

# Contribution of cyanobacterial alkane production to the ocean hydrocarbon cycle

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Hydrocarbons are ubiquitous in the ocean, where alkanes such as pentadecane and heptadecane can be found even in waters minimally polluted with crude oil. Populations of hydrocarbon-degrading bacteria, which are responsible for the turnover of these compounds, are also found throughout marine systems, including in unpolluted waters. These observations suggest the existence of an unknown and widespread source of hydrocarbons in the oceans. Here, we report that strains of the two most abundant marine cyanobacteria, *Prochlorococcus* and *Synechococcus*, produce and accumulate hydrocarbons, predominantly C15 and C17 alkanes, between 0.022 and 0.368% of dry cell weight. Based on global population sizes and turnover rates, we estimate that these species have the capacity to produce 2–540 pg alkanes per mL per day, which translates into a global ocean yield of ~308–771 million tons of hydrocarbons annually. We also demonstrate that both obligate and facultative marine hydrocarbon-degrading bacteria can consume cyanobacterial alkanes, which likely prevents these hydrocarbons from accumulating in the environment. Our findings implicate cyanobacteria and hydrocarbon degraders as key players in a notable internal hydrocarbon cycle within the upper ocean, where alkanes are continually produced and subsequently consumed within days. Furthermore we show that cyanobacterial alkane production is likely sufficient to sustain populations of hydrocarbon-degrading bacteria, whose abundances can rapidly expand upon localized release of crude oil from natural seepage and human activities.

cyanobacteria | hydrocarbons | hydrocarbon cycle | hydrocarbon-degrading bacteria | oil remediation

Hydrocarbons are ubiquitous in the oceans, where natural seepage and human activities are estimated to release between 0.4 and 4.0 million tons of crude oil into the ocean ecosystem annually (1). Even in minimally polluted marine surface waters, alkanes such as pentadecane and heptadecane have been found at concentrations ranging from 2 to 130 pg/mL (2, 3), although their sources remain unclear. A small proportion of alkanes, from 1 to 60 fg/mL, is associated with particulate matter >0.7 μm in diameter (4). Larger amounts may be associated with particulate matter <0.7 μm in diameter, because ocean concentrations are higher than the solubility of pentadecane and heptadecane, which is ~10 pg/mL and 1 pg/mL, respectively (2). Populations of hydrocarbon-degrading bacteria, referred to as hydrocarbonoclastic bacteria, including many species that cannot use other carbon sources, are present in marine systems and play an important role in turnover of these compounds (5–9). Because obligate hydrocarbon-degrading bacteria are found in waters without significant levels of crude oil pollution, these organisms must use an alternate hydrocarbon source (9–11).

Here, we investigate the extent to which cyanobacteria may contribute to these marine hydrocarbon pools. Cyanobacteria (oxygenic photosynthetic bacteria) can synthesize C15 to C19 hydrocarbons via two separate pathways. The first produces alkanes, predominantly pentadecane, heptadecane, and methyl-heptadecane, in addition to smaller amounts of alkenes, via acyl-ACP reductase (FAR) and aldehyde deformylating oxygenase (FAD) enzymes (12).

The second pathway generates alkenes, primarily nonadecene and 1,14-nonadecadiene, via a polyketide synthase enzyme (Ols) (13). The abundance and ubiquity of cyanobacteria in the marine environment suggests hydrocarbon production in the oceans could be considerable and broadly distributed geographically (14, 15).

We focused our studies on the two most abundant marine cyanobacteria, *Prochlorococcus* and *Synechococcus* (16). These genera have estimated global population sizes of  $2.9 \pm 0.1 \times 10^{27}$  and  $7.0 \pm 0.3 \times 10^{26}$  cells, respectively (14), and are together responsible for approximately a quarter of marine net primary production (14). These are also the only cyanobacterial genera for which global population size estimates have been compiled (14). Although the distribution patterns of both genera overlap (14, 17), *Prochlorococcus* cells dominate low-nutrient open-ocean areas between 40°N and 40°S and can be found at depths of up to 200 m (16, 18). *Synechococcus* are more numerous in coastal and temperate regions where conditions and nutrient levels are more variable (14, 16) but are still widely distributed in high abundance.

