B-vitamin requirements and phycosphere interactions for the HAB-forming dinoflagellate, *Pyrodinium bahamense* var. *bahamense*

Lydia Ruggles Howe[1], Lauren D' Amore[1,2], Cary Lopez[2], Sugandha Shankar[2], Margaret Mars Brisbin[1]*

[1] College of Marine Science, University of South Florida, St. Petersburg, FL, U.S.A.

[2]Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, St. Petersburg, FL, U.S.A.

*Corresponding Author:

830 1st Street South, St. Petersburg, Florida, U.S.A.

mmarsbrisbin@usf.edu

Keywords: Cobalamin, dinoflagellate, *Pyrodinium*, Harmful Algal Bloom, HAB, Phycosphere, phytoplankton-bacteria interactions, B-vitamins, B_{12}

Running title: *Pyrodinium* phycosphere and vitamin needs

Abstract:

Pyrodinium bahamense is a saxitoxin-producing dinoflagellate found in tropical and subtropical estuarine waters globally, with the var. *bahamense* variety forming recurrent harmful algal blooms (HABs) in Florida estuaries. The intensity and duration of bloom peaks in Florida estuaries vary interannually, but life cycle transitions, estuarine water residence time, water temperature, salinity, and nutrient concentrations appear to contribute to bloom formation. However, the impact of B-vitamin availability on *P. bahamense* bloom formation has not been investigated, despite the knowledge that many HAB dinoflagellates require B-vitamins for growth and bloom development. In this study, we determined that of the three B-vitamins most commonly required by marine phytoplankton—biotin (B_7), thiamine (B_1), and cobalamin (B_{12})—*P. bahamense* strictly requires an exogenous B_{12} source to sustain growth. As bacterial interactions can fulfill phytoplankton B_{12} requirements, we tested whether bacterial communities recruited from two Florida estuaries could rescue *P. bahamense* from B_{12} limitation. We demonstrate that bacteria from the Indian River Lagoon (IRL) estuary collected during a *P. bahamense* bloom restored *P. bahamense* growth in media missing B_{12}, while bacteria present in Old Tampa Bay (OTB) water collected when *P. bahamense* was not present did not restore *P. bahamense* growth in media missing B_{12}. Putative bacterial B_{12} producers exclusively recruited from IRL included Enterobacteraceae genera that likely have anthropogenic sources. Our findings suggest B_{12} is essential for fueling *P. bahamense* blooms, and that specific bacterial communities may contribute to bloom development through B_{12} production.

Introduction

Phytoplankton are critical to aquatic ecosystem functioning and global biogeochemical cycles [1–3], but phytoplankton can also negatively impact aquatic ecosystems through the formation of harmful algal blooms (HABs). During a HAB, phytoplankton species undergo mass proliferation and may also produce toxins, leading to water column hypoxia, benthic anoxia, wildlife mortalities, and human illnesses [4, 5]. Thus, it is imperative to fully understand the physiology of HAB-forming phytoplankton species to build predictive models for bloom initiation, duration, and intensity. This is increasingly important as changing climate and weather patterns are amplifying the effects of HABs on coastal ecosystems and communities [6].

B-vitamins—such as thiamine (B_1), biotin (B_7), and cobalamin (B_{12})—are integral to the growth of many phytoplankton species, as they are essential cofactors for key enzymes involved in cellular metabolism and reproduction [7]. Genomic surveys indicate that more than half of all phytoplankton and up to 90% of HAB dinoflagellates are only equipped with the B_{12}-dependent methionine synthase (MetH) [8], making them fully reliant on external B_{12} sources for essential amino acid synthesis. Through a comprehensive set of bioassays, Tang et al. (2012) demonstrated that 26 out of 27 HAB species (41 strains) required B_{12} and 20 required B_1 [9], further suggesting B-vitamin availability is especially critical to the growth of HAB-forming phytoplankton. However, the extent of the role B-vitamin availability plays in promoting or limiting HABs is not fully elucidated [10].

Despite reliance on B vitamins, many HAB species bloom in coastal waters where measurable dissolved B-vitamin concentrations are below phytoplankton growth requirements, suggesting that B vitamins may be limiting nutrients in coastal estuaries [10]. Moreover, studies in Long Island estuaries demonstrated a drawdown of vitamins B_1 and B_{12} during dinoflagellate blooms, and B_1 and B_{12} amendments in concurrent incubation experiments increased dinoflagellate biomass [11]. Bacterial production of B vitamins stands to be the major source of these critical resources to marine ecosystems. Roughly 25% of sequenced bacterial genomes contain complete B_{12}-synthesis pathways [12], but most B_{12} producers do not freely share the resource [13]. Thus, specific relationships between phytoplankton and bacteria inhabiting the phycosphere may be particularly important for fulfilling phytoplankton B_{12} requirements [14, 15]. Indeed, positive interactions between several HAB dinoflagellates and specific B-vitamin producing bacteria have been identified [16–18].

The saxitoxin-producing HAB dinoflagellate, *Pyrodinium bahamense* var. *bahamense,* forms dense assemblages (> 100,000 cells L^{-1}) in Florida estuaries, including in the northern

segment of Tampa Bay, Old Tampa Bay (OTB), on Florida's west coast and the Indian River Lagoon (IRL) estuary on the east coast [19] (Fig. 1). B vitamins have long been hypothesized to be contributing factors in supporting the high concentrations of *P. bahamense* observed in the bioluminescent bays of Puerto Rico, where the dinoflagellate blooms carry ecological, cultural, and economic significance [20]. However, the role of B-vitamin availability and bacterial interactions in supporting *P. bahamense* blooms in Florida estuaries has yet to be directly investigated, making it plausible that characterizing these interactions may improve our understanding of bloom intensity and duration in the region. This study directly investigates the B-vitamin requirements of a *P. bahamense* strain isolated from OTB and determines whether bacterial communities recruited from OTB and IRL seawater can support *P. bahamens*e growth in the absence of vitamin B_{12}.

Materials and Methods

P. bahamense *strains used in experiments*

P. bahamense strains FWC1006—originating from OTB in 2023—and FWC1003—originating from the IRL in 2017—were acquired from the Florida Fish and Wildlife Conservation Commission (FWC) Fish and Wildlife Research Institute (FWRI) for use in this study (Fig. 1). Both strains were established by germinating resting cysts from sediment samples. Germinated cells were then isolated and grown into unialgal cultures using aseptic techniques, but were not deliberately rendered axenic. Upon transfer to our lab, both strains were maintained in L1/4 media prepared with sterile 25 PSU artificial seawater (ASW; Instant Ocean) [21] at 24ºC on a 12:12 h light: dark cycle with 50% illumination (~115 µM photons m^{-2} s^{-1}) in a Percival I-36 LLVL incubator.

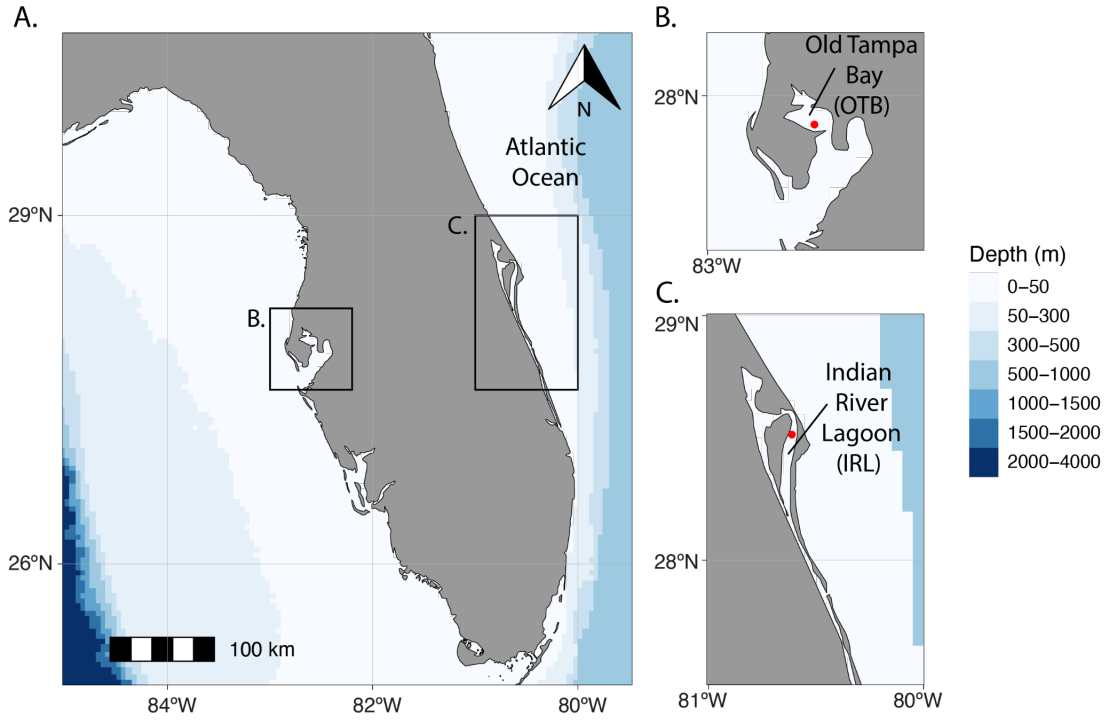


Fig. 1 Map depicting regions of Florida (U.S.A.) with recurrent *Pyrodinium bahamense* blooms and where samples were collected for this study. **A** Map of Florida with inset regions indicated (Tampa Bay and OTB; **B** and Indian River Lagoon; **C**). Red points in panels **B** and **C** indicate the locations where seawater was collected for bacterial community transfers to FWC1006.

