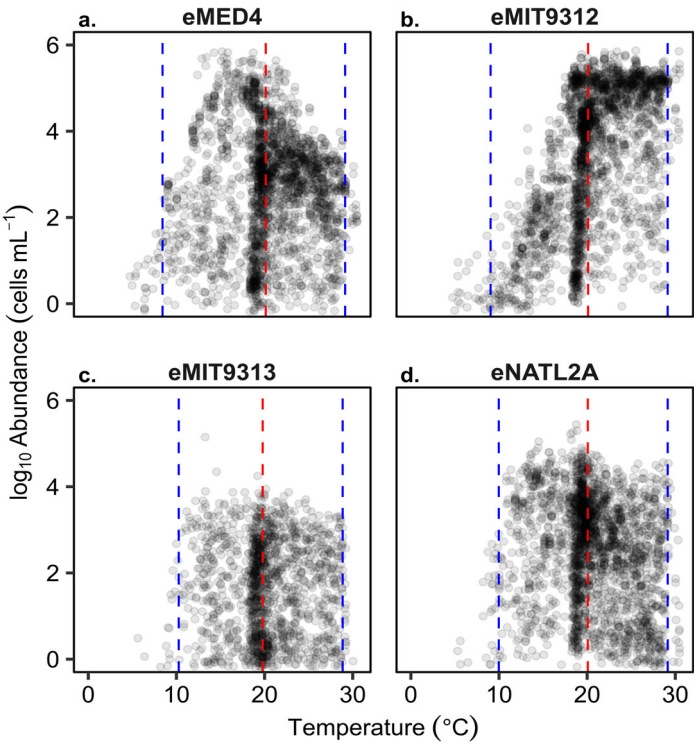

Fig 4. Realized temperature niche for (**a**) eMED4, (**b**) eMIT9312, (**c**) eMIT9313, and (**d**) eNATL2A ecotypes. The filled gray circles are individual observations across all cruises for each ecotype. The optimal temperature niche as observed in the field ($\tilde{T}_{obs}$) for each ecotype are calculated as the 50th percentile of the temperature distribution (dashed red lines), and the niche width (W_{obs}) is the range from the 1st to the 99th percentile (dashed blue lines).

Statistical tests for differences in niche metrics

We next describe our statistical approaches for assessing differences in estimated temperature niche optima and widths between: (a) lab and field data for each ecotype and (b) field data between ocean basins for each ecotype. We describe this process for comparing niche central tendencies, but repeated it for niche widths. For (a), we first created a bootstrapped null distribution of $\tilde{T}_{obs}$ by randomly sampling 80% of the available field observations for each ecotype and region; and calculating the realized temperature niche median ($\tilde{T}_{obs}$) of the subset over 1000 iterations. We then calculated the number of iterations in the bootstrapped distribution of $\tilde{T}_{obs}$ that were less than T_{opt}, and report a significant difference between $\tilde{T}_{obs}$ and T_{opt} if >95% ($p<0.05$) of cumulative null distribution exceeded T_{opt} (Fig. 6). The process for determining whether W_{obs} differed from W was the same.

For (b), we used the Mann–Whitney U test to assess whether $\tilde{T}_{obs}$ was equivalent across ocean basins for each ecotype, using all observations for each ecotype and basin. To assess whether the niche widths (W_{obs}) were equivalent across basins for each ecotype, we compared the bootstrapped distributions for niche widths across ocean basins, again using 80% of the data, randomly selected, and repeated 1000 times. We used the Mann–Whitney U test to compare between the bootstrapped distributions of niche widths. We report the p value for the statistical comparison of each sampling region within an ecotype (Supporting Information Table S1).

Results

Fundamental temperature niches

Each of the nine strains of *Prochlorococcus* showed distinct optimum temperatures and niche widths in controlled lab conditions (Table 1, Supporting Information Fig. S1). Across all nine strains, the average optimum temperature was 24.16°C, with a SD among the strains of 2.11°C. The average niche width across all nine strains was 15.25°C and the SD among the strains was 2.96°C. The

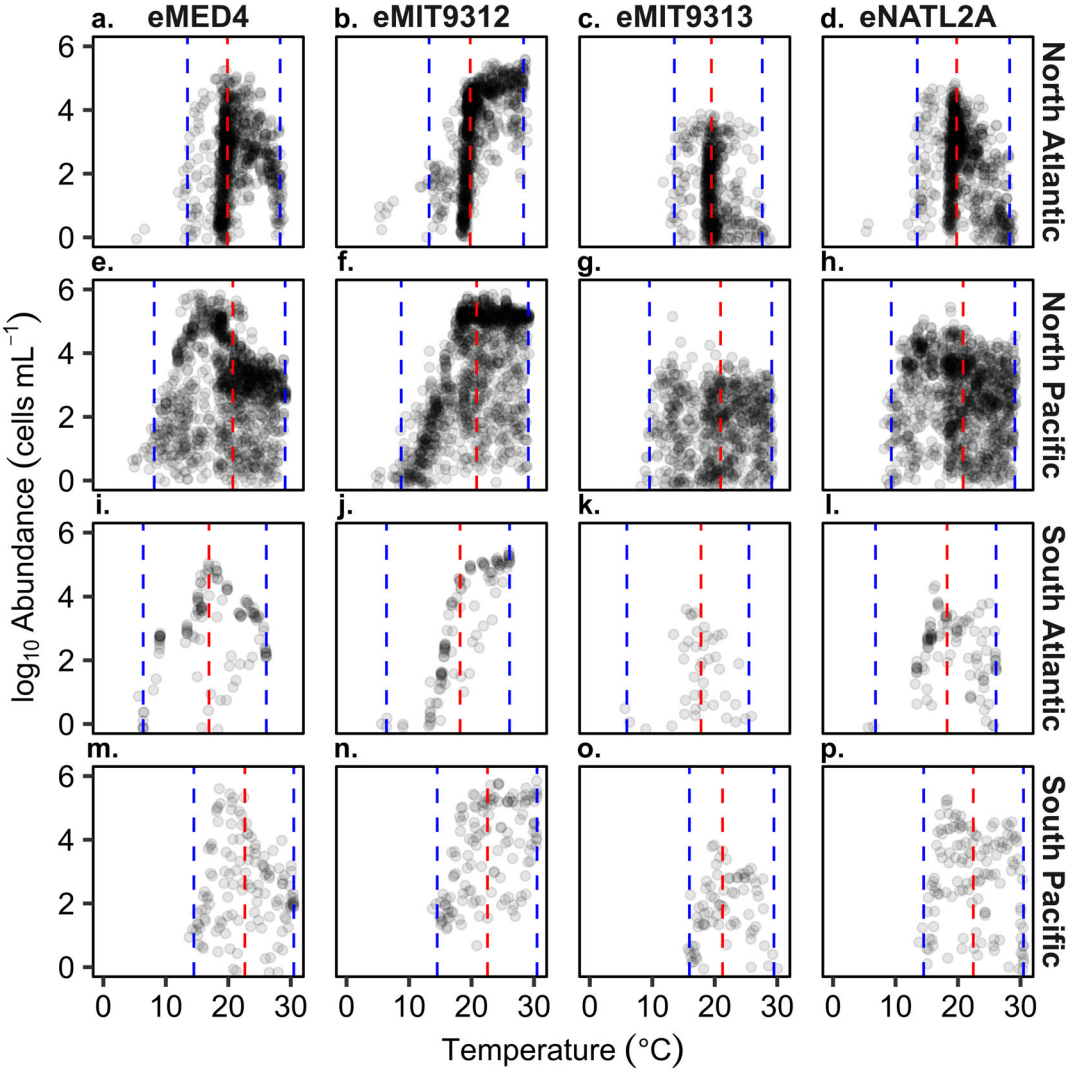

Fig 5. Realized temperature niches for each ecotype (columns) split into four different ocean basins (rows). For example, the ecotype eMED4 (far-left column) is split into four ocean basins (read top to bottom): (**a**) North Atlantic; (**e**) North Pacific; (**i**) South Atlantic; and (**m**) South Pacific. The remaining ecotypes eMIT9312, eMIT9313, and eNATL2A are split into each ocean basin, respectively: **(b- d)** North Atlantic; **(f-h)** North Pacific; **(j-l)** South Atlantic; **(n-p)** South Pacific. The filled black circles show the observations for each ecotype and region. $\tilde{T}_{obs}$ is calculated as the 50th percentile and is represented by a dashed red line. W_{obs} is the difference between the 1st and the 99th percentile and is shown in the dashed blue lines.

lowest T_{opt} value was recorded for the strain GP2 at 21.35°C with a 95% confidence interval of (21.20°C, 21.60°C) and the highest T_{opt} was recorded for the strain AS9601 at 26.97°C (18.00°C, 28.10°C; Supporting Information Fig. S1). When we group strains into their respective ecotypes (Fig. 1), we found that the ecotype eMIT9312 had the highest T_{opt} at 26.10°C (25.80°C, 26.60°C) and eNATL2A had the narrowest niche width of 10.08°C (9.21°C, 15.70°C). In comparison, the lowest T_{opt} of 22.30°C (22.00°C, 22.60°C) and largest niche width of 18.60°C (17.20°C, 19.20°C) was observed for the ecotype eMED4 (Fig. 3).