## Results and Discussion

**Marine Cyanobacteria Encode Alkane Biosynthetic Pathways.** We first examined the genetic capability for hydrocarbon production in marine cyanobacterial genomes. Previous studies have demonstrated that hydrocarbon biosynthetic pathways are conserved in sequenced cyanobacteria, encompassing isolates from marine, freshwater, and terrestrial environments (19). This suggests that

### Significance

A number of organisms synthesize hydrocarbons, but the scale at which this occurs in the environment is unknown. Here, we provide the first global estimates of hydrocarbon production by the two most abundant cyanobacteria on Earth, *Prochlorococcus* and *Synechococcus*. We suggest that these organisms represent a significant and widespread source of hydrocarbons to the world's oceans, which in turn may sustain populations of obligate hydrocarbon-degrading bacteria known to be important in consuming anthropogenic oil spills. Our study demonstrates the role cyanobacteria play in the ocean "hydrocarbon cycle" and reveals the massive scale of this process. The widespread distribution of cyanobacteria and hydrocarbon-degrading bacteria in freshwater, marine, and terrestrial environments suggests the hydrocarbon cycle is pervasive in many natural ecosystems.

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hydrocarbons have a key, as-yet-unidentified, function in cyanobacteria that is independent of the ecosystem these organisms inhabit. We extended this work by including newly sequenced *Prochlorococcus* strains, isolated from diverse regions of the ocean (20). All 36 *Prochlorococcus* and 15 marine *Synechococcus* strains examined have the predicted capacity to synthesize alkanes via FAR/FAD enzymes (Table S1). With the exception of *Lep- tolyngbya* sp. PCC7376 and *Moorea producens* 3L, which encode Ols homologs, all of the other marine cyanobacteria including *Cyanobium*, *Acaryochloris*, *Crocospaera*, *Trichodesmium*, *Lyngbya*, *Oscillatoria*, *Nodularia*, and *Microcoleus* species encode FAR/FAD homologs (15, 19). Thus, it is likely that alkanes are the predominant hydrocarbons released by cyanobacteria into the marine environment. Homologs of FAR/FAD and Ols were not identified in any other bacterial, plant, or algal species, suggesting that these pathways for hydrocarbon production are unique to cyanobacteria.

**Prochlorococcus and Synechococcus Accumulate Predominantly Heptadecane and Pentadecane.** Next, we measured the hydrocarbon contents of cultured *Prochlorococcus* and marine *Synechococcus* cells via gas chromatography-mass spectrometry (GC-MS). Hydrocarbon content has been quantified in a wide range of freshwater and terrestrial cyanobacteria, all of which produced either alkanes or alkenes, ranging between 0.024 and 0.262% of dry cell weight (19). However, the hydrocarbon content of *Prochlorococcus* and marine *Synechococcus* species has not been quantified. Our analysis included axenic cultures of three *Prochlorococcus* strains: CCMP1986 (MED4) and MIT9312—both high-light-adapted strains, representative of the most numerically abundant *Prochlorococcus* ecotypes (21)—and one low-light-adapted strain, *Prochlorococcus* MIT9313. Axenic cultures of three diverse marine *Synechococcus* strains, WH7803, WH7805, and WH8102 (16), and one estuarine strain, WH5701, were also examined.

In all *Prochlorococcus* strains examined, pentadecane was the dominant hydrocarbon, ~96% of the total, with the remainder consisting of heptadecane (Table 1 and Fig. S1). Pentadecane was previously identified as the dominant hydrocarbon in *Prochlorococcus*

