

## BRIEF COMMUNICATION

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Vitamin B<sub>12</sub> conveys a protective advantage to phycosphere-associated bacteria at high temperaturesMargaret Mars Brisbin<sup>1,2</sup>✉, Alese Schofield<sup>1,3</sup>, Matthew R. McIlvin<sup>1</sup>, Arianna I. Krinos<sup>2,4</sup>, Harriet Alexander<sup>1,2</sup> and Mak A. Saito<sup>1</sup>✉

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Many marine microbes require vitamin B<sub>12</sub> (cobalamin) but are unable to synthesize it, necessitating reliance on other B<sub>12</sub>-producing microbes. Thus, phytoplankton and bacterioplankton community dynamics can partially depend on the production and release of a limiting resource by members of the same community. We tested the impact of temperature and B<sub>12</sub> availability on the growth of two bacterial taxa commonly associated with phytoplankton: *Ruegeria pomeroyi*, which produces B<sub>12</sub> and fulfills the B<sub>12</sub> requirements of some phytoplankton, and *Alteromonas macleodii*, which does not produce B<sub>12</sub> but also does not strictly require it for growth. For B<sub>12</sub>-producing *R. pomeroyi*, we further tested how temperature influences B<sub>12</sub> production and release. Access to B<sub>12</sub> significantly increased growth rates of both species at the highest temperatures tested (38 °C for *R. pomeroyi*, 40 °C for *A. macleodii*) and *A. macleodii* biomass was significantly reduced when grown at high temperatures without B<sub>12</sub>, indicating that B<sub>12</sub> is protective at high temperatures. Moreover, *R. pomeroyi* produced more B<sub>12</sub> at warmer temperatures but did not release detectable amounts of B<sub>12</sub> at any temperature tested. Results imply that increasing temperatures and more frequent marine heatwaves with climate change will influence microbial B<sub>12</sub> dynamics and could interrupt symbiotic resource sharing.

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## INTRODUCTION

Vitamin B<sub>12</sub> (cobalamin) is required by many marine bacteria and unicellular eukaryotes [1, 2] but is scarce throughout broad regions of the global ocean, forcing microbes that cannot synthesize B<sub>12</sub> to rely on others that can [3, 4]. Many phytoplankton fulfill their B<sub>12</sub> requirements through interactions with B<sub>12</sub>-producing bacteria in the phycosphere [5, 6]. Some phycosphere bacteria, like *Ruegeria pomeroyi*, are known B<sub>12</sub> producers and require B<sub>12</sub> for growth [6]. Other phycosphere inhabitants, like *Alteromonas macleodii*, cannot produce B<sub>12</sub> and do not strictly require it for growth but benefit from its availability [7], potentially competing with phytoplankton for B<sub>12</sub> as has been demonstrated for nitrate [8]. Climate-change-induced temperature increases will influence bacterial growth rates in the oceans [9], but it is unclear how temperature will impact B<sub>12</sub> quotas and dynamics or downstream effects on microbial communities and interactions. We investigated how temperature stress interacts with B<sub>12</sub> limitation in phycosphere residents with flexible (*A. macleodii* MIT1002) and absolute (*R. pomeroyi* DSS-3) B<sub>12</sub> requirements and how temperature stress impacts production and release of B<sub>12</sub> by a B<sub>12</sub>-producer (*R. pomeroyi*).

To determine the interaction effect of temperature and B<sub>12</sub> availability on growth, *A. macleodii* and *R. pomeroyi* were grown in a minimal media prepared with (replete) and without (–B<sub>12</sub>) B<sub>12</sub> across a range of temperatures from 15 °C to 40 °C (Supplementary Information; SI Table 1, SI Fig. 1). Lack of exogenous B<sub>12</sub> significantly diminished *A. macleodii* growth at all temperatures, with the largest

effect at the highest temperature (Fig. 1). *A. macleodii* biomass was reduced by 57% when grown without B<sub>12</sub> at the highest temperature in trial 1 (Fig. 1B, SI Fig. 2), and by 22% in trial 2 (Fig. 1A, B). Withholding B<sub>12</sub> also significantly decreased *A. macleodii*'s mean maximum growth rate ( $\mu_{\max}$ ; Trial 2):  $\mu_{\max}$  decreased by 0.32 at the highest temperature (27%;  $p < 0.05$ ), by 0.14 at the mid temperature (14%;  $p < 0.05$ ), and by 0.13 at the cool temperature (18%;  $p < 0.05$ ) (Fig. 1C). Cell size was largely stable across treatments, but a significant increase was observed at 24 h for cells grown without B<sub>12</sub> at the highest temperature in both trials (SI Figs. 6, 7), which is consistent with a reduced growth rate [10] or an arrested cell cycle [11].