Realized temperature niches

Realized temperature niches calculated across all depths in the euphotic zone differed between ecotypes and within ecotypes across different ocean basins. Across all regions and ecotypes, the ecotypic realized niches have an average optimum temperature of 20.15°C and SD across all regions and ecotypes of 0.85°C. The average niche width was 17.45°C with a SD across all regions and ecotypes of 1.27°C (Fig. 4). eMIT9313 has the lowest $\tilde{T}_{obs}$ at 19.71°C (19.62°C, 19.75°C) and eMED4 has the highest $\tilde{T}_{obs}$ at 20.19°C (20.12°C, 20.27°C; Fig. 4). In addition, eMED4 had the largest niche width (W_{obs}) at 20.72°C (20.42°C, 21.09°C) and eMIT9313 had the narrowest niche width at 18.54°C (18.00°C, 18.95°C).

The realized temperature niches vary across ocean basins for each ecotype. For example, within the ecotype eMED4, the observed optimum temperature in the South Atlantic was

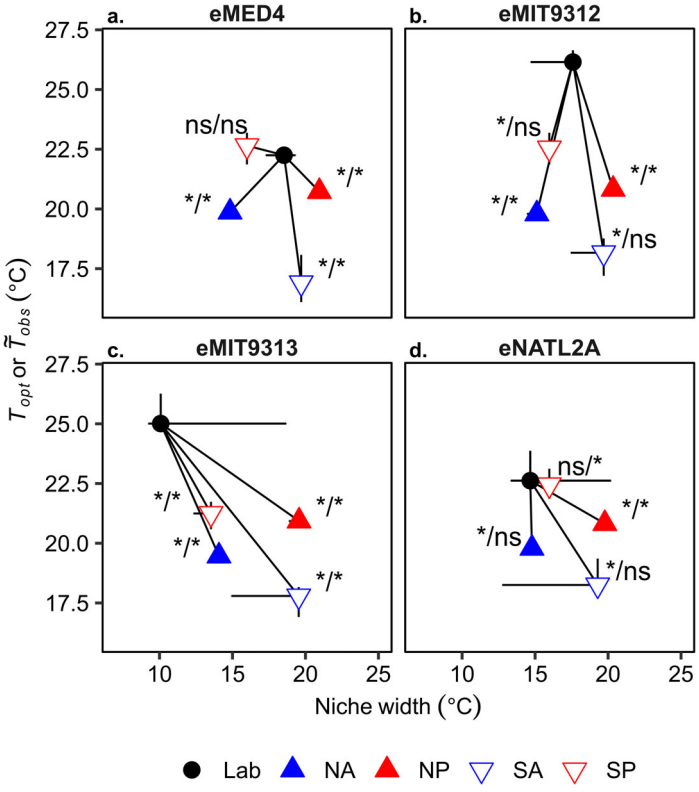

Fig 6. Differences between lab-based fundamental and field-based realized niches for each ocean basin for (**a**) eMED4, (**b**) eMIT9312, (**c**) eMIT9313, and (**d**) eNATL2A. On the x-axis, the niche widths (99th–1st percentiles) are compared, and on the y-axis, the central tendencies for the fundamental and realized niches are compared (T_{opt} and $\tilde{T}_{obs}$, respectively). The filled black circle in each panel shows the fundamental niche optimum temperature (T_{opt}) and width (W). The red and blue colors represent the Pacific and Atlantic oceans, respectively. Filled arrows pointing up represent the northern hemisphere, and open arrows pointing down represent the southern hemisphere. The black line connecting the black circle to the color triangles shows the vector of niche change between fundamental and realized niche in each ocean basin for each ecotype. The reported 95% confidence intervals for the fundamental and realized temperature niche optima and widths are the 2.5th and 97.5th percentiles of the bootstrapped distributions for the niche optimal temperature and niche width. The results of the Mann–Whitney U test between the fundamental and realized temperature niche means and widths are reported as mean/width with the notation as follows: * ($p < 0.05$), and ns ($p > 0.05$).

16.91°C (16.10°C, 18.07°C), whereas the observed optimum temperature in the South Pacific was 22.65°C (21.86°C, 23.19°C; Fig. 5). In addition, the niche width of the ecotype eMED4 was estimated at 15.99°C (15.42°C, 16.15°C) in the South Pacific and 20.97°C (20.09°C, 21.43°C) in the North Pacific. Similar regional variability in temperature niches was seen for eMIT9312, eMIT9313, and eNATL2A (Fig. 5).

Comparison between fundamental and realized temperature niches

The optimum temperatures of the realized niche ($\tilde{T}_{obs}$) were significantly lower than temperature optima of the fundamental niche (T_{opt}) for most ocean basins and ecotypes (Fig. 6). However, the differences in temperature niche central tendencies ($\tilde{T}_{obs} - T_{opt}$) vary considerably by ecotype and ocean. Across all oceans and ecotypes, *Prochlorococcus* realized temperature niches had optima that were on average 3.84°C ± 1.18°C (mean ± SD) colder than the fundamental temperature niches. Across all ecotypes, on average, the South Atlantic had the greatest difference between T_{opt} and $\tilde{T}_{obs}$ (6.22°C colder in the field than in the lab) and the South Pacific had the smallest shift (1.78°C colder in the field). Across all regions, eMIT9312 had the largest difference between T_{opt} and $\tilde{T}_{obs}$ (5.76°C colder in the field) and eMED4 had the smallest difference (2.14°C colder in the field). For eMED4 and eNATL2A, $\tilde{T}_{obs}$ in three out of four ocean basins was significantly colder than T_{opt}. For eMIT9312 and eMIT9313, $\tilde{T}_{obs}$ was colder than T_{opt} in all oceans (Fig. 6). In total, across all ecotypes split into different ocean basins, 14 out of 16 estimated realized niches were significantly colder than the fundamental niches, with two out of 16 not being statistically different.

The width of the realized temperature niche (W_{obs}) was significantly wider than the width of the fundamental niche (W) for most ocean basins and ecotypes (Fig. 6). However, the temperature niche width differences ($W_{obs} - W$) vary considerably by ecotype and ocean. On average across all ecotypes and ocean basins, the realized temperature niche width (W_{obs}) was 2.15°C ± 1.89°C (mean ± SD) wider than the fundamental temperature niche width (W). The South Pacific had the smallest average difference in niche width across all ecotypes, with the realized temperature niche being slightly narrower than the fundamental temperature niche (0.15°C). The North Pacific had the greatest average difference in niche width across all ecotypes (4.96°C wider in the field compared to lab). eMIT9313 had the greatest difference in temperature niche width (6.58°C wider in the field on average across ocean basins) and eMIT9312 had the smallest mean difference in temperature niche width (0.21°C wider in the field). The greatest shift in temperature niche width between the fundamental and realized niches occurred in the South Atlantic for eMIT9313 (9.48°C wider in the field). In total, across all ecotypes split into different ocean basins, nine out of 16 estimated realized temperature niches were significantly wider than the fundamental niches, two out of 16 were significantly narrower, and five out of 16 were not significantly different from the fundamental temperature niches. Low light ecotypes, eMIT9313 and eNATL2A, generally have wider realized temperature niches than fundamental temperature niches whereas for high light ecotypes, eMED4 and eMIT9312, the difference between fundamental and realized niche widths varies more strongly by ocean basin.