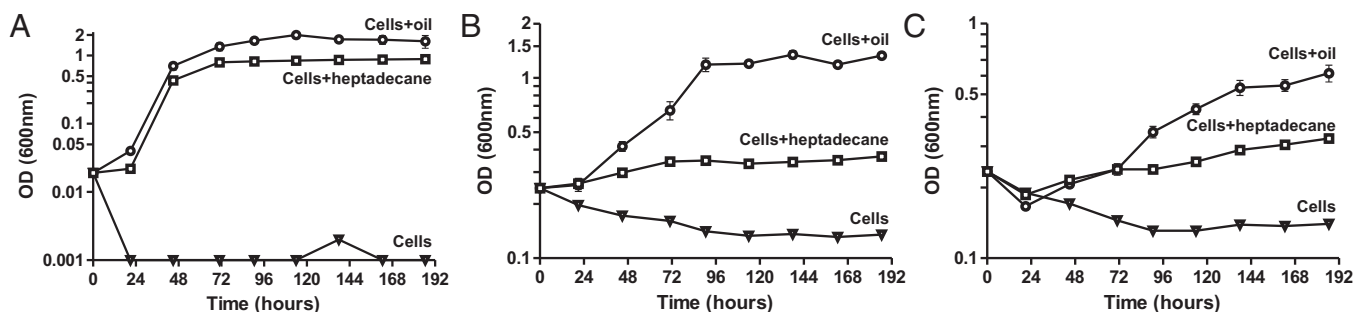
CCMP1986, although the presence of heptadecane was not observed (12). Total hydrocarbon contents were between 0.350 and 0.711 fg per cell or 0.149–0.368% of dry cell weight (Table 1). Pentadecane was also the dominant hydrocarbon in *Synechococcus* sp. WH7803, WH7805, and WH8102, ranging between 79 and 92% of total hydrocarbons (Table 1 and Fig. S1). The remainder consisted of 8-heptadecene. The hydrocarbon composition of *Prochlorococcus* and marine *Synechococcus* species differs from that of other alkane-producing cyanobacteria, where heptadecane was the dominant hydrocarbon (19). The hydrocarbon composition of *Synechococcus* sp. WH5701 was significantly different from the marine strains, consisting of 3.7% pentadecane, 45.3% heptadecane, and 50.9% 8-heptadecene (Fig. S1), which may reflect its evolutionary distance from the other *Synechococcus* strains examined here (22). Total hydrocarbons in *Synechococcus* were between 0.304 and 2.580 fg per cell or 0.022–0.138% of dry cell weight (Table 1). With the exception of *Prochlorococcus* CCMP1986, which has the highest hydrocarbon yields so far observed in a cyanobacterium, hydrocarbon contents were within the range previously observed for other cyanobacteria (19).

**Significant Potential for Hydrocarbon Production by Prochlorococcus and Synechococcus in the Oceans.** Given the average measured abundances of hydrocarbons in *Prochlorococcus* and *Synechococcus* cells and their population sizes, we estimate that, at any given point in time, these cyanobacteria represent a pool of 2.12 million tons of alkanes in the oceans (Table 1). Although total population sizes of *Prochlorococcus* and *Synechococcus* remain largely stable on an annual timescale, their turnover rates are high. *Prochlorococcus* divide once every 1–2 d (23–25), with cellular losses balancing division in a quasi-steady-state manner. Cyanobacterial mortality can be mediated by a variety of factors, including predation by grazers or viruses, UV-induced lysis, or spontaneous cell death (18), resulting in release of organic carbon compounds, including alkanes, into the environment. At these cellular turnover rates, production of alkanes by *Prochlorococcus* globally is estimated to be between 269 and 539 million tons per annum (Table 1).

**Table 1. Hydrocarbon production by *Prochlorococcus* and *Synechococcus***

Strain	Pentadecane, fg per cell	Heptadecane, fg per cell	8-heptadecene, fg per cell	Total hydrocarbons, fg per cell	Total hydrocarbons, % dry cell weight	Hydrocarbons in global cellular population, million tons	Hydrocarbon production, million tons per year
<i>Prochlorococcus</i> str. CCMP1986	0.448 ± 0.223	0.018 ± 0.009	n.d.	0.466 ± 0.232	0.368 ± 0.126		
<i>Prochlorococcus</i> str. MIT9312	0.337 ± 0.343	0.013 ± 0.012	n.d.	0.350 ± 0.355	0.181 ± 0.187		
<i>Prochlorococcus</i> str. MIT9313	0.685 ± 0.548	0.026 ± 0.014	n.d.	0.711 ± 0.562	0.149 ± 0.080		
Mean <i>Prochlorococcus</i>				0.509 ± 0.383		1.48	269–539
<i>Synechococcus</i> sp. WH5701	0.096 ± 0.018	1.170 ± 0.211	1.314 ± 0.225	2.580 ± 0.454	0.138 ± 0.009		
<i>Synechococcus</i> sp. WH7803	0.396 ± 0.136	n.d.	0.035 ± 0.001	0.431 ± 0.137	0.033 ± 0.008		
<i>Synechococcus</i> sp. WH7805	0.248 ± 0.065	n.d.	0.065 ± 0.009	0.313 ± 0.074	0.024 ± 0.005		
<i>Synechococcus</i> sp. WH8102	0.261 ± 0.047	n.d.	0.043 ± 0.005	0.304 ± 0.052	0.022 ± 0.002		
Mean <i>Synechococcus</i>				0.907 ± 0.179		0.635	39–232

Cellular hydrocarbon amounts were quantified by GC-MS. Measurements are from three biological replicates. SD is indicated. Total hydrocarbons per cell were quantified by dividing the total hydrocarbon mass by the total number of cells in the pellet (Table S2). The amount of hydrocarbons as a percentage of dry cell weight was quantified by dividing the total hydrocarbon mass by the pellet cell mass (Table S2). The average mass of hydrocarbons per cell was used to quantify hydrocarbon amounts in global ocean cell populations given global population size estimates of  $2.9 \pm 0.1 \times 10^{27}$  *Prochlorococcus* and  $7.0 \pm 0.3 \times 10^{26}$  *Synechococcus* cells (14). This total and the turnover rate were used to quantify the annual production amount. n.d., not detected.