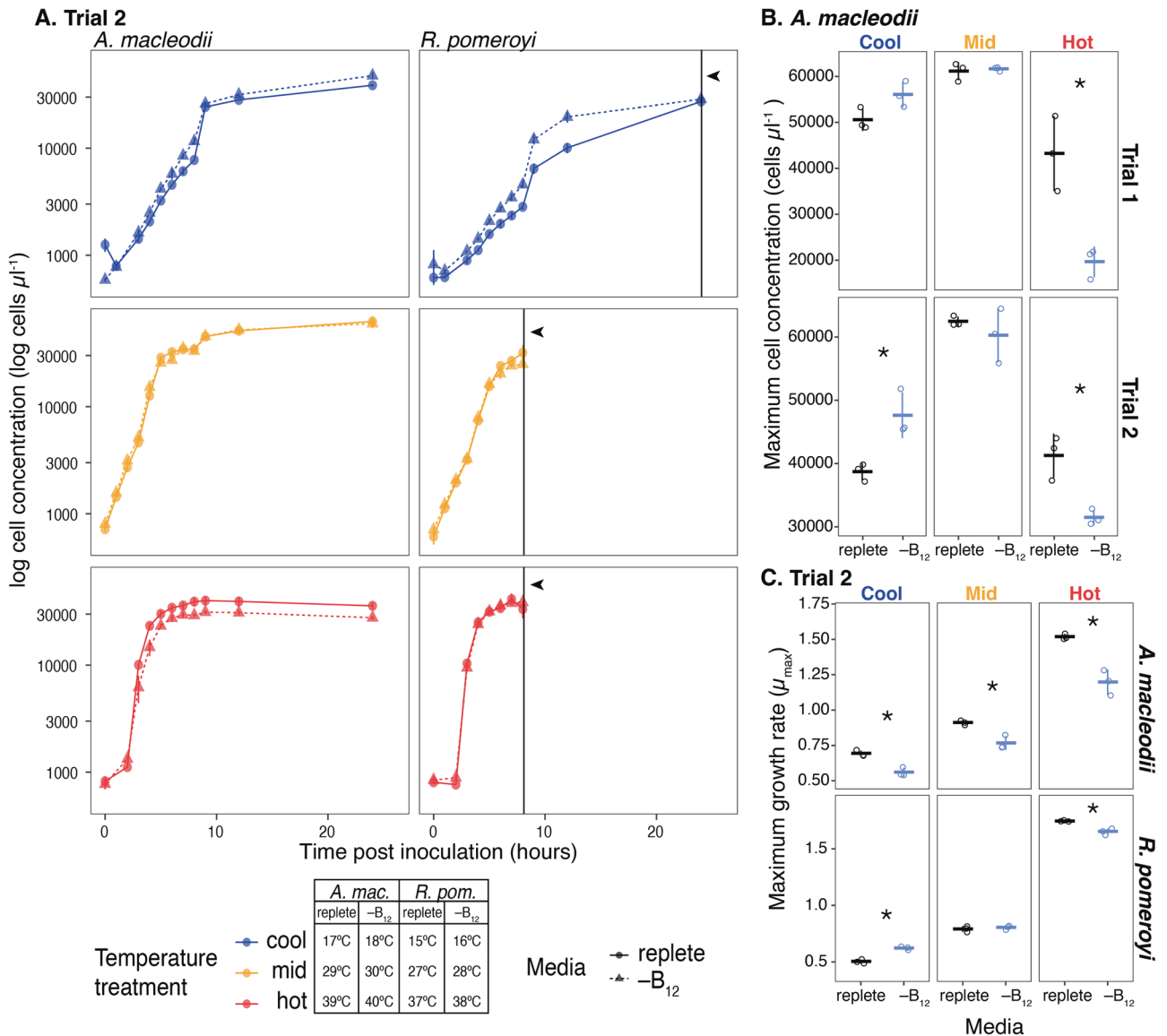
The observed changes in growth parameters suggest that B<sub>12</sub> has a protective or growth-promoting effect in *A. macleodii* at high temperatures. While such observations have not been reported in prokaryotes, B<sub>12</sub> is protective at high temperatures in the model unicellular eukaryotic alga, *Chlamydomonas reinhardtii* [12]. Like *A. macleodii*, the *C. reinhardtii* genome encodes B<sub>12</sub>-independent (MetE) and B<sub>12</sub>-dependent (MetH) methionine synthases, meaning it can grow with and without B<sub>12</sub> [13]. However, exposing *C. reinhardtii* to high temperatures (39 °C) triggers heat shock, chlorosis, and death if B<sub>12</sub> is unavailable [12]. If B<sub>12</sub> is available, *C. reinhardtii* exhibits enhanced thermal tolerance, maintaining growth at 42 °C. At high temperatures, *C. reinhardtii* MetE had decreased activity, indicating MetH is more temperature-stable and suggesting a mechanism for thermal protection [12]. This may also hold true for *A. macleodii*. Methionine, however, conveyed a smaller boost in *C. reinhardtii* thermal tolerance than B<sub>12</sub>,