We also compared temperature niche metrics within ecotypes across ocean basins, and found that all differences in $\tilde{T}_{obs}$ and niche width across ocean regions were statistically significant (Supporting Information Table S1).

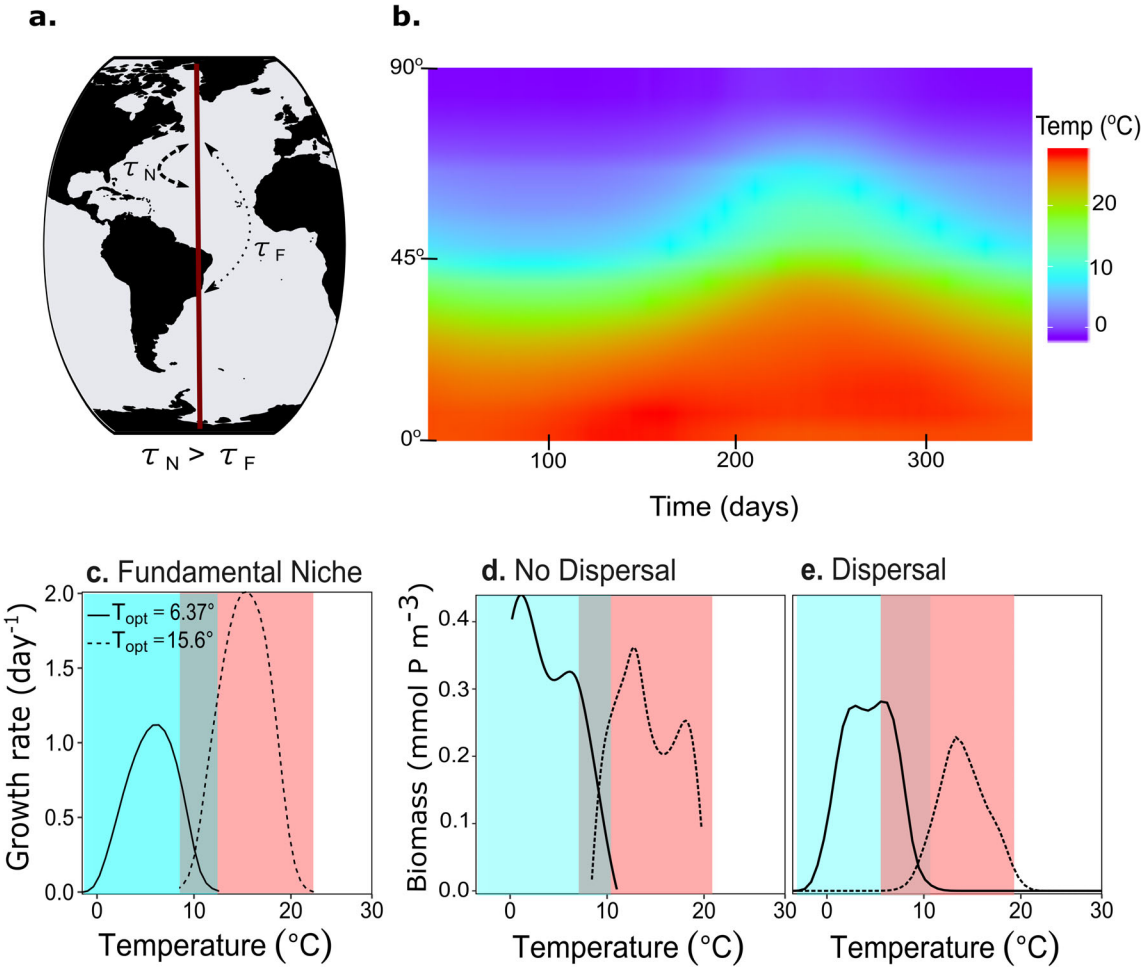

Fig 7. Schematic of simple model dynamics testing how dispersal controls realized temperature niches. (**a**,**b**) Across 30 latitudinal bands in the northern hemisphere with unique seasonal temperature cycles, each of 15 species has a distinct fundamental temperature niche with a species-specific optimum temperature and a common niche width (10°C). The magnitude of dispersal between boxes is a function of the latitudinal distance, meaning dispersal between neighboring (N) boxes (τ_N) is greater than dispersal between boxes further (F) apart (τ_F); see Supplemental Material for more information. For two species in the model, one adapted to colder temperatures (T_{opt} = 6.37°C, solid line) and one adapted to slightly warmer temperatures (T_{opt} = 15.6°C, dashed line), (**c**) we show the relationship between the fundamental temperature niche, and (**d**) the realized temperature niches without and (**e**) with dispersal. The red- and blue-shaded areas represent the range of the fundamental temperature niche (calculated as the difference between the 1st and the 99th percentile of the temperature distribution).

Discussion

Here, we discuss possible reasons why the temperature niche optima and widths differ between lab and field. First, we focus on potential methodological and observational biases. We then consider how other physical and ecological factors may cause differences between lab and field niche optima and widths. In addition, we discuss why sampling in different ocean basins results in differences in the realized temperature niche within an ecotype.

Potential methodological and observational biases

These analyses of lab and field data included both experimental and observational data from seven different lab growth rate studies and 10 different cruises. This dataset allowed us to quantify and compare the fundamental and realized niches for four ecotypes of *Prochlorococcus*. Here, we discuss uncertainties in these analyses, including errors introduced by culture bias, data limitations, and curve fitting.

There are general issues with sustaining laboratory cultures that should be noted, although they do not discount the value of experimental culture data. Culture bias (e.g., enrichment bias) pertains to selection bias that occurs when culturing and maintaining isolated strains. The transfer of cultures in exponential phase is known to result in a selection toward faster growing strains in a sample, even within the same microbial phenotype (Weissman et al. 2020). Despite media enrichment being used to favor a specific organism (e.g., low

concentrations of ammonium added to media to select for *Prochlorococcus*), the sub-level micro-diversity within an organism would lead to the strain most fit for the chosen enrichment conditions to dominate (e.g., strains of *Prochlorococcus* that only utilize ammonium whereas nitrate utilizing strains exist) (Berube et al. 2015). In the case of strains of *Prochlorococcus*, these enrichment conditions would also include the temperature of isolation since slight alterations in the temperature regime of a culture would select for different strains. If the incubation temperature of the isolate is warmer than the collection temperature, the isolation conditions could select for a subset of the strain population that prefers warmer temperatures. This selection would skew the fundamental temperature niche toward warmer temperatures.

Additionally, in the maintenance of long-term *Prochlorococcus* cultures, there is some genetic change that could be selected over many generations due to drift or selection (Osburne et al. 2011). A study of *Escherichia coli* comparing the relative fitness of isolates adapted to high temperatures, in this case 47°C, to the control group maintained at the ancestral selection temperature of 37°C, found that the high-temperature adapted lineages had increased fitness after only 200 generations (Bennett et al. 1990). Here, relative fitness was defined at the ratio of doublings in the high-temperature adapted clones over the control lineages. This suggests that mutations allowing for increased fitness in the high-temperature adapted lines appear in the population quickly (Bennett et al. 1990; Listmann et al. 2016). A later study of the same organism looked at strains adapted to 42.2°C and compared the relative fitness between the ancestral clone and the high-temperature adapted strains at 20°C and 37°C (Rodríguez-Verdugo et al. 2014). Fitness trade-offs were most apparent at 20°C where the high-adapted lineages had, on average, a 9% decline in fitness relative to the ancestral lineage (kept at 37°C). They found that this value had a high amount of variance suggesting not all individuals are adapting in the same way. Rodríguez-Verdugo et al. (2014) explored two different pathways that result in the observed temperature niche adaptation: niche expansion and niche shift. All clones seemed to have adapted to higher temperatures by shifting the upper limit of their temperature niche, however only 49% of the population had an equivalent shift in the lower temperature niche boundary. Their look into the relationship between genetic mutation, phenotypic changes, and the resulting temperature adaptations reveals a complex system in which there are multiple pathways to temperature adaptations each with their own trade-offs (Rodríguez-Verdugo et al. 2014).