**Fig. 1.** Hydrocarbon-degrading bacteria can grow on both oil and heptadecane. Growth of (A) *Alcanivorax borkumensis* SK2, (B) *Acinetobacter baylyi* ADP1, and (C) *Marinobacter aquaolei* VT8 in ASW medium (triangles), ASW medium with heptadecane (7.77 mg/mL) (squares), and ASW medium with oil (circles). Values represent the mean and SD from three biological replicates. The growth rate constants ( $\mu$ ) on oil and heptadecane were, respectively, 0.12 and 0.12  $\text{h}^{-1}$  for *Alcanivorax*, 0.019 and 0.006  $\text{h}^{-1}$  for *Acinetobacter*, and 0.010 and 0.004  $\text{h}^{-1}$  for *Marinobacter*.

*Synechococcus* maximum growth rates are comparable to or slighter faster than those of *Prochlorococcus* (25, 26), with similar mechanisms of mortality (27, 28). However, estimates of in situ specific growth rates are broader, between 1–6 d (29, 30), due to the varying environments *Synechococcus* occupies. Therefore, total annual production of hydrocarbons by *Synechococcus* could vary between 39 and 232 million tons per annum (Table 1).

Natural oil seepage from within marine sediments is estimated to be between 0.2 and 2 million tons per annum and is geographically concentrated at the continental margins (1). An equivalent release of hydrocarbons into the ocean is thought to result from human activities such as oil leakage from drilling rigs and shipping vessels (1). Localized events such as the *Deepwater Horizon* oil spill, which released  $\sim 0.435$  million tons of oil (7), can further increase anthropogenic hydrocarbon inputs into the marine environment. In contrast, the 308–771 million tons of hydrocarbons produced annually by *Prochlorococcus* and *Synechococcus* significantly exceeds the inputs from these other natural and anthropogenic sources and, due to the broad distribution of cyanobacteria across the world's surface oceans, is more widespread (14). As mentioned above, measurements of pentadecane and heptadecane in marine surface waters indicate that they are present only at femtogram- to picogram-per-mL levels (2, 4, 31). From our analyses, we estimate that the amounts of hydrocarbons produced by marine cyanobacteria to be on the order of 2–540  $\text{pg}\cdot\text{mL}^{-1}\cdot\text{d}^{-1}$  (*Materials and Methods*), indicating that the majority of pentadecane and heptadecane in surface waters may derive from cyanobacteria and that these compounds do not accumulate significantly; hence, the continuous production of cyanobacterial hydrocarbons must be balanced by degradation or loss. Some hydrocarbon exchange occurs between the ocean and the atmosphere (2, 32), but not at rates sufficient to balance estimated cyanobacterial production rates. Given that the temperature-dependent half-life of pentadecane and heptadecane is between 0.8 and 5 d at surface ocean temperatures, a significant fraction of the hydrocarbons will likely escape these abiotic loss pathways (31). Therefore, biological degradation is likely to be responsible for the majority of turnover (31).

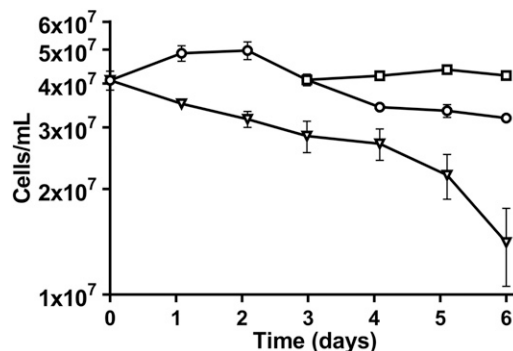
**Both Obligate and Facultative Hydrocarbon-Degrading Bacteria Can Metabolize Cyanobacterial Alkanes.** Alkane degradation pathways have not been identified in grazers of cyanobacteria (33). Therefore, the majority of cyanobacterially produced hydrocarbons are likely to be released into the environment and subsequently degraded by bacteria (34). Obligate hydrocarbon-degrading bacteria, including *Cycloclasticus*, *Thalassolituus*, *Oleiphilus*, *Oleispira*, and *Alcanivorax* species, have been isolated from geographically diverse coastal and open-ocean regions in all seas, in sediments and both surface and deep waters, including areas with minimal oil pollution (9–11, 35). *Alcanivorax* species are typically among the dominant bacteria found metabolizing crude oil during large spill events (34–

38). Other bacteria, including *Marinobacter*, *Pseudomonas*, and *Acinetobacter* species, which can use hydrocarbons in addition to a broader range of carbon sources, have also been detected in oil-polluted ocean samples (10, 34).