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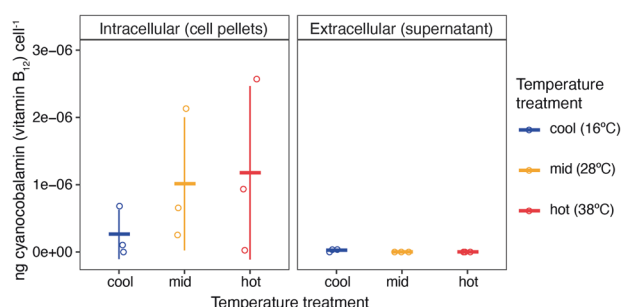
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**Fig. 1** Growth parameters for *Alteromonas macleodii* and *Ruegeria pomeroyi* grown in replete minimal media and minimal media without a vitamin B<sub>12</sub> source across a range of temperature treatments. **A** Growth curves for both species from experimental trial 2. Colors represent temperature treatments, with the exact temperature for each treatment included in the legend. Point and line shapes represent the media treatment: replete (replete minimal media; circles and solid lines) and -B<sub>12</sub> (minimal media without vitamin B<sub>12</sub>; triangles and dashed lines). Each point is the mean log cell concentration of three biological replicates determined by flow cytometry, with error bars representing one standard deviation of the mean. Black vertical lines indicated by arrowheads designate time points where *R. pomeroyi* cultures were harvested for B<sub>12</sub> measurements by mass spectrometry. **B** Maximum cell concentrations (biomass) reached by *A. macleodii* in experimental trials 1 and 2. Horizontal marks represent the mean cell concentration for each treatment; vertical error bars are one standard deviation of the mean; open circles are individual data points. The statistical significance of media treatment at each temperature was tested by t-test and  $p < 0.05$  is indicated on the plots by an asterisk (\*). There was a statistically significant reduction in maximum biomass by 57% and 22% in trials 1 and 2, respectively, when *A. macleodii* was grown without vitamin B<sub>12</sub> at the hottest temperature tested. **C** Maximum growth rates ( $\mu_{\text{max}}$ ) for *A. macleodii* and *R. pomeroyi* in each temperature and media treatment combination in experimental trial 2. Growth rates were calculated from individual growth curves using the 'growthrates' package in the R computing environment. The statistical significance of media treatment on mean maximum growth rate at each temperature was tested by t-test and  $p < 0.05$  is indicated on the plots by an asterisk (\*). *A. macleodii* cultures grown in replete media had a significantly higher maximum growth rate at all temperatures but the difference in mean maximum growth rate ( $\mu_{\text{max}}$ ) between media treatments was largest in the hot temperature treatment (0.32 (27%), compared to 0.14 (14%) in mid and 0.13 (18%) in cool). The impact of media treatment on maximum growth rate was more varied for *R. pomeroyi* with the maximum growth rate significantly higher in replete media only at the highest temperature treatment.

advancing the hypothesis that B<sub>12</sub> enhances thermal tolerance through additional pathways [12]. Notably, B<sub>12</sub> increases growth in bacteria exposed to other stressors, including oxidative stress [14], low-temperature, and copper stress [15], demonstrating that methionine synthesis at higher temperatures is not the only growth-promoting benefit provided by B<sub>12</sub> [16].