In the context of strains of *Prochlorococcus*, if a culture is kept at a specific temperature outside of the optimum, a strain could adapt over time to have a higher fitness at the given temperature. This means that the strains used describe the fundamental niches might not have the same physiological response to temperature as the populations they were isolated from. However, a wealth of literature suggests that the ecotypes of *Prochlorococcus* are differentiated primarily in their physiological responses to light and temperature in correspondence to their evolutionary history, suggesting these traits are less plastic than nutrient acquisition (for a review see Biller et al. 2015).

The filters used to select data for our study ensure that the experiments were neither nutrient nor light limited, to ensure maximum growth rates across temperatures. We recognize that experimental conditions between studies differ in light levels, nutrients, and acclimation time periods. By combining data from multiple studies, we created a more robust dataset with less directional bias. Because we looked at lab data that is neither nutrient nor light limited to obtain the maximum growth rates, we believe that looking at all available depths provides the same standard, especially in regard to the low light adapted ecotypes. The extent of field data is limited as it is not collected at all depths, during all times of the year, or across all locations in the ocean. This study only focuses on the effect of temperature on biogeography, but previous studies have shown that strains exhibit a similar relationship with light (Moore et al. 1995; Zinser et al. 2007). To capture a realized temperature niche that spans the full range of possible temperatures, we chose to use data from all measured depths. In doing so, we included measurements down to 200 m which we acknowledge could include dead or dying cells that have retained some chlorophyll that is being detected by the qPCR methods. Low light adapted ecotypes have their maximum abundance at sub-euphotic depths due to the balance between their light-tolerance and their temperature-tolerance niches (Zinser et al. 2007; Malmstrom et al. 2010). We understand that combining data from below and above the mixed layer depth is confounding with respect to the different light and nutrient regimes. However, we argue that restricting the realized temperature niche, especially for low light adapted ecotypes, to only the mixed layer would lead to a mischaracterization of the actual realized temperature niche.

We also considered the temporal mass effect, a possible measurement bias that takes into account the lag between timescales of environmental variation and phytoplankton population response. When measuring ecotype abundance, we are associating that abundance value with the temperature at which it was collected. In reality, abundance could be a lagged response to growth at a different temperature that occurred sometime prior to the sampling event. In addition, due to horizontal and vertical displacement, or a spatial mass effect, a change in abundance could be a result of local dispersal processes transporting a population to or from a distant location. We see evidence of this in our dataset where some samples collected in warmer, well-lit surface waters were characterized by high abundances of eMIT9313, a low light adapted ecotype. A previous study showed that the temperature range an organism experiences if tracked in a Lagrangian perspective (that is, following the fluid) is greater (up to 10°C) than compared to variations in temperature in

stationary frame (Doblin and Van Sebille 2016). This source of error in temperature niche estimates could be minimized in future studies by calculating thermal niches following water parcels, though that is not possible with the available qPCR data.

This specific discrepancy with eMIT9313, while attributable to the temporal mass effect, could also be an artifact of errors in the fundamental niche estimate. eMIT9313 is a low light adapted ecotype and is observed in deep waters but has a high fundamental optimal temperature (Fig. 3). The estimation of the fundamental temperature niche in this study ignores the possible trade-off between temperature tolerance and photoinhibition. Previous studies have shown that eMIT9313, which experiences photoinhibition at high light intensities (Kulk et al. 2012), are not commonly found in high irradiance surface waters despite having a high fundamental optimal temperature (Zinser et al. 2007). In contrast, it's low light adapted sister ecotype eNATL2A is commonly found in high irradiance surface waters which suggests some underlying difference in photoregulation, such as the ability to dissipate high light induced cell excitation energy as heat in order to protect from photoinhibition (i.e., "quenching"; Zinser et al. 2007). For eMIT9313 and other low light adapted ecotypes of *Prochlorococcus*, Moore and Chisholm (1999) found that the irradiance at which growth was saturated was about half that of the high light ecotypes (around 20 μmol quanta m^{-2} s^{-1} compared to about 50 μmol quanta m^{-2} s^{-1}). eMIT9312, specifically, has shown the ability to grow at colder temperatures when presented with irradiances lower than used in the experiments referenced in this study (Kulk et al. 2012). This means that relative to the light levels used in the referenced experiments, the low light adapted ecotypes were cultured at saturating irradiances and thus may not present accurate representations of the true fundamental niche relative to both light and temperature. In addition, eMIT9313 has shown increased electron transport rates and enhanced photoacclimation at high temperature with high irradiance exposure but it is hypothesized that other processes such as the Calvin cycle or DNA replication would limit growth at suboptimal temperature resulting in a warmer fundamental optimal temperature (Kulk et al. 2012).

Why is the realized temperature niche optimum typically colder than observed in the lab?

For most ecotypes of *Prochlorococcus* across ocean basins, we found that the optimum temperatures in the field were lower than the optimum temperatures in the lab by 3.84°C with a SD across all ocean basins an ecotypes of 1.18°C (Fig. 6). Other studies of marine phytoplankton have found a similar pattern (Thomas et al. 2012). For example, eMIT9312 in the North Atlantic has a realized temperature niche optimum that is 6.31°C colder than its fundamental niche optimum temperature (Figs. 5 and 6). This common discrepancy (colder realized niche compared to fundamental niche) likely occurs, at least in part, because the fundamental niche is generally described as being left-skewed where growth rates decrease faster at higher temperatures than they increase at lower temperatures (Thomas et al. 2012). This shape is a result of the effect of thermal sensitivity of biologically important chemical reactions (Kingsolver 2009). For a given phytoplankton, time-averaged growth is higher when they are living at a temperature slightly lower than their lab-measured optimum. In other words, in a variable environment it is risky to live too close to their fundamental thermal optimum (T_{opt}). Similar patterns have been observed across a range of organisms and ecosystems (Kingsolver et al. 2013; Vasseur et al. 2014).

The effects of unresolved diversity on phytoplankton temperature niches

An additional reason why the fundamental and realized temperature niche optima differ could be unresolved diversity within ecotypes. The methods used to classify organisms in the field (qPCR) provide ecotype-level genetic resolution measurements. While the strain variations for some ecotypes (like eMIT9312; see Fig. 1) are better characterized than others, there is still a wealth of unknown diversity. Previous genetic studies have revealed diversity that breaks down the known ecotype distinctions (eMED4, eMIT9312, etc.; *see* Fig. 1) into necessary sub-clades that have distinct genetic lineages have found novel sub-clades and novel clades based on RNA clustering (Martiny et al. 2009; Kashtan et al. 2014, 2017; Larkin et al. 2016). For example, one such study (Kashtan et al. 2014) found hundreds of genetically distinct sub-populations that showed distinct seasonal diversity patterns in the Atlantic Ocean. A more recent study (Kashtan et al. 2017) compared the Atlantic and Pacific sub-populations and found that the Pacific Ocean had greater sub-population richness with more unique sub-populations and no single dominant sub-population compared to the Atlantic Ocean. In addition, the genetic diversity within a single clade, across the two ocean basins, suggests that the clades had distinct evolutionary trajectories that resulted in two sub-populations separated by ocean basin (Kashtan et al. 2017).

We demonstrate the effect of strain diversity on the fundamental temperature niche effect for eMIT9312, which is comprised of six different strains, by randomly removing one to five strains, refitting the subset data with the left-skewed curve and recalculating T_{opt} and W, repeating this process 1000 times. As a result, the temperature niche optimum, on average, was shifted warmer by 0.23°C and the realized temperature niche width decreased by 0.35°C (Supporting Information Fig. S2). In other words, removing strain diversity within the ecotype results in warmer and narrower fundamental temperature niches. Here, unresolved strain diversity underestimates the true ecotypic fundamental niche width and overestimates the optimal temperature. A similar process may influence realized niches in the field, though we are not able to assess the magnitude of the effect with the available data. Given the small size of the changes in niche parameters

due to subsampling of strains (Supporting Information Fig. S2), we do not think these potential biases can explain the consistent cold discrepancy between fundamental and realized temperature niches (Fig. 6).

What role could competition and dispersal play in shaping the realized temperature niche?