Characterization of bacterial communities in P. bahamense *cultures*

Bacteria associated with phytoplankton can provide B vitamins to support phytoplankton [7, 16, 22], making it imperative to characterize bacteria co-cultured with *P. bahamense* before experimentation. The presence of bacteria in *P. bahamense* strains FWC1006 and FWC1003 was initially evaluated by transferring aliquots into two test broths for bacterial growth: Difco Marine Broth and an artificial seawater tryptone broth (ASWT) matching the salinity of algal culture media [23]. ASWT broth was prepared from 1 L of 25 PSU Instant Ocean ASW amended with 5 g tryptone, 3 g yeast extract, and 3 mL 50% glycerol (in water, RICCA Chemical Company), and sterilized by autoclaving and filtering with a 0.2-μm pore-size filter [23]. Aliquots of each isolate (100 μl) were transferred to triplicate wells containing 1 mL of test broth in a sterile 24-well plate. Triplicate negative controls for each test broth were included. Plates were incubated at room temperature, and bacterial growth was visually monitored after 72 h and checked under 400 x magnification with an inverted microscope.

117 Bacterial communities present in cultures of the two *P. bahamense* strains were further
118 characterized through metabarcode sequencing. Biomass was collected from each strain by
119 sequentially filtering cultures through 10.0-µm and 0.2-µm pore-size filters under gentle vacuum
120 pressure. *P. bahamense* cells and associated bacteria were retained on 10.0-µm pore-size
121 filters, and free-living bacteria were retained on 0.2-µm pore-size filters. RNA was extracted
122 from filters using the RNeasy PowerWater Kit (Qiagen) according to the manufacturer's protocol
123 with the suggested 10-minute 65ºC incubation for improved cell lysis. RNA was reverse
124 transcribed following the manufacturer's protocols for the High-Capacity RNA-to-cDNA Kit
125 (Applied Biosystems). The V3–V4 hypervariable regions of the reverse-transcribed 16S rRNA
126 were amplified following the "16S Metagenomic Sequencing Library Preparation" Illumina
127 protocol, including the recommendations for primers [24] and PCR conditions. Metabarcode
128 libraries were sequenced on an Illumina NextSeq2000 2x300-bp P1 flow cell. Amplicons were
129 processed with the QIIME2 pipeline [25], utilizing the DADA2 denoising algorithm [26], and
130 representative sequences were taxonomically classified using a Naive Bayes classifier [27]
131 trained on the SILVA Ref 99 database v138.1 [28]. Amplicon Sequence Variants (ASVs) were
132 further processed using the phyloseq [29] package in R Studio [30]. Based on results from
133 bacterial growth tests and metabarcode analyses, the FWC1006 *P. bahamense* isolate from
134 OTB was selected for subsequent experiments.
135
136 *Evaluating the B-vitamin requirements of* P. bahamense
137 Given the difficulty of growing dinoflagellates axenically, dinoflagellate cultures with
138 reduced or knocked-down microbiomes have been used in experiments to minimize the impact
139 of bacterial processes on experimental results [17]**.** Here, we propose that the low-diversity
140 bacterial community associated with the FWC1006 *P. bahamense* strain from OTB represents a
141 reduced bacterial community and an opportunity for testing the B-vitamin requirements of *P.*
142 *bahamense* without subjecting cultures to extensive antibiotic treatment.
143 To determine the B-vitamin requirements for *P. bahamense*, the OTB strain was grown
144 in L1/4 media missing one of the three major B-vitamins—B_{12} (cobalamin), B_1 (thiamine), or B_7
145 (biotin)—and in replete L1/4 (i.e., containing all three B vitamins) as a positive control. All media
146 formulations were made using sterile 25 PSU ASW amended with $NaNO_3$, NaH_2PO_4, and trace
147 metal solution from the NCMA L1 Medium kit at 1/4 the concentration of L1 medium [21].
148 Individual B vitamins (Sigma-Aldrich) were added to media at 1/4 the concentration in L1
149 medium [21]. Experimental cultures were set up with 1 mL of the OTB isolate and 45 mL of each

150 media treatment in triplicate 50 mL cell culture flasks and incubated as described above [31].
151 Growth was monitored by measuring *in vivo* Chlorophyll-*a* fluorescence (RFU) with a Trilogy
152 fluorometer (Turner Designs) every 48 h. The experiment was maintained for four trials, with 1
153 mL serial transfers made in the late exponential growth phase or early stationary phase. The –
154 B_{12} treatment was not serially transferred because these cultures failed to grow. Instead, 1 mL
155 of the culture in replete media was also transferred to –B_{12} media at the beginning of each new
156 trial. Growth rates were calculated from Chlorophyll-*a* fluorescence using the fit_easylinear
157 function from the growthrates R package (h = 5, quota = 1) [32].
158 To better characterize the minimum B_{12} requirement of *P. bahamense*, the OTB isolate
159 was grown in L1/4 media with varying final concentrations of vitamin B_{12} from 369 pM—the
160 concentration in L1 medium [21]—through 0.0369 pM, prepared through 10% serial dilutions.
161 Culture conditions matched those described above, and *in vivo* Chlorophyll-*a* fluorescence was
162 measured every 48 h for a single 40-day trial.
163
164 *Evaluation of bacterial community potential to supplement B_{12} and support* P. bahamense
165 *blooms*
166 Next, we tested the ability of bacterial communities from OTB and IRL to produce
167 vitamin B_{12} and promote *P. bahamense* growth. OTB surface seawater was collected on May 2,
168 2024 (27.9055ºN, –82.6046ºW) and IRL surface seawater was collected on July 10, 2024
169 (28.5136ºN, –80.6125ºW) by FWRI (Fig. 1). At the time of collection, no *P. bahamense* cells
170 were detected at the OTB site, whereas *P. bahamense* cells were detected at concentrations
171 exceeding the FWC-designated bloom level at the IRL site (113,333 cells L^{-1}, bloom-level:
172 >100,000 cells L^{-1}). Seawater from both sites and a *P. bahamense* culture (IRL FWC1003) were
173 sequentially filtered through 10.0-µm, 0.8-µm, and 0.45-µm pore-size filters to remove
174 eukaryotic phytoplankton, but particle-associated bacteria and larger or filamentous bacteria
175 were likely also removed or reduced during filtering. A 250 mL aliquot of the final filtrate for each
176 recruitment treatment was filtered through a 0.2-µm pore-size filter, and the remaining filtrate for
177 each recruitment treatment was diluted 50% with L1/4 media and used in recruitment trials. The
178 10.0-µm and 0.2-µm filters were flash frozen and stored at –80ºC for DNA extraction.
179 A 1-mL aliquot of OTB *P. bahamense* culture was transferred into 45 mL of each of the
180 three recruitment treatment filtrates (OTB seawater, IRL seawater, IRL culture) and incubated
181 for two weeks to allow equilibration of the introduced bacterial communities. Subsequently, 1 mL
182 aliquots of cultures with recruited bacterial communities were transferred in triplicate to 45 mL of

183 replete L1/4 and media without B_{12} (L1/4 –B_{12}), and grown and monitored as described above
184 for two trials. For the second trial, cultures were serially transferred if growth occurred, which
185 was the case for all cultures grown in replete media but only for the IRL seawater-treated culture
186 grown in L1/4 –B_{12}. The OTB seawater- and IRL culture-treated cultures in L1/4 –B_{12} were
187 restarted from the matching treatment in replete media in trial 2, since they failed to grow in trial
188 1. All culture replicates from growth trial 2 were sequentially filtered through 10.0-µm and 0.2-
189 µm pore-size filters under gentle vacuum pressure when cultures began entering stationary
190 growth phase. Filters were flash frozen and stored at –80ºC for DNA extraction.
191
192 *Nanopore full-length 16S metabarcoding and bioinformatics*
193 The bacterial communities introduced from seawater sources to OTB *P. bahamense* and
194 the established "recruited" communities from each source (IRL *P. bahamense* culture, IRL
195 seawater, and OTB seawater) were assessed through full-length 16S rRNA gene sequencing
196 on the Oxford Nanopore MinION platform. DNA was extracted from 10.0-µm and 0.2-µm pore-
197 sized filters with the Qiagen DNeasy PowerSoil Pro kit according to the manufacturer's protocol,
198 including the additional 65°C heating step. The full-length 16S rRNA gene was amplified using
199 forward primer S-D-008-c-S-20 (5′-AGRGTTYGATYMTGGCTCAG-3′) [24] and reverse primer
200 1492R (5′-RGYTACCTTGTTACGACTT-3′) [33] with KAPA2G Robust HotStart (Roche) in the
201 following PCR program: 95ºC/ 3min, 35x (95ºC/ 20sec, 57ºC/ 20sec, 72ºC/ 30sec), 72ºC/ 1min,
202 and 4ºC/ ∞ [33]. Sequencing libraries were prepared using the Oxford Nanopore Native
203 Barcoding Kit 24 v14 following the manufacturer's protocol and sequenced on two MinION v10.4
204 flow cells and one Flongle flow cell. Sequence data were processed (min. length = 1300 bp,
205 max. length = 2000 bp), quantified, and classified using Kraken v2.1.2 [34] within the wf-16s
206 nextflow pipeline in the EPI2ME platform [35] with the SILVA v138.1 taxonomic database [28].
207 Results were exported to R Studio for further processing.
208
209 **Results**
210
211 *Assessment of bacterial communities co-cultured with* P. bahamense *isolates.*
212 There was no visible bacterial growth (broth turbidity or observable cells by microscopy)
213 in Difco Marine Broth or ASWT broth 72 h after inoculation with FWC1006 *P. bahamense*
214 culture. Similarly, there was no visible bacterial growth in Difco Marine Broth or in salinity-