Exogenous B<sub>12</sub> had a smaller effect on *R. pomeroyi*'s growth, presumably because it is a B<sub>12</sub>-producer. Withholding B<sub>12</sub> did not impact the maximum biomass reached by *R. pomeroyi* at any temperature (SI Fig. 3) but did significantly decrease growth rates at the highest temperature (Fig. 1C). We detected elevated intracellular B<sub>12</sub> levels in mid and hot temperatures compared to



**Fig. 2** Concentrations of intracellular and extracellular vitamin B<sub>12</sub> normalized to cell counts in *Ruegeria pomeroyi* cultures grown without an exogenous B<sub>12</sub> source across three temperature treatments. *R. pomeroyi* cultures in early stationary phase were harvested for cyanocobalamin (B<sub>12</sub>) measurements by mass spectrometry. Measured values were normalized to the number of cells in the originating culture volume (i.e., the number of cells in a cell pellet or the number of cells removed from a supernatant). Horizontal marks represent the mean B<sub>12</sub> concentration per cell for each treatment; vertical error bars are one standard deviation of the mean; open circles are individual data points. Pelleted cells contained significantly more B<sub>12</sub> than was present in supernatants ( $p < 0.05$ , t-test). While not a statistically significant difference, cells grown in the mid and hot-temperature treatments tended to have higher intracellular vitamin B<sub>12</sub> concentrations than cells grown in the cool-temperature treatment.

the cool treatment, although not statistically significant (Fig. 2). Thus, *R. pomeroyi* may produce more B<sub>12</sub> at warmer temperatures to maintain similar biomass and growth rates as when exogenous B<sub>12</sub> is supplied, but B<sub>12</sub> synthesis cannot keep up with growth requirements at extremely high temperatures. This suggests B<sub>12</sub> plays a similar growth-promoting or protective role in *R. pomeroyi* as observed for *A. macleodii*. In future studies, this could be tested by growing *R. pomeroyi* mutants incapable of synthesizing B<sub>12</sub> at high temperatures and determining if growth is diminished when B<sub>12</sub> is withheld. Of note, extracellular B<sub>12</sub> was not detected in any of the warm or hot treatment replicates and only trace amounts were detected in two cool treatment replicates (Fig. 2). These results imply that little to no B<sub>12</sub> is released by *R. pomeroyi* in our experimental conditions and that temperature does not have a measurable effect on B<sub>12</sub> release. While many B<sub>12</sub>-producing bacteria do not release B<sub>12</sub> [17], these results were surprising because *R. pomeroyi* fulfills the B<sub>12</sub> requirement of the diatom *Thalassiosira pseudonana* when grown in co-culture [6]. While co-culture with *T. pseudonana* does not influence *R. pomeroyi* expression of the B<sub>12</sub> biosynthetic pathway [6], our study suggests that a cue from symbiotic phytoplankton may be required for *R. pomeroyi* to release B<sub>12</sub>.

This study demonstrates that B<sub>12</sub> conveys a protective or growth-promoting effect at high temperatures for two bacterial species commonly associated with phytoplankton. While the highest temperatures in the study are rare in the current global ocean, they are found in tide pools in subtropical and tropical regions [18], and summer sea surface temperatures (SST) in the Persian Gulf regularly exceed 37 °C [19]. Marine heatwaves—such as the 2023 heatwave affecting the Florida Keys, the Bahamas, and Cuba that caused SST to reach 38 °C (ndbc.noaa.gov)—are expected to become more frequent and severe due to climate change [20]. Our results suggest that increasing temperatures will increase the biochemical need for B<sub>12</sub> among marine microbial consortia. Shifting B<sub>12</sub> dynamics may impact symbiotic relationships that sustain phytoplankton and other organisms. Future work should investigate protective mechanisms for B<sub>12</sub> in marine microbes and the impact of inter-species interactions on B<sub>12</sub> production and release with changing temperatures.

## DATA AVAILABILITY

The raw flow cytometry data generated for this project are publicly available from <https://doi.org/10.5281/zenodo.8133026>. Vitamin B<sub>12</sub> mass spectrometry data, intermediate data products, and code used for this study are available in the GitHub repository <https://github.com/maggimars/bactB12>. The full analysis pipeline is further available as an interactive document: [https://maggimars.github.io/bactB12/Flow\\_Cytometry\\_Analysis.html](https://maggimars.github.io/bactB12/Flow_Cytometry_Analysis.html).

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## AUTHOR CONTRIBUTIONS

MMB, HA, and MAS designed the experiments. MMB, AS, and MRM performed experiments and collected data. MMB and AIK analyzed data. MMB wrote the manuscript with input from all authors.

## COMPETING INTERESTS

The authors declare no competing interests.

## ADDITIONAL INFORMATION

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s43705-023-00298-6>.

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