We found that *Prochlorococcus* temperature niches are on average 2.15°C wider in the field than in the lab with a SD across all ocean basins and ecotypes of 1.89°C (Fig. 6). Our expectation was that competition between related *Prochlorococcus* ecotypes and other phytoplankton would lead to narrower realized than fundamental niches. Strong overlap in temperature niche space between competing species should lead to competitive dominance of each species in a subrange of temperatures where they have higher average fitness than other competitors (Hutchinson 1957; Thomas et al. 2012; Soberón and Arroyo-Peña 2017). *Prochlorococcus* ecotypes have distinct biogeographical patterns due to nutrients, light, and temperature; for example, eMED4 has a slightly cooler fundamental temperature than does eMIT9312, and consequently is found in somewhat colder regions than eMIT9312 (Johnson et al. 2006). However, at least for the ecotypes and regions we examined, we do not see a dramatic narrowing of realized temperature niches relative to fundamental niches that we expected due to competition.

One possible explanation for the surprising width of realized temperature niches is dispersal. Ocean currents and mixing are capable of moving phytoplankton great distances in the ocean. Over a matter of days, *Prochlorococcus* in the Gulf Stream, for example, can be moved hundreds of kilometers and ultimately encounter conditions outside their expected thermal tolerance (Clayton et al. 2014; Doblin and Van Sebille 2016). *Prochlorococcus* have been found in northward flowing waters of the Gulf Stream, out of their expected range (Cavender-Bares et al. 2001). In the context of our study, each sampling site measured the population of each ecotype within the larger metapopulation at a particular moment (Leibold et al. 2004). Dispersal from nearby sites, each perhaps with some degree of local adaptation or site specific differences in *Prochlorococcus* diversity, could produce a realized temperature niche that appears wider than seen in the lab (Chandler et al. 2016; Hellweger et al. 2016).

We illustrate the roles of competition and dispersal in shaping realized temperature niches using a conceptual metacommunity model (Fig. 7) representing competing phytoplankton populations across a latitudinal gradient of temperature where locations are connected via dispersal. Briefly, the simple model includes 15 species across 30 boxes arrayed from north to south in the Northern Hemisphere. The boxes represent regions of the ocean, and the annual cycle of temperature in each box is determined by a yearly seasonal cycle of daily averages from the time period 1981–2010 (NOAA OISST; *NOAA High Resolution SST data provided by the NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, from their web site at* https://www.esrl.noaa.gov/psd/). Model phytoplankton species differ only in terms of their optimal temperature for growth. Nutrients are supplied from ocean depths via a constant dilution term approximating constant diapycnal mixing. Dispersal can be turned on, or off. Details of the model, which we use to demonstrate the potential roles of competition and dispersal in shaping realized temperature niches, are fully outlined in the Supporting Information.

With this conceptual model we compare the realized temperature niches of two species with overlapping fundamental temperature niches, with and without dispersal. One species is adapted to colder conditions ($T_{opt} = 6.73$°C), and the other to slightly warmer conditions ($T_{opt} = 15.6$°C; Fig. 7c). Each species dominates in the model box where the temperature is closest to their optimum. Without dispersal, competition between the phytoplankton types reduces the width of the realized compared to fundamental temperature niche (Fig. 7d). When phytoplankton are dispersed across ocean zones (nutrients are not dispersed in the model), the realized temperature niches for both species are wider relative to the fundamental temperature niche (Fig. 7e). For example, when there is no dispersal between boxes, the species adapted to colder temperatures ($T_{opt} = 6.37$°C) has a niche width of 9.82°C, calculated as the difference between the 1st and the 99th percentiles (Fig. 7d.), however, when dispersal is allowed between latitudes, the niche width of this species increases to 29.2°C (Fig. 7e). We therefore hypothesize that the increased width of *Prochlorococcus* realized temperature niches relative to fundamental temperature niches may be driven, at least in part, by dispersal in the ocean.

In addition to affecting the width of the realized temperature niches, there is evidence that advection could impact the optimal temperature of realized niches. One study (Hellweger et al. 2016) created a global model of phytoplankton competition that allows the model to track the temperature a microbe experiences along its trajectory. The output shows that in two different locations along the Gulf Stream for the most abundant species, a model with advection allowed for a 3.2°C increase in optimal temperature compared to a model without advection. This suggests that advection increases the range of temperature an organism experiences and results in a shift in the realized temperature niche compared to a regime without advection. In regard to *Prochlorococcus* ecotypes, Hellweger et al. (2016) used observational data from several sites in highly advective areas that deviate from the trend of temperature increase resulting in an increase in the ratio of eMIT9312 to eMED4 abundance. By using their model to correct for potential optimal temperature differences resulting from advective processes, they found that accounting for advection improved the correlation between temperature and the ratio of eMIT9312 to eMED4. Additionally, their study also showed that the model which accounts for advection improves the correlation between the modeled optimal temperature of the

most dominant species in the model and the expected optimal temperature of that species based off of experimental growth rate data (i.e., the modeled realized niche and the fundamental niche). This suggests that while temperature is a main driver in *Prochlorococcus* biogeography, advection plays an important role in shaping realized temperature niches. While we draw some comparisons between our data and this example, our data includes more depths, more sampling effort, more ecotypes, and focuses on the comparison between the fundamental and realized temperature niches.

Differences in niche width across ecotypes

On average, the fundamental niche width (W) for high light ecotypes (eMED4 and eMIT9312) is greater than for low light ecotypes (eMIT9313 and eNATL2A; Fig. 6). We have also found that the difference in niche widths between realized and fundamental niches ($W_{obs} - W$) is greater for low light ecotypes than for high light ecotypes (Fig. 6). It is plausible that the low light ecotype widths are narrower because these organisms are adapted to habitats deeper in the water column with less variability in temperature. These observations may account for the consistently greater differences between niche widths (i.e., $W_{obs} - W$) between low light and high light ecotypes.

Interactive ecological and environmental factors

An organism's realized niche is defined by trade-offs between multiple traits as well as biological interactions with competitors, predators, and other organisms (Litchman and Klausmeier 2008). Here, we have focused only on the roles of temperature, but other environmental factors and biotic interactions clearly play a role in shaping the realized niches of each ecotype. Marine microbes respond to a complex range of environmental drivers, and can achieve equivalent fitness under multiple combinations of factors such as light, nutrients, CO_2, and temperature (Boyd and Brown 2015; Boyd et al. 2018). Interactions among *Prochlorococcus* ecotypes are likely to shape realized temperature niches as well. For example, the low light clades eNATL2A and eMIT9313 grow well at warmer temperatures in the lab (e.g., Fig. 3, Table 1), but are typically founder deeper (and colder) in the water column than are the high light adapted clades eMED4 and eMIT9312 (Johnson et al. 2006; Zinser et al. 2007). The realized niches of the low light clades appear colder than in the lab due to variations in the light in the water column. Interactions with other picophytoplankton (Luis Otero-Ferrer et al. 2018) and protist grazers (Guillou et al. 2001) are likely to cause changes in the realized niches of *Prochlorococcus* ecotypes. Heterotrophic bacteria can protect *Prochlorococcus* from oxidative stress, and this cross-protection can influence the growth of *Prochlorococcus* at temperature extremes (Ma et al. 2018). The interaction of *Prochlorococcus* ecotypes, other phytoplankton, and predators varies across spatial and temporal gradients, as well as across ocean basins (Zwirglmaier et al. 2008; Thomas et al. 2012; Sohm et al. 2016).

Why is the realized niche different in different ocean basins?

Local adaptation may explain why realized niches differ across ocean basins. Generally speaking, local adaptation implies that an organism has higher fitness in its local environment than when transplanted to other habitats (Blanquart et al. 2013). While it is difficult to quantify and demonstrate local adaptation from field observations of abundance alone, there is increasing evidence of local adaptation in microbial populations (Kraemer and Boynton 2017; Yvon-Durocher et al. 2017; Martiny et al. 2019). For *Prochlorococcus*, dispersal timescales across ocean basins are long, on the order of decades (Martiny et al. 2009), but the turnover timescale is short, suggesting local adaptation is likely to occur. For example, Martiny et al. (2019) found that there is a strong negative relationship between phosphorus concentration and the number of phosphorus uptake genes in *Prochlorococcus*, implying that *Prochlorococcus* adapt to local macronutrient availability. Within the context of the available data, and without reciprocal transplant experiments, it is difficult to quantify the extent of local adaptation. However, we believe that the strong differences in realized niche for each ecotype across ocean basins is consistent with local adaptation and is likely given the rapid generation times and large population sizes of *Prochlorococcus*.

