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Vitamin B₁₂ conveys a protective advantage to phycosphere-associated bacteria at high temperatures

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Many marine microbes require vitamin B₁₂ (cobalamin) but are unable to synthesize it, necessitating reliance on other B₁₂-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 B₁₂ availability on the growth of two bacterial taxa commonly associated with phytoplankton: *Ruegeria pomeroyi*, which produces B₁₂ and fulfills the B₁₂ requirements of some phytoplankton, and *Alteromonas macleodii*, which does not produce B₁₂ but also does not strictly require it for growth. For B₁₂-producing *R. pomeroyi*, we further tested how temperature influences B₁₂ production and release. Access to B₁₂ 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 B₁₂, indicating that B₁₂ is protective at high temperatures. Moreover, *R. pomeroyi* produced more B₁₂ at warmer temperatures but did not release detectable amounts of B₁₂ at any temperature tested. Results imply that increasing temperatures and more frequent marine heatwaves with climate change will influence microbial B₁₂ dynamics and could interrupt symbiotic resource sharing.

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INTRODUCTION

Vitamin B₁₂ (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 B₁₂ to rely on others that can [3, 4]. Many phytoplankton fulfill their B₁₂ requirements through interactions with B₁₂-producing bacteria in the phycosphere [5, 6]. Some phycosphere bacteria, like *Ruegeria pomeroyi*, are known B₁₂ producers and require B₁₂ for growth [6]. Other phycosphere inhabitants, like *Alteromonas macleodii*, cannot produce B₁₂ and do not strictly require it for growth but benefit from its availability [7], potentially competing with phytoplankton for B₁₂ 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 B₁₂ quotas and dynamics or downstream effects on microbial communities and interactions. We investigated how temperature stress interacts with B₁₂ limitation in phycosphere residents with flexible (*A. macleodii* MIT1002) and absolute (*R. pomeroyi* DSS-3) B₁₂ requirements and how temperature stress impacts production and release of B₁₂ by a B₁₂-producer (*R. pomeroyi*).

To determine the interaction effect of temperature and B₁₂ availability on growth, *A. macleodii* and *R. pomeroyi* were grown in a minimal media prepared with (replete) and without (−B₁₂) B₁₂ across a range of temperatures from 15 °C to 40 °C (Supplementary Information; SI Table 1, SI Fig. 1). Lack of exogenous B₁₂ 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 B₁₂ at the highest temperature in trial 1 (Fig. 1B, SI Fig. 2), and by 22% in trial 2 (Fig. 1A, B). Withholding B₁₂ also significantly decreased *A. macleodii*'s mean maximum growth rate (μ_{\max} ; Trial 2): μ_{\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 B₁₂ 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 B₁₂ has a protective or growth-promoting effect in *A. macleodii* at high temperatures. While such observations have not been reported in prokaryotes, B₁₂ is protective at high temperatures in the model unicellular eukaryotic alga, *Chlamydomonas reinhardtii* [12]. Like *A. macleodii*, the *C. reinhardtii* genome encodes B₁₂-independent (MetE) and B₁₂-dependent (MetH) methionine synthases, meaning it can grow with and without B₁₂ [13]. However, exposing *C. reinhardtii* to high temperatures (39 °C) triggers heat shock, chlorosis, and death if B₁₂ is unavailable [12]. If B₁₂ 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 B₁₂,