To determine whether cyanobacterial alkanes can support the growth of both obligate as well as nonobligate hydrocarbon-degrading bacteria, we tested the ability of three axenic and well-characterized oil degraders (5, 8) to grow on either heptadecane or crude oil: *Alcanivorax borkumensis* SK2, an obligate hydrocarbon-degrading bacterium (39), and *Acinetobacter baylyi* ADP1 and *Marinobacter aquaolei* VT8, two strains that can facultatively grow on crude oil. We observed that all three strains could grow in media containing either oil or heptadecane as the only source of reduced carbon (Fig. 1). These findings suggest that diverse groups of marine hydrocarbon degraders may contribute to the degradation of the hydrocarbons produced by *Prochlorococcus* and *Synechococcus*.

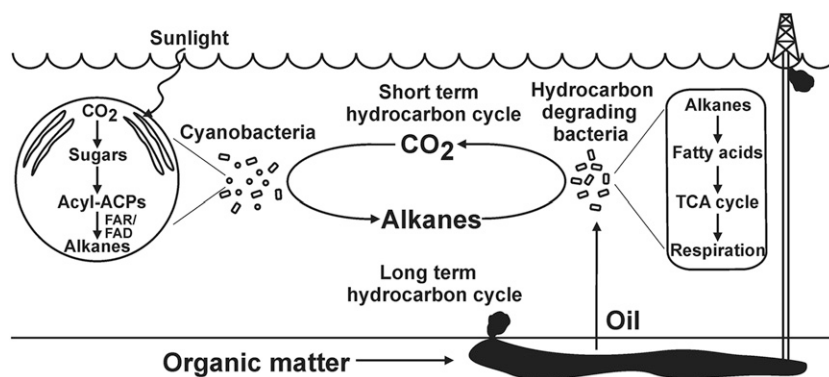
#### Cyanobacterial Hydrocarbon Production Can Support Populations of

***A. borkumensis* SK2.** *A. borkumensis* SK2 has previously been demonstrated to use a broad range of alkanes, with similar growth rates observed when either pentadecane or heptadecane was added as the sole carbon source (40). However, in both this study and the previous report (40) an excess of alkanes, similar to concentrations in oil spills, was used (41). Whether cyanobacterial alkane production is sufficient to support hydrocarbon-degrading



**Fig. 2.** Marine heptadecane concentrations support the growth of *A. borkumensis* SK2. Growth of *A. borkumensis* SK2 was quantified in ASW medium (triangles) or ASW medium supplemented with 40  $\mu\text{g}/\text{mL}$  heptadecane (squares and circles). The cultures containing heptadecane were split and an additional 40  $\mu\text{g}/\text{mL}$  heptadecane added to one set at day 3 (squares). Results are from three biological replicates. SD is indicated. Control cultures containing only ASW medium or ASW medium and heptadecane (i.e., without added bacteria) showed no evidence of cell contamination.





**Fig. 3.** The hydrocarbon cycle. A schematic representation of the short-term hydrocarbon cycle, which occurs over days, and the long-term hydrocarbon cycle, which takes place over thousands to millions of years. A simplified process showing the conversion of CO<sub>2</sub> to alkanes using energy derived from photosynthesis is detailed in the enlarged cyanobacterial cell on the left. A simplified process showing the metabolism of alkanes is detailed in the enlarged hydrocarbon degrading bacterial cell on the right.

bacterial populations in minimally polluted seawater has not been determined.