CONCLUSION

This study compared the fundamental and realized temperature niches for multiple ecotypes of *Prochlorococcus* across ocean basins. We find that the realized temperature niches for *Prochlorococcus* ecotypes are, on average, colder and wider than their fundamental temperature niches. The cold bias of realized compared to fundamental niches may be due to the left-skewed nature of the fundamental temperature niche. In a variable thermal environment, an organism that has an optimum too close to the mean temperature is more likely to encounter detrimentally high temperatures than an organism living in an environment with a mean temperature below the optimum. The wider niches in the field compared with the lab may arise due to a range of factors, but we speculate that competition and dispersal may play important roles. Whereas competition between phytoplankton would likely make realized temperature niches narrower than fundamental niches, dispersal of phytoplankton outside of their thermal optimum would tend to have the opposite effect. Therefore, ocean currents and mixing likely play a key role in shaping the observed realized niches of *Prochlorococcus*.

We also found that the realized temperature niches of *Prochlorococcus* ecotypes differ between ocean basins. There are a range of possible explanations for this observation, but because of the rapid generation times and large population sizes of *Prochlorococcus* and dispersal limitation between ocean basins, we speculate that local adaptation to the physical

environment or differences in biological interactions between the ocean basins may explain the temperature niche differences.

Our findings have a range of implications for the study of *Prochlorococcus* and other marine microbes. Firstly, most ecological models that forecast the distribution of *Prochlorococcus* in current and future ocean conditions have low diversity of picoplankton (e.g., one or a few groups of small picoplankton) and generally little to no intraspecific diversity within *Prochlorococcus* (e.g., the model does not resolve ecotypes or strains of *Prochlorococcus*). Models lacking diversity among the smallest phytoplankton in the ocean would likely provide only rough estimates of the distribution of these key phytoplankton in the ocean. Models simulating how marine phytoplankton respond to climate change would particularly benefit from resolving some of the diversity within *Prochlorococcus* (e.g., Follows et al. 2007), as changes in ocean conditions are likely change the distribution of different ecotypes. Second, most ecological models assume that species traits are fixed through time. However, recent work shows that phytoplankton have a high degree of physiological plasticity and capacity for evolution in response to environmental changes (Schaum et al. 2013; Collins et al. 2014). In order to simulate the differences in *Prochlorococcus* temperature niches between ocean basins, it is necessary to allow for some degree of plasticity or evolution in ecological models (Ward et al. 2021). Finally, species distribution models rarely resolve the diversity within *Prochlorococcus*. A species distribution model builds an empirical relationship between the abundance of a particular taxa and associated environmental and biotic conditions (Elith et al. 2011; Irwin et al. 2012; Flombaum et al. 2013; Merow et al. 2014). This model can then be used to estimate the future distribution of a species in response to climate change, assuming the realized niche remains fixed through time (Araujo and Peterson 2012; Barton et al. 2016). Species distribution models for *Prochlorococcus* that do not resolve intraspecific diversity may not give accurate estimates of future distributions of *Prochlorococcus* and may also mask important underlying differences among ecotypes present in the current ocean.

This study highlights the need for additional effort to understand that mechanisms that control the differences between realized and fundamental niches of *Prochlorococcus* across ocean basins. These differences are significant and have broad relevance to how we interpret and model *Prochlorococcus* biogeography in the current and future climates.

CONFLICTS OF INTEREST

None declared.

Author Contributions

A.N.S, G.M.M.H., and A.D.B. designed the study. A.N.S. conducted data analysis and wrote the manuscript with input from all authors. E.R.Z., B.C.C., and J.W.C. collected, curated, and provided data for the study.

References

Araújo, M. B., and Peterson, A. T. 2012. Uses and misuses of bioclimatic envelope modeling. Ecology **93**: 1527–1539. http://dx.doi.org/10.1890/11-1930.1

Barton, A. D., S. Dutkiewicz, G. Flierl, J. Bragg, and M. J. Follows. 2010. Patterns of diversity in marine phytoplankton. Science **327**: 1509–1512. doi:10.1126/science.1184961

Barton, A. D., A. J. Irwin, Z. V. Finkel, and C. A. Stock. 2016. Anthropogenic climate change drives shift and shuffle in North Atlantic phytoplankton communities. Proc. Natl. Acad. Sci. USA **113**: 2964–2969. doi:10.1073/pnas.1519080113

Bennett, A. F., K. M. Dao, and R. E. Lenski. 1990. Rapid evolution in response to high-temperature selection. Nature **346**: 79–81. doi:10.1038/346079a0

Berube, P. M., S. J. Biller, A. G. Kent, and others. 2015. Physiology and evolution of nitrate acquisition in *Prochlorococcus*. ISME J. **9**: 1195–1207. doi:10.1038/ismej.2014.211

Bestion, E., C.-E. Schaum, and G. Yvon-Durocher. 2018. Nutrient limitation constrains thermal tolerance in freshwater phytoplankton. Limnol. Oceanogr. Lett. **3**: 436–443. doi:10.1002/lol2.10096

Biller, S. J., P. M. Berube, D. Lindell, and S. W. Chisholm. 2015. *Prochlorococcus*: The structure and function of collective diversity. Nat. Rev. Microbiol. **13**: 13–27. doi:10.1038/nrmicro3378

Blanquart, F., O. Kaltz, S. L. Nuismer, and S. Gandon. 2013. A practical guide to measuring local adaptation. Ecol. Lett. **16**: 1195–1205. doi:10.1111/ele.12150

Boyd, P. W., and C. J. Brown. 2015. Modes of interactions between environmental drivers and marine biota. Front. Mar. Sci. **2**: 1–8. doi:10.3389/fmars.2015.00009

Boyd, P. W., S. Collins, S. Dupont, and others. 2018. Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change: A review. Glob. Chang. Biol. **24**: 2239–2261. doi:10.1111/gcb.14102

Braschler, B., and others, 2020. Realised rather than fundamental thermal niches predict site occupancy: Implications for climate change forecasting. J. Anim. Ecol. **89**: 2863–2875. http://dx.doi.org/10.1111/1365-2656.13358

Burson, A., M. Stomp, E. Greenwell, J. Grosse, and J. Huisman. 2018. Competition for nutrients and light: Testing advances in resource competition with a natural phytoplankton community. Ecology **99**: 1108–1118. doi:10.1002/ecy.2187

Cavender-Bares, K. K., Karl, D.M., and Chisholm, S. W. 2001. Nutrient gradients in the western North Atlantic Ocean: Relationship to microbial community structure and comparison to patterns in the Pacific Ocean. Deep. Res. Part I:

Oceanogr. Res. Pap. **48**: 2373–2395. http://dx.doi.org/10.1016/s0967-0637(01)00027-9

Chandler, J. W., Y. Lin, P. J. Gainer, A. F. Post, Z. I. Johnson, and E. R. Zinser. 2016. Variable but persistent coexistence of *Prochlorococcus* ecotypes along temperature gradients in the ocean's surface mixed layer. Environ. Microbiol. Rep. **8**: 272–284. doi:10.1111/1758-2229.12378

Clayton, S., Nagai, T., and Follows, M. J. 2014. Fine scale phytoplankton community structure across the Kuroshio Front. J. Plankton Res. **36**: 1017–1030. http://dx.doi.org/10.1093/plankt/fbu020

Collins, S., B. Rost, and T. A. Rynearson. 2014. Evolutionary potential of marine phytoplankton under ocean acidification. Evol. Appl. **7**: 140–155. doi:10.1111/eva.12120

Colwell, R. K., and T. F. Rangel. 2009. Hutchinson's duality: The once and future niche. Proc. Natl. Acad. Sci. USA **106**: 19651–19658. doi:10.1073/pnas.0901650106

Doblin, M. A., and E. Van Sebille. 2016. Drift in ocean currents impacts intergenerational microbial exposure to temperature. Proc. Natl. Acad. Sci. USA **113**: 5700–5705. doi:10.1073/pnas.1521093113

Edwards, K. F., E. Litchman, and C. A. Klausmeier. 2013. Functional traits explain phytoplankton community structure and seasonal dynamics in a marine ecosystem. Ecol. Lett. **16**: 56–63. doi:10.1111/ele.12012

Edwards, K. F., M. K. Thomas, C. A. Klausmeier, and E. Litchman. 2012. Allometric scaling and taxonomic variation in nutrient utilization traits and maximum growth rate of phytoplankton. Limnol. Oceanogr. **57**: 554–566. doi:10.4319/lo.2012.57.2.0554

Elith, J., S. J. Phillips, T. Hastie, M. Dudík, Y. E. Chee, and C. J. Yates. 2011. A statistical explanation of MaxEnt for ecologists. Divers. Distrib. **17**: 43–57. doi:10.1111/j.1472-4642.2010.00725.x

Eppley, R. W. 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. **70**: 1063–1085.