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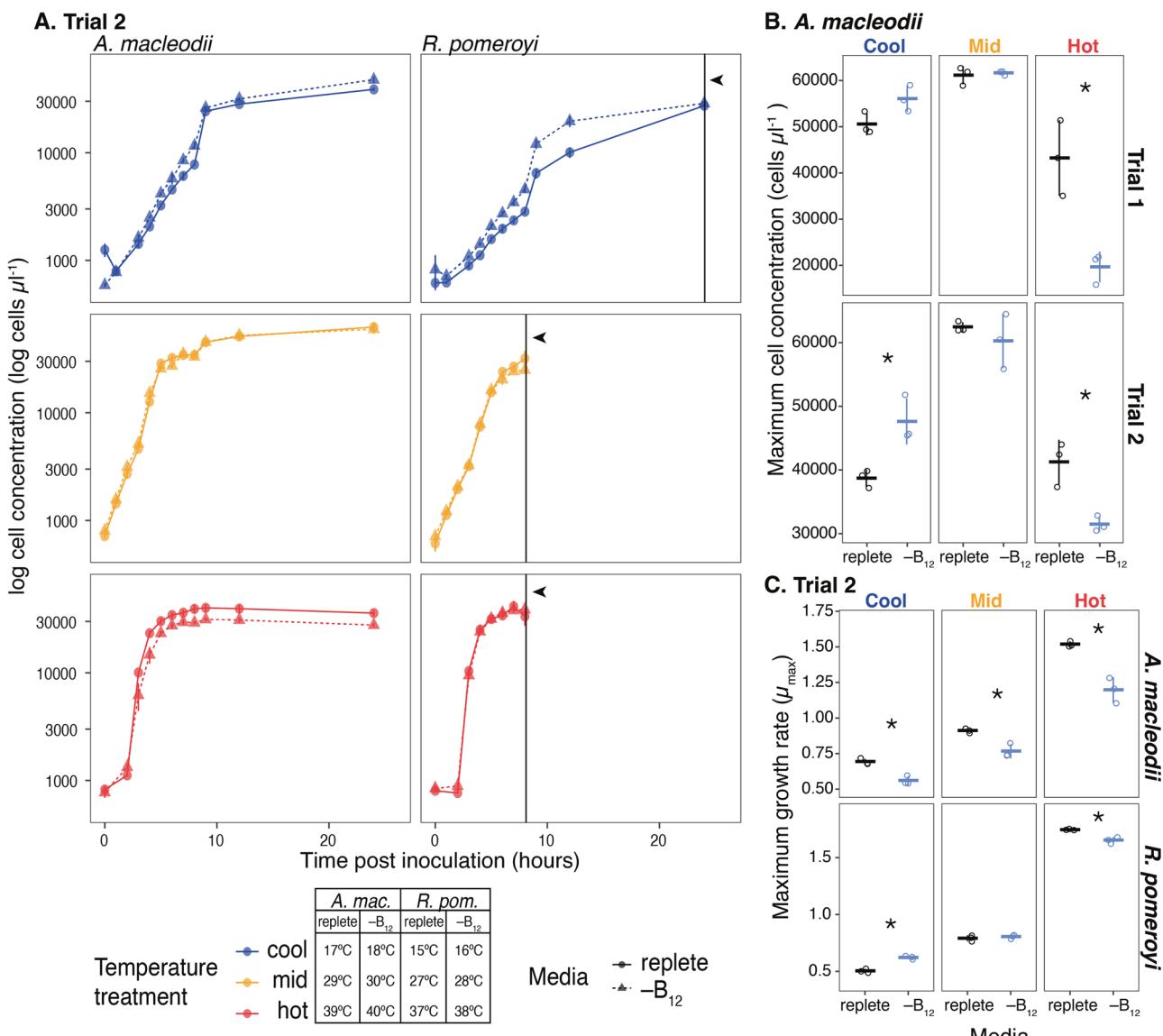


Fig. 1 Growth parameters for *Alteromonas macleodii* and *Ruegeria pomeroyi* grown in replete minimal media and minimal media without a vitamin B₁₂ source across a range of temperature treatments. **A** Growth curves for both species from experimental trial 2. Colors represent temperature treatments, 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 -B₁₂ (minimal media without vitamin -B₁₂; 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 B₁₂ 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 B₁₂ at the hottest temperature tested. **C** Maximum growth rates (μ_{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 (μ_{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 B₁₂ enhances thermal tolerance through additional pathways [12]. Notably, B₁₂ 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 B₁₂ [16].

Exogenous B₁₂ had a smaller effect on *R. pomeroyi*'s growth, presumably because it is a B₁₂-producer. Withholding B₁₂ 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 B₁₂ levels in mid and hot temperatures compared to

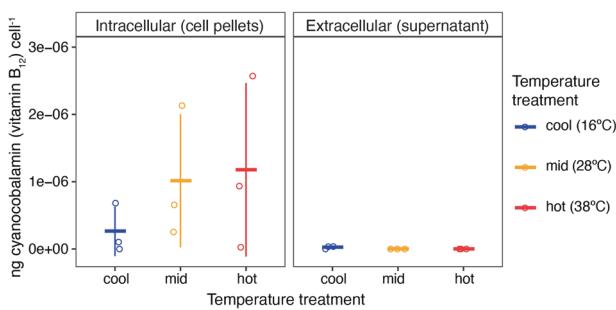


Fig. 2 Concentrations of intracellular and extracellular vitamin B₁₂ normalized to cell counts in *Ruegeria pomeroyi* cultures grown without an exogenous B₁₂ source across three temperature treatments. *R. pomeroyi* cultures in early stationary phase were harvested for cyanocobalamin (B₁₂) measurements 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 B₁₂ 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 B₁₂ 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 B₁₂ concentrations than cells grown in the cool-temperature treatment.

the cool treatment, although not statistically significant (Fig. 2). Thus, *R. pomeroyi* may produce more B₁₂ at warmer temperatures to maintain similar biomass and growth rates as when exogenous B₁₂ is supplied, but B₁₂ synthesis cannot keep up with growth requirements at extremely high temperatures. This suggests B₁₂ 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 B₁₂ at high temperatures and determining if growth is diminished when B₁₂ is withheld. Of note, extracellular B₁₂ 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 B₁₂ is released by *R. pomeroyi* in our experimental conditions and that temperature does not have a measurable effect on B₁₂ release. While many B₁₂-producing bacteria do not release B₁₂ [17], these results were surprising because *R. pomeroyi* fulfills the B₁₂ 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 B₁₂ biosynthetic pathway [6], our study suggests that a cue from symbiotic phytoplankton may be required for *R. pomeroyi* to release B₁₂.

This study demonstrates that B₁₂ 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 B₁₂ among marine microbial consortia. Shifting B₁₂ dynamics may impact symbiotic relationships that sustain phytoplankton and other organisms. Future work should investigate protective mechanisms for B₁₂ in marine microbes and the impact of inter-species interactions on B₁₂ 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 B₁₂ 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.