matched ASWT broth in the negative control wells after the 72-hour observation period. In contrast, there was visible bacterial growth (high turbidity) in both Difco Marine Broth and salinity-matched ASWT broth in wells inoculated with FWC1003 *P. bahamense* culture. Further investigation into the bacterial community composition in cultures of both *P. bahamense* strains through metabarcoding also revealed stark differences. The bacterial community in the OTB *P. bahamense* culture had very low alpha diversity (Shannon Index) compared to the communities in IRL *P. bahamense* cultures (Fig. 2A). The IRL *P. bahamense* culture hosted bacteria from 11 taxonomic orders, including typical phytoplankton-associated groups such as Alteromonadales, Caulobacterales, and Rhodobacterales (Fig. 2B). Conversely, the OTB *P. bahamense* culture hosted bacteria from just three orders: Microtrichales, Oceanospirillales, and Rhizobiales (Fig. 2B). At the amplicon sequence variant (ASV) level, only nine ASVs were detected among OTB *P. bahamense* cultures and just six ASVs made up > 99% of sequencing reads (Table S1). Further investigation of these ASVs through BLAST searches revealed that they were previously found in soils, marine sediments, and aquatic plant rhizospheres (Table S1). Thus, the OTB *P. bahamense* culture has a reduced bacterial community compared to the IRL *P. bahamense* culture. Moreover, the bacteria associated with the OTB *P. bahamense* culture likely do not represent water column bacteria that *P. bahamense* would interact with during blooms of vegetative cells.

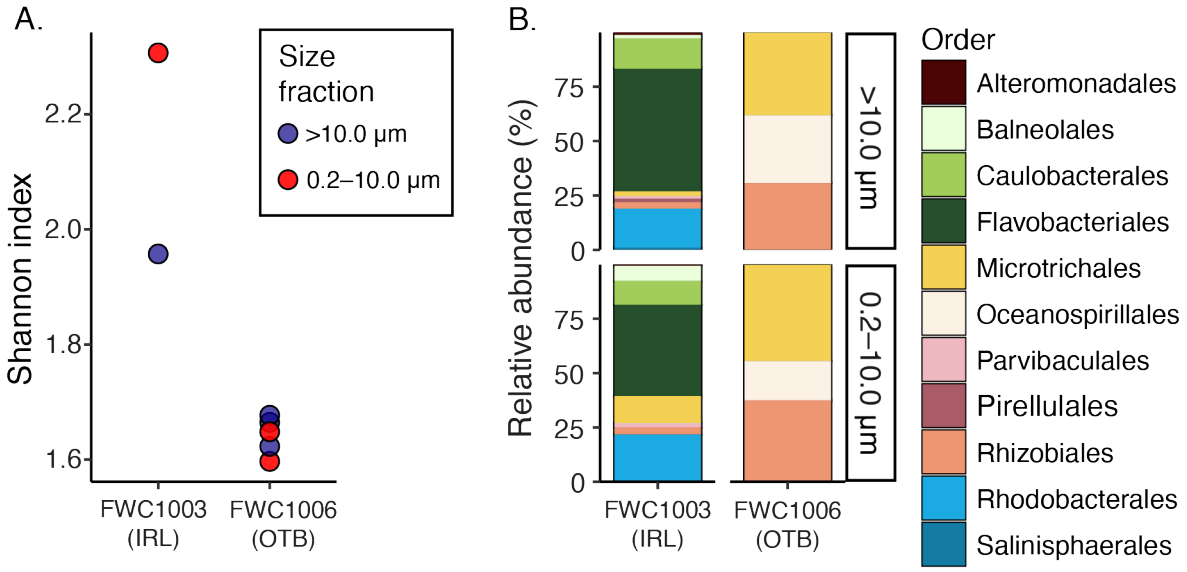


Fig. 2 Alpha diversity and composition of bacterial communities in *P. bahamense* cultures. **A** Shannon diversity index for particle-associated (>10.0-µm size fraction; blue) and free-living (0.2–10.0-µm size

fraction; red) bacterial communities in cultures of FWC1003 and FWC1006 *P. bahamense* isolated from Indian River Lagoon and Old Tampa Bay, respectively. **B** Relative abundance of bacterial orders in size-fractioned communities in *P. bahamense* cultures.

B-vitamin requirements of OTB P. bahamense

Given the low-diversity bacterial community detected in the OTB *P. bahamense* culture, this strain was used in experiments testing the B-vitamin requirements of *P. bahamense*. We hypothesized that the low-diversity sediment-derived bacteria in the OTB culture would be less likely to symbiotically provide B-vitamins than the higher-diversity community observed in the IRL culture, which was more similar to communities observed in other phytoplankton cultures (e.g., [36–38]).

Growth curves for OTB *P. bahamense* appeared similar across four serial transfers in replete L1/4 media, L1/4 –thiamine, and L1/4 –biotin (Fig. 3A). In contrast, OTB *P. bahamense* immediately ceased growing when transferred into L1/4 media missing vitamin B_{12} (L1/4 –$B_{12,}$ Fig. 3A). As a result, serial transfers could not be made for this treatment, and culture from the replete treatment was transferred to L1/4 –B_{12} in each transfer round. Results from a one-way ANOVA indicated that the effect of media formulation on maximum chlorophyll-*a* fluorescence, a proxy for biomass, was significant (p = .0000, F = 45.35), and a Tukey HSD multiple comparisons of means test showed that the maximum fluorescence was significantly lower in L1/4 –B_{12} media than in replete (*p-adj* = .0000), –thiamine (*p-adj* = .0000), and –biotin media (*p-adj* = .0000). Of note, the maximum fluorescence reached in media missing thiamine was significantly greater than in replete media (*p-adj* = .0000)or in –biotin media (*p-adj* = 0.0006). Since *P. bahamense* ceased growing when transferred into media missing B_{12}, reliable growth rates could not be determined for this treatment. Aside from the –B_{12} treatment, media formulation did not have a large effect on growth rate (Fig. 3C, S1); a one–way ANOVA excluding the –B_{12} treatment indicated that media formulation (i.e., –thiamine, –biotin, replete) did not significantly affect growth rate (μ; p = 0.172, F = 1.861).

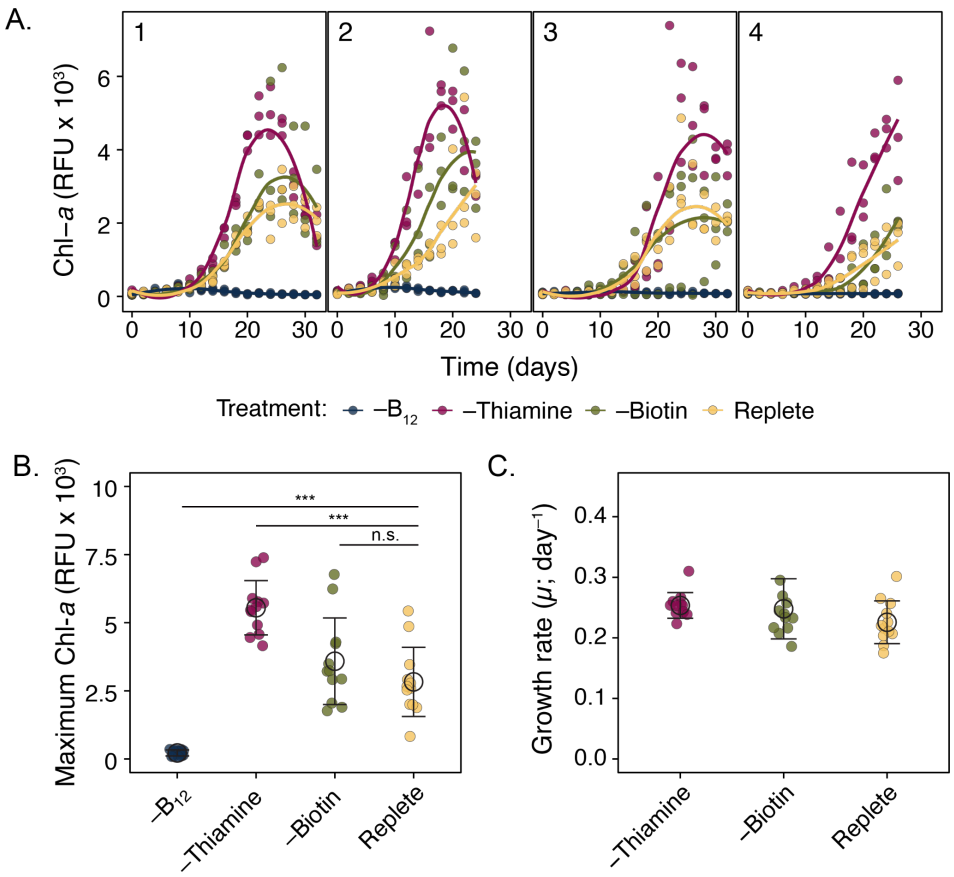
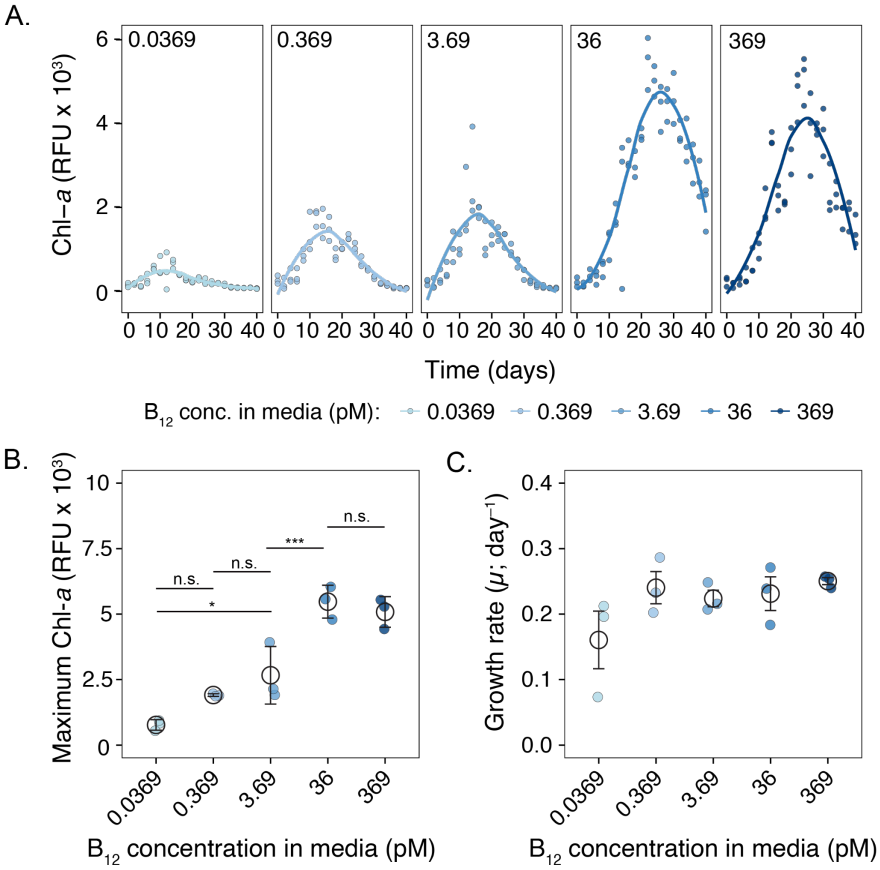