Falkowski, P. G., R. T. Barber, and V. Smetacek. 1998. Biogeochemical controls and feedbacks on ocean primary production. Science **281**: 200–206. doi:10.1126/science.281.5374.200

Falkowski, P. G., and M. J. Oliver. 2007. Mix and match: How climate selects phytoplankton. Nat. Rev. Microbiol. **5**: 813–819. doi:10.1038/nrmicro1751

Flombaum, P., J. L. Gallegos, R. A. Gordillo, and others. 2013. Present and future global distributions of the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. Proc. Natl. Acad. Sci. **110**: 9824–9829. doi:10.1073/pnas.1307701110

Follows, M. J., Dutkiewicz, S., Grant, S., and Chisholm, S. W. 2007. Emergent biogeography of microbial communities in a model ocean. Science **315**: 1843–1846. http://dx.doi.org/10.1126/science.1138544

Geider, R. J., H. L. MacIntyre, and T. M. Kana. 1998. A dynamic regulatory model of phytoplanktonic acclimation to light, nutrients, and temperature. Limnol. Oceanogr. **43**: 679–694. doi:10.4319/lo.1998.43.4.0679

Guillou, L., S. Jacquet, M. J. Chrétiennot-Dinet, and D. Vaulot. 2001. Grazing impact of two small heterotrophic flagellates on *Prochlorococcus* and *Synechococcus*. Aquat. Microb. Ecol. **26**: 201–207. doi:10.3354/ame026201

Hellweger, F. L., E. Van Sebille, B. C. Calfee, J. W. Chandler, E. R. Zinser, B. K. Swan, and N. D. Fredrick. 2016. The role of ocean currents in the temperature selection of plankton: Insights from an individual-based model. PLoS One **11**: 1–19. doi:10.1371/journal.pone.0167010

Hutchinson, G.E. 1957. Concluding Remarks. Cold Spring Harbor Symposia on Quantitative Biology **22**: 415–427. http://dx.doi.org/10.1101/sqb.1957.022.01.039

Ibarbalz, F. M., N. Henry, M. C. Brandão, and others. 2019. Global trends in marine plankton diversity across kingdoms of life. Cell **179**: 1084–1097.e21. doi:10.1016/j.cell.2019.10.008

Irwin, A. J., Z. V. Finkel, F. E. Müller-Karger, and L. T. Ghinaglia. 2015. Phytoplankton adapt to changing ocean environments. Proc. Natl. Acad. Sci. U. S. A. **112**: 5762–5766. doi:10.1073/pnas.1414752112

Irwin, A. J., A. M. Nelles, and Z. V. Finkel. 2012. Phytoplankton niches estimated from field data. Limnol. Oceanogr. **57**: 787–797. doi:10.4319/lo.2012.57.3.0787

Johnson, Z. I., E. R. Zinser, A. Coe, N. P. Mcnulty, E. S. Malcolm, S. W. Chisholm, E. M. S. Woodward, and S. W. Chisholm. 2006. Partitioning among *Prochlorococcus* ecotypes along environmental gradients. Science **311**: 1737–1740. doi:10.1126/science.1118052

Jönsson, B. F., and J. R. Watson. 2016. The timescales of global surface-ocean connectivity. Nat. Commun. **7**: 1–6. doi:10.1038/ncomms11239

Kashtan, N., S. E. Roggensack, J. W. Berta-Thompson, M. Grinberg, R. Stepanauskas, and S. W. Chisholm. 2017. Fundamental differences in diversity and genomic population structure between Atlantic and Pacific *Prochlorococcus*. ISME J. **11**: 1997–2011. doi:10.1038/ismej.2017.64

Kashtan, N., S. E. Roggensack, S. Rodrigue, and others. 2014. Coexisting subpopulations in wild *Prochlorococcus*. Science **344**: 416–420. doi:10.1126/science.1248575

Kingsolver, J. G. 2009. The well-temperatured biologist. Am. Nat. **174**: 755–768. doi:10.1086/648310

Kingsolver, J. G., S. E. Diamond, and L. B. Buckley. 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. Funct. Ecol. **27**: 1415–1423. doi:10.1111/1365-2435.12145

Kraemer, S. A., and P. J. Boynton. 2017. Evidence for microbial local adaptation in nature. Mol. Ecol. **26**: 1860–1876. doi:10.1111/mec.13958

Kremer, C. T., M. K. Thomas, and E. Litchman. 2017. Temperature- and size-scaling of phytoplankton population growth rates: Reconciling the Eppley curve and the metabolic

theory of ecology. Limnol. Oceanogr. **62**: 1658–1670. doi:10.1002/lno.10523

Kulk, G., P. De Vries, W. H. Van De Poll, R. J. W. Visser, and A. G. J. Buma. 2012. Temperature-dependent growth and photophysiology of prokaryotic and eukaryotic oceanic picophytoplankton. Mar. Ecol. Prog. Ser. **466**: 43–55. doi:10.3354/meps09898

Larkin, A. A., S. K. Blinebry, C. Howes, Y. Lin, S. E. Loftus, C. A. Schmaus, E. R. Zinser, and Z. I. Johnson. 2016. Niche partitioning and biogeography of high light adapted *Prochlorococcus* across taxonomic ranks in the North Pacific. ISME J. **10**: 1555–1567. doi:10.1038/ismej.2015.244

Leibold, M. A., M. Holyoak, N. Mouquet, and others. 2004. The metacommunity concept: A framework for multi-scale community ecology. Ecol. Lett. **7**: 601–613. doi:10.1111/j.1461-0248.2004.00608.x

Listmann, L., M. LeRoch, L. Schlüter, M. K. Thomas, and T. B. H. Reusch. 2016. Swift thermal reaction norm evolution in a key marine phytoplankton species. Evol. Appl. **9**: 1156–1164. doi:10.1111/eva.12362

Litchman, E., and C. A. Klausmeier. 2008. Trait-based community ecology of phytoplankton. Annu. Rev. Ecol. Evol. Syst. **39**: 615–639. doi:10.1146/annurev.ecolsys.39.110707.173549

Luis Otero-Ferrer, J., P. Cermeño, A. Bode, and others. 2018. Factors controlling the community structure of picoplankton in contrasting marine environments. Biogeosciences **15**: 6199–6220. doi:10.5194/bg-15-6199-2018

Ma, L., B. C. Calfee, J. J. Morris, Z. I. Johnson, and E. R. Zinser. 2018. Degradation of hydrogen peroxide at the ocean's surface: The influence of the microbial community on the realized thermal niche of *Prochlorococcus*. ISME J. **12**: 473–484. doi:10.1038/ismej.2017.182

Malmstrom, R. R., A. Coe, G. C. Kettler, A. C. Martiny, J. Frias-Lopez, E. R. Zinser, and S. W. Chisholm. 2010. Temporal dynamics of *Prochlorococcus* ecotypes in the Atlantic and pacific oceans. ISME J. **4**: 1252–1264. doi:10.1038/ismej.2010.60

Malmstrom, R. R., S. Rodrigue, K. H. Huang, and others. 2013. Ecology of uncultured *Prochlorococcus* clades revealed through single-cell genomics and biogeographic analysis. ISME J. **7**: 184–198. doi:10.1038/ismej.2012.89