REFERENCES

- Rodionov DA, Vitreschak AG, Mironov AA, Gelfand MS. Comparative genomics of the vitamin B₁₂ metabolism and regulation in prokaryotes. *J Biol Chem*. 2003;278:41148–59.
- Tang YZ, Koch F, Gobler CJ. Most harmful algal bloom species are vitamin B₁ and B₁₂ auxotrophs. *Proc Natl Acad Sci USA*. 2010;107:20756–61.
- Sañudo-Wilhelmy SA, Cutter LS, Durazo R, Smail EA, Gómez-Consarnau L, Webb EA, et al. Multiple B-vitamin depletion in large areas of the coastal ocean. *Proc Natl Acad Sci USA*. 2012;109:14041–5.
- Bertrand EM, Saito MA, Rose JM, Riesselman CR, Lohan MC, Noble AE, et al. Vitamin B₁₂ and iron colimitation of phytoplankton growth in the Ross Sea. *Limnol Oceanogr*. 2007;52:1079–93.
- Mars Brisbin M, Mitairi S, Saito MA, Alexander H. Microbiomes of bloom-forming *Phaeocystis* algae are stable and consistently recruited, with both symbiotic and opportunistic modes. *ISME J*. 2022;16:2255–64.
- Durham BP, Sharma S, Luo H, Smith CB, Amin SA, Bender SJ, et al. Cryptic carbon and sulfur cycling between surface ocean plankton. *Proc Natl Acad Sci USA*. 2015;112:453–7.
- Biller SJ, Coe A, Chisholm SW. Torn apart and reunited: impact of a heterotroph on the transcriptome of *Prochlorococcus*. *ISME J*. 2016;10:2831–43.
- Diner RE, Schwenck SM, McCrow JP, Zheng H, Allen AE. Genetic manipulation of competition for nitrate between heterotrophic bacteria and diatoms. *Front Microbiol*. 2016;7:880.
- Veazza L, Grande C, Reid PC, Hélaouët P, Edwards M, Höfle MG, et al. Climate influence on *Vibrio* and associated human diseases during the past half-century in the coastal North Atlantic. *Proc Natl Acad Sci USA*. 2016;113:E5062–71.
- Allman R, Hann AC, Phillips AP, Martin KL, Lloyd D. Growth of *Azotobacter vinelandii* with correlation of Coulter cell size, flow cytometric parameters, and ultrastructure. *Cytometry*. 1990;11:822–31.
- Lefort-Tran M, Bre MH, Poupouli P, Manigault P. DNA flow cytometry of control *Euglena* and cell cycle blockade of vitamin B₁₂-starved cells. *Cytometry*. 1987;8:46–54.
- Xie B, Bishop S, Stessman D, Wright D, Spalding MH, Halverson LJ. *Chlamydomonas reinhardtii* thermal tolerance enhancement mediated by a mutualistic interaction with vitamin B₁₂-producing bacteria. *ISME J*. 2013;7:1544–55.
- Helliwell KE, Wheeler GL, Leptos KC, Goldstein RE, Smith AG. Insights into the evolution of vitamin B₁₂ auxotrophy from sequenced algal genomes. *Mol Biol Evol*. 2011;28:2921–33.
- Ferrer A, Rivera J, Zapata C, Norambuena J, Sandoval Á, Chávez R, et al. Cobalamin protection against oxidative stress in the acidophilic iron-oxidizing bacterium *Leptospirillum* group II CF-1. *Front Microbiol*. 2016;7:748.
- Vásquez L, Parra A, Quesada-Villalobos AM, Gálvez G, Navarrete P, Latorre M, et al. Cobalamin cbiP mutant shows decreased tolerance to low temperature and copper stress in *Listeria* monocytogenes. *Biol Res*. 2022;55:9.
- Romine MF, Rodionov DA, Maezato Y, Anderson LN, Nandhikonda P, Rodionova IA, et al. Elucidation of roles for vitamin B₁₂ in regulation of folate, ubiquinone, and methionine metabolism. *Proc Natl Acad Sci USA*. 2017;114:E1205–E1214.
- Sultana S, Bruns S, Wilkes H, Simon M, Wienhausen G. Vitamin B₁₂ is not shared by all marine prototrophic bacteria with their environment. *ISME J*. 2013;17:836–45.
- Vinagre C, Mendonça V, Cereja R, Abreu-Afonso F, Dias M, Mizrahi D, et al. Ecological traps in shallow coastal waters—Potential effect of heat-waves in tropical and temperate organisms. *PLoS One*. 2018;13:e0192700.
- Alosairi Y, Alsulaiman N, Rashed A, Al-Houti D. World record extreme sea surface temperatures in the northwestern Arabian/Persian Gulf verified by in situ measurements. *Mar Pollut Bull*. 2020;161:111766.
- Jacox MG, Alexander MA, Amaya D, Becker E, Bograd SJ, Brodie S, et al. Global seasonal forecasts of marine heatwaves. *Nature*. 2022;604:486–90.

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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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