Information on population sizes of specific hydrocarbon degraders in the environment is limited, with estimates of *Alcanivorax* varying between  $10^1$  and  $5 \times 10^3$  cells/mL (35, 41). However, in most studies *Alcanivorax* cell numbers are below the detection limit (34), as assayed via quantitative PCR (5, 6) or in situ hybridization (36), and their population dynamics in the oligotrophic ocean are unknown. To determine the yield of *A. borkumensis* SK2 on cyanobacterial alkanes, cells were grown in artificial sea water (ASW) medium either without any organic carbon or containing only the minimum amount of heptadecane that could be added to the culture. A small increase in cell number was observed in cultures containing heptadecane, before declining to the original cell number by day 3 (Fig. 2). The addition of heptadecane to this culture sustained cell numbers for a further 3 d, although a smaller increase in population size was observed compared with the initial growth period, suggesting that some other component of the medium became limiting. Based on these data, we find that the yield of *A. borkumensis* SK2 is 0.1 pg dry cell weight per pg heptadecane (*Materials and Methods*)—lower than other yields previously reported for other hydrocarbon-degrading bacteria, which range from 0.5 to 1.77 pg/pg hydrocarbon (42). Thus, we expect that a marine cyanobacterial hydrocarbon production rate of  $2\text{--}540 \text{ pg}^{-1}\cdot\text{mL}\cdot\text{d}^{-1}$  could support a population of  $1.5 \times 10^1$  to  $4.1 \times 10^3$  *A. borkumensis* SK2 cells per mL if used as the only source of reduced carbon, comparable to the population of obligate hydrocarbon-degrading bacteria observed in natural environments.

In the cultures to which no heptadecane was added, cell numbers decreased significantly over the course of the experiment. This suggests that populations of *Alcanivorax*, and possibly other obligate hydrocarbon-degrading bacteria in the oceans, may be unable to persist for long in the absence of a constant hydrocarbon supply. Given the widespread distribution of both *Prochlorococcus* and marine *Synechococcus*, it is highly likely that hydrocarbon production is continuous and therefore hydrocarbon-degrading bacteria are constantly supplied with a fresh source of alkanes. Although pyruvate is present in the oceans (43, 44), and *A. borkumensis* SK2 can use it as an energy and carbon source (40), it likely faces greater competition from other heterotrophs for pyruvate than for alkanes (43, 44). Other compounds, predominantly hydrocarbons but possibly other unidentified substrates, may also contribute to the pool available to sustain obligate hydrocarbon-degrading species. These include pristane, a C<sub>19</sub> saturated terpenoid alkane produced in significant quantities by some algal species (45), which *A. borkumensis* SK2 can use (10), and a branched C<sub>21</sub> alkane produced by some algae (45).

**The Ocean Hydrocarbon Cycle.** Catabolic degradation of hydrocarbons by marine bacteria produces significant amounts of CO<sub>2</sub>, which can be incorporated back into alkanes within cyanobacteria (33). Based on our estimates of cyanobacterial alkane production rates and observed concentrations in the ocean, we expect that this “short-term hydrocarbon cycle” (46) occurs on the order of days (Fig. 3). Given the significant contribution of *Prochlorococcus* and *Synechococcus* to marine carbon flux, the levels of hydrocarbon production we have measured indicate that there must be a notable biogeochemical cycle of these compounds in the world’s upper oceans. In terms of annual hydrocarbon fluxes within the ocean, the short-term hydrocarbon cycle occurs at rates several orders of magnitude greater than the “long-term hydrocarbon cycle,” whereby organic matter is converted to oil in sediments over a period of thousands to millions of years before release into the marine environment by natural seepage or human activity and subsequent breakdown.

The short- and long-term hydrocarbon cycles are linked in that hydrocarbon-degrading bacteria are responsible for removing both cyanobacterial alkanes and crude oil from the environment. Upon release of crude oil, a significant increase in hydrocarbon-degrading bacteria is observed (5–7, 41). Crude oil is a complex mixture of ~20,000 compounds broadly grouped into four categories: saturated hydrocarbons, predominantly C<sub>5</sub>–C<sub>40</sub> alkanes (40–60%), aromatic hydrocarbons (20–40%), resins (5–20%), and asphaltenes (1–10%) (8, 34). It is notable that after release of crude oil into the environment, saturated hydrocarbons are the first to be degraded by bacteria (7, 9, 34). This suggests that populations of bacteria, which normally degrade cyanobacterial alkanes and other hydrocarbons derived from biological sources, quickly acclimate to metabolize crude oil hydrocarbons, followed by rapid population expansion to use this new energy source.

Although cyanobacterial hydrocarbons are only a small proportion (0.00032%) of the estimated 662 billion tons of dissolved organic carbon (DOC) present in the ocean at any point in time (47), only a relatively small fraction of this bulk DOC (~0.2 billion tons) is turned over within days (48). Cyanobacterial hydrocarbons, with an estimated pool of 2.12 million tons, likely belong to the labile subset of rapidly cycled DOC and constitute a notable proportion (~1%) of that bioavailable fraction. The short-term hydrocarbon cycle should therefore be considered a component of the “microbial loop” within marine food webs (49, 50).