Marãnón, E., P. Cermeño, M. Huete-Ortega, D. C. López-Sandoval, B. Mouriño-Carballido, and T. Rodríguez-Ramos. 2014. Resource supply overrides temperature as a controlling factor of marine phytoplankton growth. PLoS One **9**: 20–23. doi:10.1371/journal.pone.0099312

Martiny, A. C., L. Ma, C. Mouginot, J. W. Chandler, and E. R. Zinser. 2016. Interactions between thermal acclimation, growth rate, and phylogeny influence *Prochlorococcus* elemental stoichiometry. PLoS One **11**: 1–12. doi:10.1371/journal.pone.0168291

Martiny, A. C., A. P. K. Tai, D. Veneziano, F. Primeau, and S. W. Chisholm. 2009. Taxonomic resolution, ecotypes and the biogeography of *Prochlorococcus*. Environ. Microbiol. **11**: 823–832. doi:10.1111/j.1462-2920.2008.01803.x

Martiny, A. C., L. Ustick, C. A. Garcia, and M. W. Lomas. 2019. Genomic adaptation of marine phytoplankton populations regulates phosphate uptake. Limnol. Oceanogr. **00**: 1–11. doi:10.1002/lno.11252

Merow, C., J. P. Dahlgren, C. J. E. Metcalf, and others. 2014. Advancing population ecology with integral projection models: A practical guide. Methods Ecol. Evol. **5**: 99–110. doi:10.1111/2041-210X.12146

Moore, L. R., R. Goericke, and S. W. Chisholm. 1995. Comparative physiology of *Synechococcus* and *Prochlorococcus*: Influence of light and temperature on growth, pigments, fluorescence and absorptive properties. Mar. Ecol. Prog. Ser. **116**: 259–276. doi:10.3354/meps116259

Moore, L. R., and Chisholm, S. W. 1999. Photophysiology of the marine cyanobacterium Prochlorococcus: Ecotypic differences among cultured isolates. Limnology and Oceanography **44**: 628–638. http://dx.doi.org/10.4319/lo.1999.44.3.0628

Morán, X. A. G., Á. López-Urrutia, A. Calvo-Díaz, and W. K. W. LI. 2010. Increasing importance of small phytoplankton in a warmer ocean. Glob. Chang. Biol. **16**: 1137–1144. doi:10.1111/j.1365-2486.2009.01960.x

Morel, F. M. M. 1987. Kinetics of nutrients uptake and growth in phytoplankton. J. Phycol. **23**: 137–150. doi:10.1111/j.1529-8817.1987.tb04436.x

Norberg, J. 2004. Biodiversity and ecosystem functioning: A complex adaptive systems approach. Limnol. Oceanogr. **49**: 1269–1277. doi:10.4319/lo.2004.49.4_part_2.1269

Osburne, M. S., B. M. Holmbeck, A. Coe, and S. W. Chisholm. 2011. The spontaneous mutation frequencies of *Prochlorococcus* strains are commensurate with those of other bacteria. Environ. Microbiol. Rep. **3**: 744–749. doi:10.1111/j.1758-2229.2011.00293.x

Partensky, F., W. R. Hess, and D. Vaulot. 1999. *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. Microbiol. Mol. Biol. Rev. **63**: 106–127. doi:10.1128/MMBR.63.1.106-127.1999

Rocap, G., F. W. Larimer, J. Lamerdin, and others. 2003. Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. Nature **424**: 1042–1047. doi:10.1038/nature01947

Rodríguez-Verdugo, A., D. Carrillo-Cisneros, A. Gonza⊠ez-González, B. S. Gaut, and A. F. Bennett. 2014. Different tradeoffs result from alternate genetic adaptations to a common environment. Proc. Natl. Acad. Sci. USA **111**: 12121–12126. doi:10.1073/pnas.1406886111

Rost, B., I. Zondervan, and D. Wolf-Gladrow. 2008. Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: Current knowledge, contradictions and research

directions. Mar. Ecol. Prog. Ser. **373**: 227–237. doi:10.3354/meps07776

Schaum, E., B. Rost, A. J. Millar, and S. Collins. 2013. Variation in plastic responses of a globally distributed picoplankton species to ocean acidification. Nat. Clim. Chang. **3**: 298–302. doi:10.1038/nclimate1774

Smayda, T. J. 1958. Biogeographical studies of marine phytoplankton. Oikos **9**: 158–191. doi:10.2307/3564763

Snell-Rood, E., R. Cothran, A. Espeset, P. Jeyasingh, S. Hobbie, and N. I. Morehouse. 2015. Life-history evolution in the anthropocene: Effects of increasing nutrients on traits and trade-offs. Evol. Appl. **8**: 635–649. doi:10.1111/eva.12272

Soberón, J., and B. Arroyo-Peña. 2017. Are fundamental niches larger than the realized? Testing a 50-year-old prediction by Hutchinson. PLoS One **12**: 1–14. doi:10.1371/journal.pone.0175138

Sohm, J. A., N. A. Ahlgren, Z. J. Thomson, C. Williams, J. W. Moffett, M. A. Saito, E. A. Webb, and G. Rocap. 2016. Co-occurring *Synechococcus* ecotypes occupy four major oceanic regimes defined by temperature, macronutrients and iron. ISME J. **10**: 333–345. doi:10.1038/ismej.2015.115

Stawiarski, B., E. T. Buitenhuis, and C. Le Quéré. 2016. The physiological response of picophytoplankton to temperature and its model representation. Front. Mar. Sci. **3**: 1–13. doi:10.3389/fmars.2016.00164

Thomas, M. K., C. T. Kremer, C. A. Klausmeier, and E. Litchman. 2012. A global pattern of thermal adaptation in marine phytoplankton. Science **338**: 1085–1089. doi:10.1126/science.1224836

Thomas, M. K., C. T. Kremer, and E. Litchman. 2016. Environment and evolutionary history determine the global biogeography of phytoplankton temperature traits. Glob. Ecol. Biogeogr. **25**: 75–86. doi:10.1111/geb.12387

Vasseur, D. A., J. P. DeLong, B. Gilbert, and others. 2014. Increased temperature variation poses a greater risk to species than climate warming. Proc. R. Soc. B Biol. Sci. **281**: 1–8. http://dx.doi.org/10.1098/rspb.2013.2612

Ward, B. A., B. B. Cael, S. Collins, and C. Robert Young. 2021. Selective constraints on global plankton dispersal. Proc. Natl. Acad. Sci. USA **118**: 1–7. doi:10.1073/pnas.2007388118

Weissman, J. L., Hou, S., and Fuhrman, J. A. 2021. Estimating maximal microbial growth rates from cultures, metagenomes, and single cells via codon usage patterns. Proc. Nat. Acad. Sci. **118**: 1-12. http://dx.doi.org/10.1073/pnas.2016810118

West, N. J., and D. J. Scanlan. 1999. Niche-partitioning of *Prochlorococcus* populations in a stratified water column in the eastern North Atlantic Ocean. Appl. Environ. Microbiol. **65**: 2585–2591. doi:10.1128/aem.65.6.2585-2591.1999

Yvon-Durocher, G., C. E. Schaum, and M. Trimmer. 2017. The temperature dependence of phytoplankton stoichiometry: Investigating the roles of species sorting and local adaptation. Front. Microbiol. **8**: 1–14. doi:10.3389/fmicb.2017.02003

Zinser, E. R., A. Coe, Z. I. Johnson, A. C. Martiny, N. J. Fuller, D. J. Scanlan, and S. W. Chisholm. 2006. *Prochlorococcus* ecotype abundances in the North Atlantic Ocean as revealed by an improved quantitative PCR method. Appl. Environ. Microbiol. **72**: 723–732. doi:10.1128/AEM.72.1.723-732.2006

Zinser, E. R., Z. I. Johnson, A. Coe, E. Karaca, D. Veneziano, and S. W. Chisholm. 2007. Influence of light and temperature on *Prochlorococcus* ecotype distributions in the Atlantic Ocean. Limnol. Oceanogr. **52**: 2205–2220. doi:10.4319/lo.2007.52.5.2205

Zwirglmaier, K., L. Jardillier, M. Ostrowski, and others. 2008. Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. Environ. Microbiol. **10**: 147–161. doi:10.1111/j.1462-2920.2007.01440.x

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