Fig. 3 Growth responses of *P. bahamense* when grown in replete L1/4 media and in media missing B_{12}, thiamine, or biotin. All data are plotted with color representing media treatment. **A** *In vivo* Chlorophyll *a* fluorescence (Chl-*a*) was used to track *P. bahamense* growth across four trials. Solid lines indicate the smoothed conditional mean, plotted with the geom_smooth function in the R package ggplot2 (method = 'loess'). **B** Maximum Chl-*a* values in each replicate by media formulation. Results from post-hoc comparisons to replete are shown with the following significance codes: *p-adj* > 0.05; 'n.s.' and *p-adj* < 0.001; '***'. **C** Growth rate of each replicate in –thiamine, –biotin, and replete media. Growth rates could not be calculated for replicates in $–B_{12}$ media due to the lack of growth (Fig. S1). Means are plotted as black open circles, and error bars represent one standard deviation of the mean.

P. bahamense *growth response to varying B_{12} availability.*

Given that withholding B_{12} rapidly diminished *P. bahamense* growth (Fig. 3), we further evaluated the *P. bahamense* growth response to varying B_{12} concentrations using 10% serial dilutions of B_{12} in L1/4 media. *P. bahamense* growth showed a typical dose-response to increasing B_{12} availability (Fig. 4A). Maximum biomass (Chl-*a* fluorescence) increased with increasing B_{12} availability (Fig. 4B), with significant increases in maximum fluorescence between

282 0.0369 and 3.69 pM B_{12} and between 3.69 and 36.9 pM B_{12} (*p-adj* = 0.025 and 0.0000,
283 respectively; Tukey HSD). However, when enough B_{12} was available to support growth, the
284 growth rates were fairly consistent (Fig. 4C, S2). Growth rate was depressed in media with
285 0.0369 pM B_{12} (Fig 4C), but one-way ANOVA results did not indicate that B_{12} concentration
286 significantly affected growth rate ($p = 0.196$, $F = 1.85$, Fig 4C). Notably, Chl-*a* fluorescence in
287 0.0369 pM B_{12} media remained low and variable throughout the trial, leading to poor linear
288 model fits for estimating growth rate ($p > 0.05$, $R^2 < 0.85$, Fig. S2) that appear to overestimate
289 growth rates and skew the statistical test results.
290



291
292 **Fig. 4** Growth responses of *P. bahamense* when grown in L1/4 media with a 10% dilution series of B_{12}
293 concentrations. All data are plotted with color representing the B_{12} concentration. *In vivo* Chlorophyll-*a*
294 fluorescence (filled circles) was used to track *P. bahamense* growth in L1/4 media with five
295 concentrations of B_{12}: 0.0369 pM, 0.369 pM, 36 pM, and 369 pM. Solid lines indicate the smoothed
296 conditional mean, plotted with the geom_smooth function in the R package ggplot2 (method = 'loess'). **B**
297 Maximum Chl-*a* fluorescence values and **C** growth rates (filled circles), shown for each replicate grouped
298 by media B_{12} concentration. Means are plotted as black open circles, and error bars represent one

standard deviation of the mean. Results from key post-hoc comparisons are shown with the following significance codes: *p-adj* > 0.05; 'n.s.', *p-adj* < 0.05; '*', and *p-adj* < 0.001; '***'.

Effects of recruited bacterial communities on P. bahamense *growth*

Increased growth was detected in OTB *P. bahamense* (FWC1006) with bacteria recruited from IRL seawater compared to *P. bahamense* with bacteria recruited from IRL culture or OTB seawater when grown in L1/4 –B_{12} media (Fig. 5A). One-way ANOVA indicated that the recruited bacterial community significantly affected maximum Chl-*a* when B_{12} was withheld (Fig. 5B; $p = 0.0001$, $F = 50.27$). The maximum Chl-*a* in cultures with bacteria from IRL seawater was significantly higher than in cultures with bacteria from OTB seawater, bacteria from the IRL culture, or with the initial bacteria in the OTB culture (*p-adj* = 0.0000 for each comparison, Tukey HSD). Growth rates for *P. bahamense* with bacteria from IRL seawater were not significantly different when grown in replete media versus media missing B_{12} (two-sample t-test; $p = 0.5678$, $t(10) = -0.5907$; Fig. 5C, S3). These results indicate that at least some of the bacteria in the community recruited from IRL seawater are B_{12} producers and can support P. *bahamense* growth.

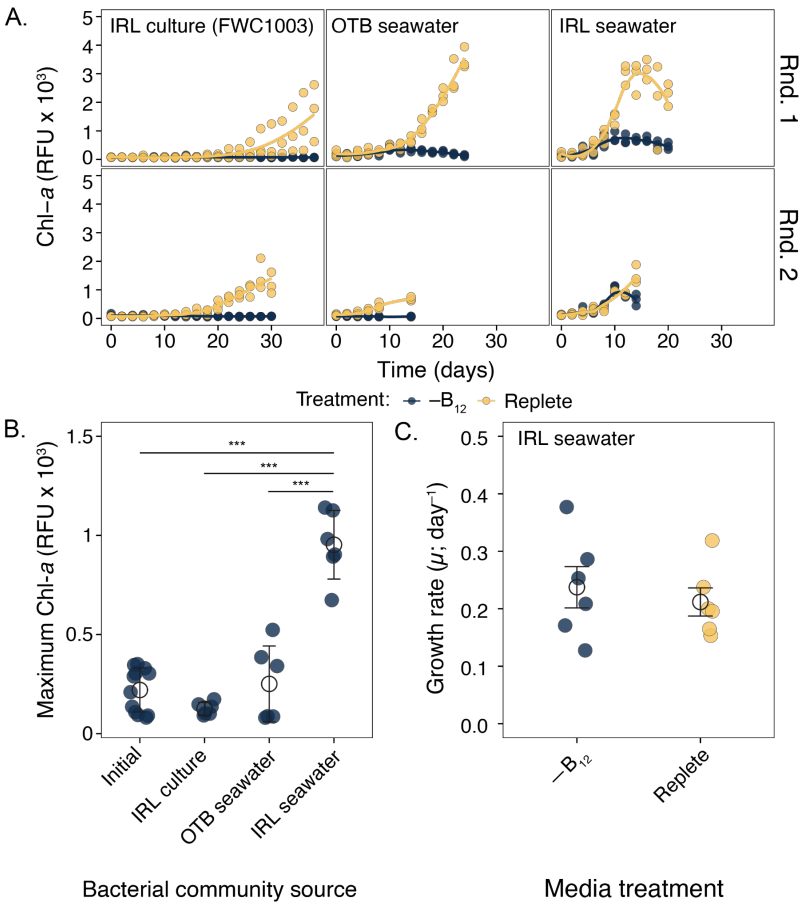
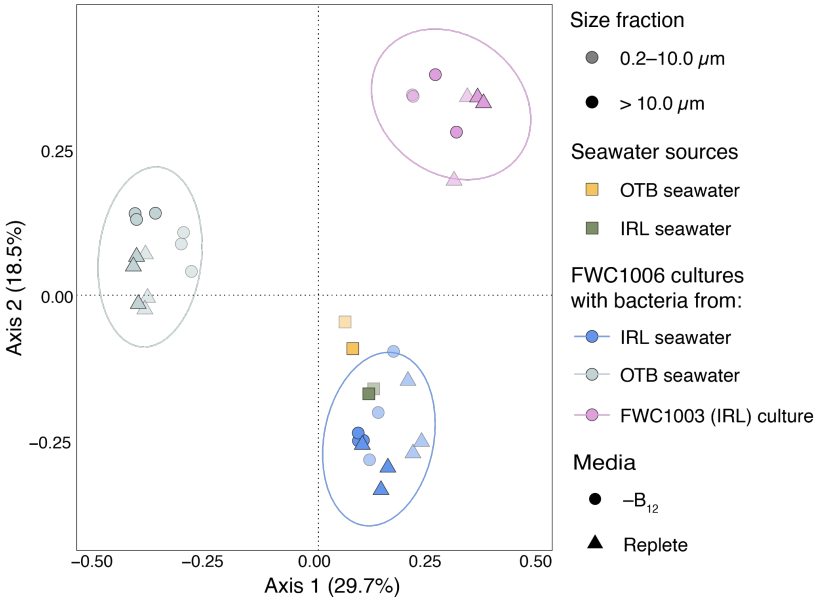