## Conclusions

This study sets a minimum estimate for the total amount of hydrocarbons produced by cyanobacteria in marine environments.

Other abundant marine cyanobacteria, notably *Trichodesmium* species (51), may add significantly to hydrocarbon output, as might some algal species. Although population sizes of freshwater and terrestrial cyanobacteria are not well constrained, given that these organisms are also capable of producing hydrocarbons (19), alkane and alkene production is likely to be considerable here as well. The widespread distribution of hydrocarbon-degrading bacteria in freshwater, marine, and terrestrial environments suggests that the short-term hydrocarbon cycle is pervasive in many natural ecosystems (9, 11).

## Materials and Methods

**Bioinformatics.** FASTA BLAST comparisons (52) were performed using inferred protein sequences for *Synechocystis* sp. PCC6803 *sll0209* (FAR) and *sll0208* (FAD) and *Synechococcus* sp. PCC7002 *Syn7002\_A1173* (Ols) (WVP\_012306795) with the 115 completed cyanobacterial genomes listed in the NCBI database ([www.ncbi.nlm.nih.gov/genome/browse/](http://www.ncbi.nlm.nih.gov/genome/browse/)) and Biller et al. (20). *Synechococcus* sp. PCC7002 was not classified as a marine species because it was isolated from mud flats in Puerto Rico, is evolutionarily distant from other *Synechococcus* species (16, 22), and has not been detected in the open ocean.

**Bacterial Strains, Media, and Growth Conditions.** Axenic cultures of *Prochlorococcus* CCMP1986 (MED4), MIT9312, and MIT9313 were used. Cultures were routinely assessed for purity by confirming a lack of turbidity after inoculation into three different purity test broths (53). Triplicate 2-L cultures of each strain were grown in Pro99 medium (54) prepared with 0.2  $\mu\text{m}$  filtered, autoclaved seawater (collected from Vineyard Sound, MA) and supplemented with 10 mM filter-sterilized sodium bicarbonate upon inoculation. Cells were grown under constant light flux (30–40  $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for CCMP1986 and MIT9312; 10–20  $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for MIT9313) at 24 °C, in acid-washed polycarbonate containers. Growth was monitored by measuring bulk culture fluorescence using a 10-AU fluorometer (Turner Designs).

All *Synechococcus* species were grown in ASW medium (55). *Synechococcus* sp. WH5701 was cultured in conical flasks at 30 °C and 40  $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  with shaking at 160 rpm. *Synechococcus* spp. WH7803, WH7805, and WH8102 were cultured in culture flasks at 24 °C and 40  $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  without shaking. Cultures were routinely assessed for purity by plating aliquots on ASW solid medium containing 0.8% wt/vol yeast extract and 1.5% wt/vol agar.

All hydrocarbon degrading bacteria were cultured at 30 °C with shaking at 160 rpm. *Acinetobacter baylyi* ADP1 (56) was routinely grown in BHI medium. *Alcanivorax borkumensis* SK2 (39) was routinely grown in *A. borkumensis* medium 809 (DSMZ). *Marinobacter aquaolei* VT8 (57) was routinely grown in *Marinobacter* medium 970 (DSMZ). To test growth on hydrocarbons cultures were grown to stationary phase and washed twice with ASW medium before being cultured in 10 mL ASW medium with no carbon source or with either 1% (vol/vol) crude oil (Nigerian bonny light crude oil) or filter-sterilized, analytical standard-grade ( $\geq 99.5\%$ ) heptadecane (100  $\mu\text{L}$  equivalent to 77.7 mg) (Sigma). Samples of ASW medium, ASW medium and crude oil, and ASW medium and heptadecane were also set up as negative controls. Growth was determined by measuring the optical density at 600 nm, and specific growth rate constants ( $\mu$ ) were calculated during exponential phase (21–45 h for *A. borkumensis*, 21–71 h for *A. baylyi*, and 21–138 h for *M. aquaolei*). Three biological replicates of each sample were measured. Cultures were routinely assessed for purity by plating aliquots on ASW solid medium containing 0.8% wt/vol yeast extract and 1.5% wt/vol agar and on either BHI medium, *A. borkumensis* medium 809, or *Marinobacter* medium 970 with 1.5% wt/vol agar for *A. baylyi* ADP1, *A. borkumensis* SK2, and *M. aquaolei* VT8 cultures, respectively.