Fig. 5 Growth responses of OTB *P. bahamense* (FWC1006) with bacterial communities recruited from IRL culture (FWC1003), OTB seawater, and IRL seawater when grown in L1/4 media missing vitamin B_{12} and replete L1/4 media. All data are plotted with color representing media treatment. **A** *In vivo* Chlorophyll-*a* fluorescence (filled circles) was used to track the growth of OTB *P. bahamense* with bacteria from three sources (IRL culture, OTB seawater, and IRL seawater) in replete L1/4 media and L1/4 media missing B_{12} (L1/4 $-B_{12}$). Solid lines indicate the smoothed conditional mean, plotted with the geom_smooth function in the R package ggplot2 (method = 'loess'). **B** Maximum Chl-*a* fluorescence values (filled circles) in L1/4 $-B_{12}$ are shown for each replicate, grouped by bacterial community source. Note, the maximum Chl-*a* reached in L1/4 $-B_{12}$ by OTB *P. bahamense* with its initial low-diversity bacterial community is included for reference (from Fig. 3). Results from key post-hoc comparisons are shown with the following significance code: *p-adj* < 0.001; '***'. **C** Growth rates (filled circles) for each replicate with bacteria from IRL seawater, grouped by media treatment. Means are plotted as black open circles, and error bars represent one standard deviation of the mean.

Sequencing results

333 Overall, this project produced 10,431,416 Nanopore full-length 16S reads that passed
334 quality filters. After applying a length filter (min = 1300 bp, max = 2000 bp), 8,526,020 reads
335 remained. Culture samples sequenced on MinION flow cells yielded 128,931–500,307 (median
336 = 322,769) reads per sample and retained 103,950–407,502 (median = 272,432) after applying
337 the length filter. Seawater samples (bacterial source cultures) sequenced on a Flongle flowcell
338 yielded 34,389–141,236 (median = 93,931) reads per sample and retained 12,221–53,990
339 (median = 37,987) reads per sample after length filtering. Alpha diversity (richness and
340 Shannon index, based on genus) was higher in the IRL seawater (richness = 1,695–1,805,
341 Shannon = 4.82–4.89) than the OTB seawater (richness = 1,056–1,208, Shannon = 3.24–4.69;
342 Fig. S5, S6). Alpha diversity was similar across recruited communities (Fig. S5, S6).
343
344 *Comparison of bacterial communities recruited from different sources*
345 Distinct bacterial communities were recruited to the FWC1006 *P. bahamense* cultures
346 from the three sources (FWC1003 *P. bahamense* cultures (IRL), IRL seawater, and OTB
347 seawater). Recruited bacterial communities formed three clusters in a principal coordinate
348 analysis based on Bray-Curtis distances (Fig. 6). PERMANOVA results further indicated that
349 bacterial source significantly affected recruited community composition (R^2 = 0.67542, F =
350 16.127, p = 0.001; 999 permutations), whereas size fraction and media formulation did not
351 significantly affect community composition ($p > 0.05$). Visualizing the relative abundance of
352 bacterial orders in each sample further demonstrates the distinct communities recruited from
353 each source (Fig. 7). Moreover, the FWC1006 *P. bahamense* culture clearly recruited bacteria
354 from the various sources; only three bacterial orders were detected in the initial FWC1006
355 culture microbiomes, despite deep Illumina metabarcode sequencing (394,399–621,150 reads
356 per sample after all filterings steps), whereas 10–12 bacterial orders were detected after
357 recruiting new bacterial communities.
358 Since only cultures with bacteria recruited from IRL seawater were able to grow in media
359 without vitamin B_{12}, we identified bacteria that were exclusively present in these cultures.
360 Alteromonadaceae, Saprospiraceae, and Enterobacteriaceae were consistently present in
361 communities recruited from IRL seawater but not from OTB seawater or IRL culture (Fig. 8).
362 *Alteromonas spp*., which typically require B_{12} but can not synthesize it [39], were abundant in
363 the smaller size fraction of communities recruited from IRL seawater when B_{12} was not included
364 in the media. *Klebsiella* and *Enterobacter*—both B_{12} producers [40, 41]—were present in all
365 communities recruited from the IRL seawater but not from OTB seawater or IRL culture.

366 Similarly, *Lewinella* was present in all communities recruited from IRL seawater but not from
367 OTB seawater or IRL culture. In contrast, *Ruegeria* and *Sulfitobacter* (Rhodobacteraceae),
368 genera often detected in phytoplankton cultures, were present in all cultures (Fig. 8).
369



370
371 **Fig. 6** Principal coordinate analysis (PCoA) of Bray-Curtis distances between the bacterial communities
372 recruited by *P. bahamense* FWC1006 and the source surface seawater bacterial communities. FWC1006
373 cultures with each bacterial community in replete L1/4 media and L1/4–B_{12} media were sacrificed for 16S
374 rRNA gene sequencing at the end of round two in Fig. 5C. Three 95% confidence ellipses drawn with the
375 ggplot2 stat_ellipse function highlight the groupings within the data. The replicates with bacteria from OTB
376 seawater separate from all other samples on the primary axis, while the samples with bacteria from the
377 FWC1003 (IRL) culture and IRL seawater separate on the secondary axis. The bacterial communities in
378 the source seawater grouped most closely with the bacterial communities in culture that were recruited
379 from IRL seawater (bottom right quadrant), with the IRL seawater source communities within the 95%
380 confidence ellipse for the communities recruited from IRL seawater.
381
382

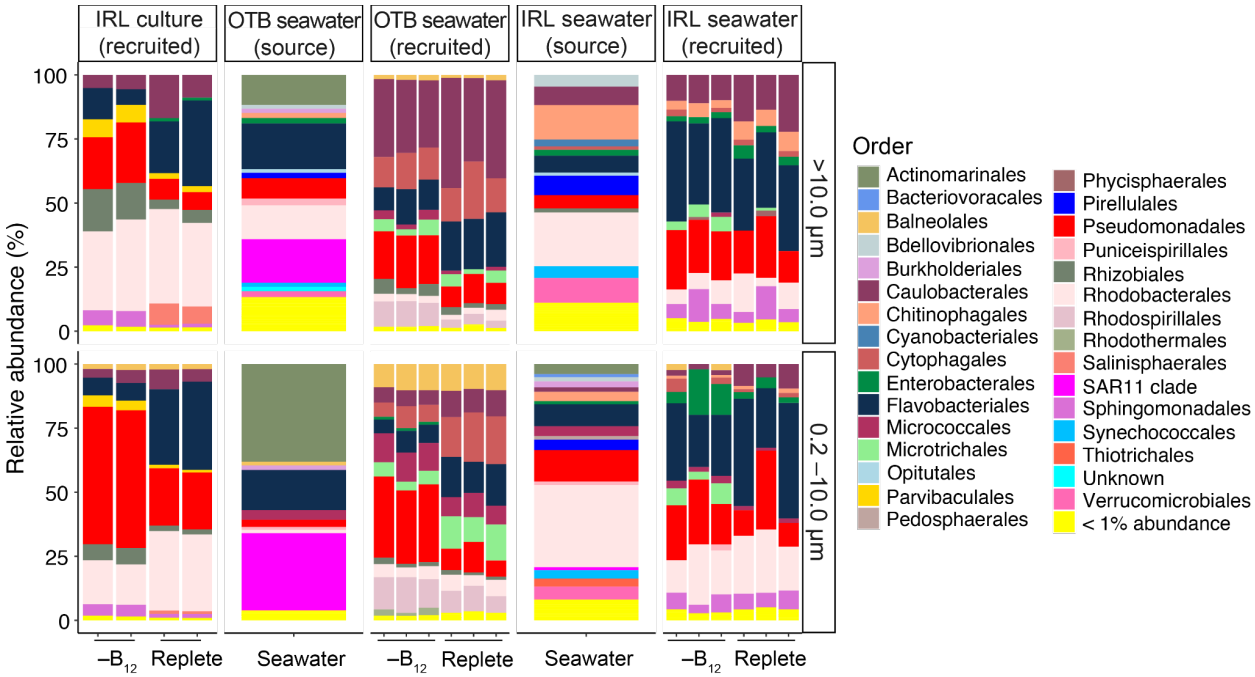


Fig. 7 Relative abundance of bacterial orders in source seawater samples and in cultures with bacterial communities recruited from IRL P. bahamense culture, OTB seawater, and IRL seawater. Orders that made up less than 1% in a given sample were combined to reduce the number of orders included in the plot.

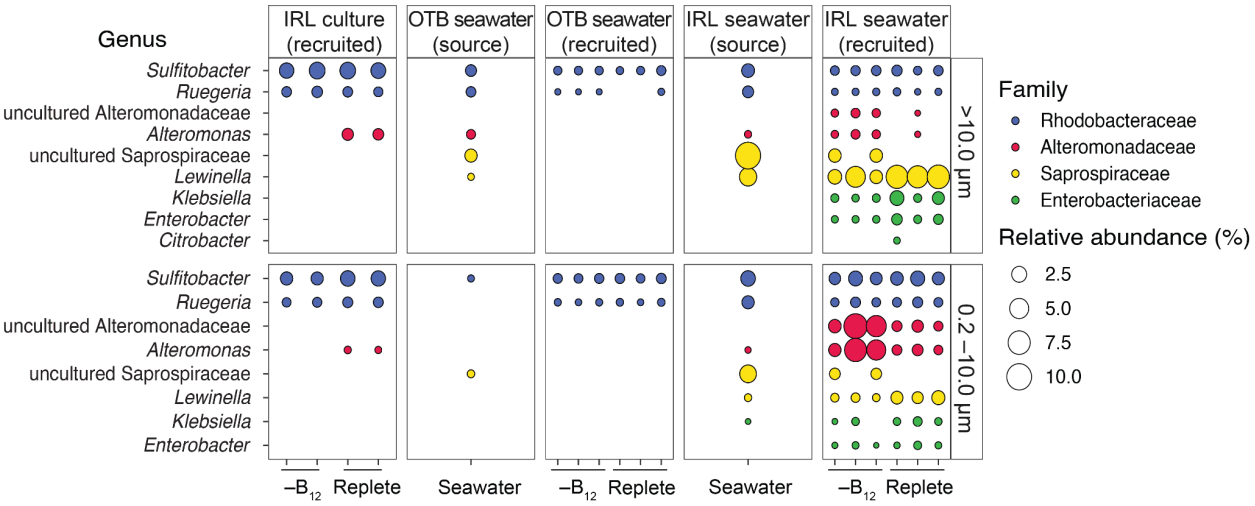


Fig. 8 Relative abundance of genera in groups detected primarily in communities recruited from IRL seawater (Alteromonadaceae, Saprospiracea, and Enterobacteracea). Rhodobacteracea is included as a comparison. Rhodobacteracea are commonly found in association with phytoplankton and other algae. *Ruegeria* and *Sulfitobacter* were detected in all sample types.

395 **Discussion**

396 HAB-forming dinoflagellates generally require B_{12} for growth and often also require B_1 or
397 B_7 [9]. Despite this, HABs frequently form in regions with undetectable B_{12} concentrations,
398 suggesting that B_{12} may be being drawn down below detection limits or that cryptic B_{12} cycling
399 from bacteria is key to supporting HABs [10]. Here, we exclusively tested the cobalamin (B_{12}),
400 thiamine (B_1), and biotin (B_7) requirements of *P. bahamense* var. b*ahamense*—a dinoflagellate
401 that causes toxic HABs in Florida estuaries [19], but whose bioluminescence makes it culturally
402 and economically important in the Caribbean [42]—by utilizing a strain isolated from Florida
403 (OTB) with a reduced culture microbiome (Fig. 1). Our results clearly demonstrate that *P.*
404 *bahamense* has an absolute B_{12} requirement for growth and bloom formation. Only the bacterial
405 community recruited from IRL seawater collected during a *P. bahamense* bloom restored *P.*
406 *bahamense* growth in media without B_{12}. These results suggest that bacteria present during *P.*
407 *bahamense* blooms may contribute to fueling blooms through B_{12} production. While bacterial
408 communities that restored P. bahamense growth (from IRL seawater) when B_{12} was withheld
409 overlapped in composition with those that did not (IRL culture and OTB seawater), several
410 specific bacterial genera were found exclusively in the growth-restoring communities. Among
411 these, *Klebsiella* and *Enterobacter* are known B_{12} producers [41, 43] and may be introduced to
412 the environment through wastewater or runoff [44, 45]. Thus, anthropogenic inputs may be
413 contributing to *P. bahamense* blooms in Florida estuaries by introducing or otherwise supporting
414 B_{12}-synthesizing bacteria.

415

416 *B-vitamin requirements of toxigenic dinoflagellates*

417 Results from this study demonstrate that *P. bahamense* from OTB requires an external
418 source of vitamin B_{12}, but not necessarily B_1 or B_7, for growth. However, since the culture used
419 in the experiments had a reduced microbiome rather than being fully axenic, it remains possible
420 that the remaining bacteria supplied B_1 or B_7 and obscured *P. bahamense*'s requirement of
421 those vitamins. These results are in line with previous genomic surveys [8] and bioassays [9],
422 indicating that most HAB dinoflagellates are B_{12} auxotrophs. While *P. bahamense* was not
423 included in these studies, Santos and Azana [46] found that maximum biomass tripled in
424 antibiotic-treated cultures of *P. bahamense* var. *compressum* when the B-vitamin concentrations
425 in f/2 media [47] were also tripled. Together, these results strengthen the case that B_{12}
426 auxotrophy is a commonly shared trait among toxigenic dinoflagellates.

427 The *P. bahamense* strain used in this study required > 0.369 pM B_{12} to reach a
428 chlorophyll fluorescence commensurate with 100,000 cells L^{-1}, the minimum cell density
429 designated by Florida state regulatory agencies as a bloom (Fig. 4, S4). The *P. bahamense*
430 growth rate stabilized at 0.369 pM B_{12} and remained stable at higher B_{12} concentrations, even
431 though higher B_{12} concentrations further increased the maximum chlorophyll fluorescence
432 reached (Fig. 4). Similarly, the saxitoxin-producing dinoflagellates *Alexandrium pacificum* and
433 *Gymnodinium catenatum* required > 0.34 and > 1. 52 pM B_{12}, respectively, for measurable
434 growth [8]. The brevetoxin-producing *Karenia brevis* requires > 0.01–1.0 pM B_{12} for measurable
435 growth, depending on the strain tested [8, 9]. Thus, in addition to having an absolute B_{12}
436 requirement, toxigenic dinoflagellates may have especially high B_{12} needs due to their unique
437 physiologies.
438 Phytoplankton are generally considered auxotrophic for B_{12} if they lack the gene
439 encoding B_{12}-independent methionine synthase, but B_{12} may support dinoflagellate growth
440 through other mechanisms as well [48]. Vitamin B_{12} is a cofactor for methylmalonyl-CoA mutase
441 (MCM)—the enzyme that converts methylmalonyl-CoA to succinyl-CoA and is required for the
442 breakdown of odd-chain fatty acids and several amino acids. The metabolites from MCM-
443 catalyzed breakdown of amino acids are directed into the Krebs cycle, supporting energy
444 production in eukaryotic cells [48, 49]. Moreover, S-adenosylmethionine (SAM), the activated
445 form of methionine, acts as a universal methyl donor. SAM cycle methylation is key for
446 regulating gene expression through DNA and histone methylation, and is also central to
447 saxitoxin biosynthesis [8]. Given the large genomes characteristic of dinoflagellates [50],
448 extensive methylation may be required for gene regulation [51], which would increase usage of
449 the SAM cycle and elevate B_{12} demand.
450
451 *Bacteria in seawater collected during a bloom supported* P. bahamense *growth*
452 Many studies have demonstrated that bacteria can fulfill phytoplankton B_{12} needs (e.g.,
453 [23, 36, 52]), including those of HAB-forming dinoflagellates (e.g., [17, 22]). Therefore, we
454 tested whether bacteria recruited from several sources could rescue *P. bahamense* from B_{12}
455 limitation. Only the bacterial community recruited from IRL seawater collected during a *P.*
456 *bahamense* bloom was able to support *P. bahamense* growth in media without B_{12}, although
457 these cultures only reached about half the total biomass of cultures in replete media (Fig. 5).
458 The maximum Chl-*a* fluorescence in these cultures was 1,140 RFU, just shy of 1,457 RFU—the
459 value corresponding to the 100,000 cells L^{-1} bloom level (Fig. S4). Moreover, the growth rates in

460 cultures with bacteria from IRL seawater were similar in replete media and media missing B_{12}.
461 Together, these results suggest that bacteria present during the *P. bahamense* bloom in IRL
462 and transferred to our culture may supply B_{12} and support bloom development.
463 To evaluate which bacteria in the IRL seawater may provide B_{12}, we identified taxa that
464 were exclusively recruited from this source. Genera in the families Saprospiraceae (*Lewinella*)
465 and Enterobacteriaceae (*Klebsiella* and *Enterobacter*) were consistently present in cultures with
466 bacteria from IRL seawater and absent in cultures with bacteria from OTB seawater or IRL *P.*
467 *bahamense* culture. *Lewinella* is not a known B_{12}-producer, but is commonly associated with
468 phytoplankton [53] and macroalgae [54], and may be particularly suited to degrading algal
469 polysaccharides [53]. *Klebsiella* species are prolific B_{12} producers and are added to fermented
470 foods for B_{12} enrichment [41]. *Enterobacter* species are similarly B_{12} producers [43]. In addition
471 to being found in foods, *Klebsiella* and *Enterobacter* reside in mammalian small intestines [55]
472 and are found in wastewater [44, 45]. *Alteromonas spp*., which are typically B_{12} auxotrophs [39],
473 were most abundant in the smaller size fraction of communities recruited from IRL seawater
474 when B_{12} was not included in the media, suggesting they may also benefit from B_{12} produced by
475 Enterobacteraceae or other bacteria recruited from IRL. In contrast, Rhodobacteraceae
476 genera—*Ruegeria* and *Sulfitobacter*—that are often found in phytoplankton cultures and
477 associated with phytoplankton blooms [56] were found in all of our cultures and were not
478 specific to those that grew without B_{12} added to media (Fig. 6, 7).
479 Surprisingly, bacteria recruited from the IRL culture did not support *P. bahamense*
480 growth in the absence of B_{12}, despite the IRL culture microbiome being fairly diverse and likely
481 representing the seawater community when the culture was initiated. The diversity of culture
482 microbiomes can diminish with culture age [56], and B_{12}-producing bacteria may be
483 outcompeted if the media is replete in B_{12}. The OTB seawater may have originally hosted B_{12}-
484 producing bacteria that were lost in the extensive filtering used to remove eukaryotic
485 phytoplankton from the inoculum, but it is also plausible that B_{12}-producing bacteria were absent
486 at the time of collection since *P. bahamense* was also absent. Notably, this study reveals that
487 distinct communities were recruited to *P. bahamense* from three sources, suggesting that *P.*
488 *bahamense* is opportunistic or a generalist in its associations with bacteria.
489 In some cases, bacteria associated with phytoplankton cultures or blooms compete for
490 essential nutrients [36, 57, 58]. Here, B_{12}-producing bacteria may have been present in the
491 communities recruited from OTB seawater, but other community members outcompeted *P.*
492 *bahamense* for the resource. For instance, *Caulobacter* can change morphology and motility to

survive oligotrophic conditions, making them especially good competitors [59]. Caulobacterales were present in all recruited bacterial communities, but were most abundant in communities recruited from OTB seawater. Thus, bacterial competition for essential resources may have affected the growth of the *P. bahamense* grown with bacteria from OTB seawater.

Conclusions & future directions

The results from this study demonstrate that *P. bahamense* is auxotrophic for B_{12} and that potentially anthropogenic bacteria present in the IRL may supply B_{12} to support bloom formation. Bacteria from OTB seawater did not support *P. bahamense* growth when B_{12} was withheld, but these bacteria were collected when *P. bahamense* was not detected in the environment. Future work should investigate B_{12} sources that support *P. bahamense* bloom formation by seeking to identify B_{12}-producing bacteria that co-bloom with *P. bahamense* or determining if B_{12} is directly introduced to the ecosystem in wastewater. OTB and IRL are highly urbanized and receive excess nutrients from wastewater treatment plants [60], and the prolonged residence times [61] in these estuaries may also lead to an accumulation of B_{12}. Given that increased B_{12} levels could exacerbate blooms, anthropogenic sources of B_{12} should be investigated in tandem with B_{12}-synthesizing bacteria. Ultimately, measuring and tracing B_{12} in OTB and the IRL could disentangle the sources of B_{12} to these ecosystems and the relationship between anthropogenic B_{12} input and B_{12}-producing bacteria with HABs. Such an effort may advance our ability to predict the severity of not just *P. bahamense* blooms, but other HABs as well.

Acknowledgements

Research was funded by the University of South Florida College of Marine Science (USF CMS), with additional support from the Phycological Society of America Grants-in-Aid of Research and the Sanibel Captiva Shell Club grant. LRH was supported by the Abby Sallenger Endowed Fellowship in Marine Science and the Anne and Werner Von Rosenstiel Fellowship in Marine Science, awarded through USF CMS. We would like to thank the Florida Fish and Wildlife Conservation Commission (FWC) Fish and Wildlife Research Institute (FWRI) for *P. bahamense* culture loans and seawater sampling. Illumina metabarcode sequencing was performed by Georgia Genomics and Bioinformatics Core at the University of Georgia (GGBC, UG Athens, GA, RRID:SCR_010994).

Author Contributions

MMB planned the project. LRH, LD, and MMB performed research. LRH and MMB analyzed data and wrote the manuscript. CL and SS reviewed and edited manuscript drafts.

Data Availability

All sequence data from this project is available on NCBI SRA under PRJNA1315153. Code, data analysis pipelines, and raw data are available in a GitHub repository: https://github.com/lruggles1/Pyrodinium_bahamense_growth.git. The reproducible R Markdown analysis pipeline is further available as an interactive HTML file: https://lruggles1.github.io/Pyrodinium_bahamense_growth/pyro_growth_meta.html.

Competing interests

The authors declare no competing interests.

References

1. Beardall J, Stojkovic S, Larsen S. Living in a high CO_2 world: impacts of global climate change on marine phytoplankton. *Plant Ecol Divers* 2009; **2**: 191–205.
2. Longhurst A, Sathyendranath S, Platt T, Caverhill C. An estimate of global primary production in the ocean from satellite radiometer data. *J Plankton Res* 1995; **17**: 1245–1271.
3. Sekerci Y, Ozarslan R. Oxygen-plankton model under the effect of global warming with nonsingular fractional order. *Chaos Solitons Fractals* 2020; **132**: 109532.
4. Glibert PM, Berdalet E, Burford MA, Pitcher GC, Zhou M. Global Ecology and Oceanography of Harmful Algal Blooms. 2018. Springer.
5. Anderson DM, Cembella AD, Hallegraeff GM. Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. *Ann Rev Mar Sci* 2012; **4**: 143–176.
6. Gobler CJ. Climate Change and Harmful Algal Blooms: Insights and perspective. *Harmful Algae* 2020; **91**: 101731.
7. Croft MT, Warren MJ, Smith AG. Algae need their vitamins. *Eukaryot Cell* 2006; **5**: 1175–1183.
8. Lin S, Hu Z, Song X, Gobler CJ, Tang YZ. Vitamin B_{12}-auxotrophy in dinoflagellates caused

559 by incomplete or absent cobalamin-independent methionine synthase genes (metE).
560 *Fundamental Research* 2022; **2**: 727–737.
561 9. Tang YZ, Koch F, Gobler CJ. Most harmful algal bloom species are vitamin B_1 and B_{12}
562 auxotrophs. *Proc Natl Acad Sci U S A* 2010; **107**: 20756–20761.
563 10. Sañudo-Wilhelmy SA, Cutter LS, Durazo R, Smail EA, Gómez-Consarnau L, Webb EA, et
564 al. Multiple B-vitamin depletion in large areas of the coastal ocean. *Proc Natl Acad Sci U S*
565 *A* 2012; **109**: 14041–14045.
566 11. Gobler CJ, Norman C, Panzeca C, Taylor GT, Sañudo-Wilhelmy SA. Effect of B-vitamins
567 (B_1, B_{12}) and inorganic nutrients on algal bloom dynamics in a coastal ecosystem. *Aquat*
568 *Microb Ecol* 2007; **49**: 181–194.
569 12. Fang H, Kang J, Zhang D. Microbial production of vitamin B_{12}: a review and future
570 perspectives. *Microb Cell Fact* 2017; **16**: 15.
571 13. Sultana S, Bruns S, Wilkes H, Simon M, Wienhausen G. Vitamin B_{12} is not shared by all
572 marine prototrophic bacteria with their environment. *ISME J* 2023; **17**: 836–845.
573 14. Wagner-Döbler I, Ballhausen B, Berger M, Brinkhoff T, Buchholz I, Bunk B, et al. The
574 complete genome sequence of the algal symbiont *Dinoroseobacter shibae*: a hitchhiker's
575 guide to life in the sea. *ISME J* 2010; **4**: 61–77.
576 15. Durham BP, Sharma S, Luo H, Smith CB, Amin SA, Bender SJ, et al. Cryptic carbon and
577 sulfur cycling between surface ocean plankton. *Proc Natl Acad Sci U S A* 2015; **112**: 453–
578 457.
579 16. Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG. Algae acquire vitamin B_{12}
580 through a symbiotic relationship with bacteria. *Nature* 2005; **438**: 90–93.
581 17. Fei C, Booker A, Klass S, Vidyarathna NK, Ahn SH, Mohamed AR, et al. Friends and foes:
582 symbiotic and Algicidal bacterial influence on *Karenia brevis* blooms. *ISME Commun* 2024;
583 ycae164.
584 18. Ren C-Z, Gao H-M, Dai J, Zhu W-Z, Xu F-F, Ye Y, et al. Taxonomic and bioactivity
585 characterizations of *Mameliella alba* strain LZ-28 isolated from highly toxic marine
586 dinoflagellate *Alexandrium catenella* LZT09. *Mar Drugs* 2022; **20**: 321.
587 19. Phlips JE, Badylak S, Bledsoe E, Cichra M. Factors affecting the distribution of *Pyrodinium*
588 *bahamense* var. *bahamense* in coastal waters of Florida. *Mar Ecol Prog Ser* 2006; **322**: 99–
589 115.
590 20. Burkholder PR, Burkholder LM. Studies on B vitamins in relation to productivity of the Bahia
591 fosforescente, Puerto Rico. *Bull Mar Sci* 1958; **8**: 201–223.

592 21. Guillard RRL, Hargraves PE. *Stichochrysis immobilis* is a diatom, not a chrysophyte.
593 *Phycologia* . 1993. , **32**: 234–236
594 22. Cruz-López R, Maske H. The Vitamins B_1 and B_{12} required by the marine dinoflagellate
595 *Lingulodinium polyedrum* can be provided by its associated bacterial community in culture.
596 *Front Microbiol* 2016; **7**: 560.
597 23. Andrew S, Wilson T, Smith S, Marchetti A, Septer AN. A tripartite model system for
598 Southern Ocean diatom-bacterial interactions reveals the coexistence of competing
599 symbiotic strategies. *ISME Commun* 2022; **2**: 97.
600 24. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of
601 general 16S ribosomal RNA gene PCR primers for classical and next-generation
602 sequencing-based diversity studies. *Nucleic Acids Res* 2013; **41**: e1.
603 25. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al.
604 Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2.
605 *Nat Biotechnol* 2019; **37**: 852–857.
606 26. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-
607 resolution sample inference from Illumina amplicon data. *Nat Methods* 2016; **13**: 581–583.
608 27. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, et al. Optimizing
609 taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-
610 classifier plugin. *Microbiome* 2018; **6**: 90.
611 28. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal
612 RNA gene database project: improved data processing and web-based tools. *Nucleic Acids*
613 *Res* 2013; **41**: D590–6.
614 29. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and
615 graphics of microbiome census data. *PLoS One* 2013; **8**: e61217.
616 30. RStudio Team. RStudio: Integrated Development for R. RStudio, PBC.
617 http://www.rstudio.com/.
618 31. Guillard RRL, Morton SL. Culture methods. *Manual on harmful marine microalgae*
619 *UNESCO, Paris* 2003; 77–97.
620 32. Petzoldt T. *growthrates: Estimate Growth Rates from Experimental Data*. R package. 2025.
621 33. Matsuo Y, Komiya S, Yasumizu Y, Yasuoka Y, Mizushima K, Takagi T, et al. Full-length
622 16S rRNA gene amplicon analysis of human gut microbiota using MinION™ nanopore
623 sequencing confers species-level resolution. *BMC Microbiol* 2021; **21**: 35.
624 34. Lu J, Salzberg SL. Ultrafast and accurate 16S rRNA microbial community analysis using

625 Kraken 2. *Microbiome* 2020; **8**: 124.
626 35. Oxford Nanopore Technologies. Native Barcoding Kit 24 V14. *Oxford Nanopore*
627 *Technologies*.
628 https://community.nanoporetech.com/docs/prepare/library_prep_protocols/ligation-
629 sequencing-amplicons-native-barcoding-v14-sqk-nbd114-
630 24/v/nba_9168_v114_revm_15sep2022/overview-of-the-protocol?devices=minion.
631 Accessed 18 Aug 2024.
632 36. Mars Brisbin M, Mitarai S, Saito MA, Alexander H. Microbiomes of bloom-forming
633 *Phaeocystis* algae are stable and consistently recruited, with both symbiotic and
634 opportunistic modes. *ISME J* 2022; **16**: 2255–2264.
635 37. Câmara Dos Reis M, Romac S, Le Gall F, Marie D, Frada MJ, Koplovitz G, et al. Exploring
636 the phycosphere of *Emiliania huxleyi*: From bloom dynamics to microbiome assembly
637 experiments. *Mol Ecol* 2023; **32**: 6507–6522.
638 38. Isaac A, Francis B, Amann RI, Amin SA. Tight adherence (tad) pilus genes indicate putative
639 niche differentiation in phytoplankton bloom associated Rhodobacterales. *Front Microbiol*
640 2021; **12**: 718297.
641 39. Mars Brisbin M, Schofield A, McIlvin MR, Krinos AI, Alexander H, Saito MA. Vitamin B_{12}
642 conveys a protective advantage to phycosphere-associated bacteria at high temperatures.
643 *ISME Commun* 2023; **3**: 88.
644 40. Lawrence JG, Roth JR. Evolution of coenzyme B_{12} synthesis among enteric bacteria:
645 evidence for loss and reacquisition of a multigene complex. *Genetics* 1996; **142**: 11–24.
646 41. Keuth S, Bisping B. Vitamin B_{12} production by *Citrobacter freundii* or *Klebsiella pneumoniae*
647 during tempeh fermentation and proof of enterotoxin absence by PCR. *Appl Environ*
648 *Microbiol* 1994; **60**: 1495–1499.
649 42. Soler-Figueroa BM, Otero E. The influence of rain regimes and nutrient loading on the
650 abundance of two dinoflagellate species in a tropical bioluminescent bay, Bahía
651 fosforescente, La Parguera, Puerto Rico. *Estuaries Coast* 2015; **38**: 84–92.
652 43. Chung HJ, Fields ML. Production of Riboflavin and Vitamin B_{12} by *Bacillus megaterium*
653 ATCC 13639 and *Enterobacter aerogenes* in Corn Meal. *J Food Sci* 1986; **51**: 1514–1517.
654 44. Rajagopalan K. Studies on vitamin B_{12} production by strains of *Klebsiella pneumoniae* and
655 *Bacillus pasteurii*. *J Appl Bacteriol* 1976; **40**: 111–114.
656 45. Kvesić M, Šamanić I, Novak A, Fredotović Ž, Dželalija M, Kamenjarin J, et al. Submarine
657 outfalls of treated wastewater effluents are sources of extensively- and multidrug-resistant

658 KPC- and OXA-48-producing Enterobacteriaceae in coastal marine environment. *Front*
659 *Microbiol* 2022; **13**: 858821.
660 46. Santos MAG, Azanza RV. Responses of *Pyrodinium bahamense* var. *compressum* and
661 associated cultivable bacteria to antibiotic treatment. *J Appl Phycol* 2012; **24**: 825–835.
662 47. Guillard RR, Ryther JH. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt,
663 and *Detonula confervacea* (cleve) Gran. *Can J Microbiol* 1962; **8**: 229–239.
664 48. Helliwell KE. The roles of B vitamins in phytoplankton nutrition: new perspectives and
665 prospects. *New Phytol* 2017; **216**: 62–68.
666 49. Forny P, Froese DS, Suormala T, Yue WW, Baumgartner MR. Functional characterization
667 and categorization of missense mutations that cause methylmalonyl-CoA mutase (MUT)
668 deficiency. *Hum Mutat* 2014; **35**: 1449–1458.
669 50. Wang H, Wu P, Xiong L, Kim H-S, Kim JH, Ki J-S. Nuclear genome of dinoflagellates: Size
670 variation and insights into evolutionary mechanisms. *Eur J Protistol* 2024; **93**: 126061.
671 51. Ho P, Kong KF, Chan YH, Tsang JSH, Wong JTY. An unusual S-adenosylmethionine
672 synthetase gene from dinoflagellate is methylated. *BMC Mol Biol* 2007; **8**: 87.
673 52. Costas-Selas C, Martínez-García S, Pinhassi J, Fernández E, Teira E. Unveiling
674 interactions mediated by B vitamins between diatoms and their associated bacteria from
675 cocultures. *J Phycol* 2024; **60**: 1456–1470.
676 53. Gómez-Letona M, Arístegui J, Riebesell U, Sebastián M. Consistent prokaryotic
677 successional dynamics across contrasting phytoplankton blooms. *Limnol Oceanogr* 2025;
678 **70**: 493–508.
679 54. Miranda LN, Hutchison K, Grossman AR, Brawley SH. Diversity and abundance of the
680 bacterial community of the red Macroalga *Porphyra umbilicalis*: did bacterial farmers
681 produce macroalgae? *PLoS One* 2013; **8**: e58269.
682 55. Albert MJ, Mathan VI, Baker SJ. Vitamin B_{12} synthesis by human small intestinal bacteria.
683 *Nature* 1980; **283**: 781–782.
684 56. Mars Brisbin M, Acord M, Davitt R, Bent S, Van Mooy BAS, Flaum E, et al. Exploring the
685 Phaeosphere: Characterizing the microbiomes of *Phaeocystis antarctica* colonies from the
686 coastal Southern Ocean and laboratory culture. *J Phycol* 2025; **61**: 1124–1139.
687 57. Cai G, Yu X, Wang H, Zheng T, Azam F. Nutrient-dependent interactions between a marine
688 copiotroph *Alteromonas* and a diatom *Thalassiosira pseudonana*. *MBio* 2023; **14**:
689 e0094023.
690 58. Calfee BC, Glasgo LD, Zinser ER. *Prochlorococcus* exudate stimulates heterotrophic

691 bacterial competition with rival phytoplankton for available nitrogen. *MBio* 2022; **13**:
692 e0257121.
693 59. Hallgren J, Koonce K, Felletti M, Mortier J, Turco E, Jonas K. Phosphate starvation
694 decouples cell differentiation from DNA replication control in the dimorphic bacterium
695 *Caulobacter crescentus*. *PLoS Genet* 2023; **19**: e1010882.
696 60. Greening H, Janicki A, Sherwood ET, Pribble R, Johansson JOR. Ecosystem responses to
697 long-term nutrient management in an urban estuary: Tampa Bay, Florida, USA. *Estuar*
698 *Coast Shelf Sci* 2014; **151**: A1–A16.
699 61. Meyers SD, Luther ME. A numerical simulation of residual circulation in Tampa bay. Part II:
700 Lagrangian residence time. *Estuaries Coast* 2008; **31**: 815–827.