For growth of *A. borkumensis* SK2 in 40  $\mu\text{g/mL}$  heptadecane,  $4 \times 10^7$  cells per mL were inoculated so that hydrocarbon amounts were equivalent to 1 pg of heptadecane per cell at the time of inoculation. This was necessary because a heptadecane concentration of 40  $\mu\text{g/mL}$ , equivalent to 0.5  $\mu\text{L}$  per 10 mL, was the minimum amount that could be used in this experiment, without diluting heptadecane in another similar solvent. These could be either used by *A. borkumensis* SK2 as an energy and carbon source or demonstrate high cellular toxicity or growth inhibition, which has been shown with alkanols (40). The higher inoculum population size was also essential to accurately count cell numbers using a Beckman Coulter Z2 particle counter. Three biological replicates of each sample were measured.

The dry cell weight of *A. borkumensis* SK2 was determined by culturing three biological replicates in ASW medium and heptadecane (100  $\mu\text{L}$  in 10 mL) to late exponential phase. Samples were washed three times with water and cell counts per milliliter determined using a Beckman Coulter Z2 particle counter. The dry cell weight was determined by first washing 10 mL of culture three times with water, filtering the solution via Whatman glass microfiber filters (GE

Healthcare), and drying the samples before measurement. Dry cell weights were recorded using an Adam PW 214 analytical balance. A mass of  $0.39 \pm 0.042$  pg per *A. borkumensis* SK2 cell was calculated via dividing the dry cell weight by the cell count. To calculate the growth yield the increase in cell population of  $1.01 \times 10^7$  observed in the first 2 d in media containing 40  $\mu\text{g/mL}$  of heptadecane was multiplied by the average mass of a cell. This amount was then divided by the mass of heptadecane per mL, resulting in a growth yield of 0.1 pg dry cell weight per pg of heptadecane.

**Cell Counting and Sample Preparation for GC-MS Analysis.** *Prochlorococcus* cultures were harvested in exponential phase by centrifugation at  $15,000 \times g$  for 15 min in a JLA-8.1000 rotor (Beckman Coulter) at 4 °C. Pellets were resuspended in fresh Pro99 medium and transferred into tared glass vials and lyophilized for 48 h. Total *Prochlorococcus* cells in each sample were enumerated by flow cytometry using an Influx Cell Sorter (BD Biosciences) as previously described (58, 59).

*Synechococcus* samples were harvested in exponential phase (Table S1). An aliquot was removed and cells counted by flow cytometry using a BD FACScan. Cell counting was performed by running 12  $\mu\text{L}$  of sample per minute through the FACS device until 100,000 particles were counted. The background particle count from the media ( $\sim 200$  counts per second) was subtracted from the total count. Cell counts were performed in triplicate for each sample. Fifteen milliliters of each sample was centrifuged at  $2,800 \times g$ , washed twice with water, and freeze-dried using a ScanVac CoolSafe Freeze Dryer. Dry cell weights were recorded using an Adam PW 214 analytical balance.

**Extraction and Analysis of Total Hydrocarbons.** All chemicals were purchased from Sigma chemicals. To extract total hydrocarbons, dichloromethane (1.5 mL for *Prochlorococcus* and 1 mL for *Synechococcus*) was added to pelleted dried cells in glass vials. Samples were placed in a sonicator bath for 30 min then centrifuged (GeneVac EZ-2; SP Scientific) for 10 min ( $1,800 \times g$ ) to pellet any remaining material. The supernatant was transferred to a glass GC sample vial and stored at  $-80$  °C. The pellets were extracted twice more in dichloromethane as above to ensure complete extraction of hydrocarbons (80–90% of hydrocarbons were extracted in the first extraction). For negative controls, extraction blanks were carried out on growth media without cyanobacteria and positive controls consisted of adding 1 mg/mL standard alkane mix (C8–C20 alkane mix; Sigma) to the extraction procedure. Three biological replicates of each sample were analyzed.

**Identification and Quantification of Hydrocarbons.** Hydrocarbons were identified by GC-MS (Thermo Scientific Trace GC 1310–ISQ LT Single Quadrupole EI MS, A1-1310 Auto sampler) with a Thermo TG-SQC GC column (15 m  $\times$  0.25 mm, 0.25- $\mu\text{m}$  film thickness). The injection volume was 1  $\mu\text{L}$  with a 10:1 split ratio with an injector temperature of 230 °C, using helium as a carrier gas at a constant flow of 1.2 mL $\cdot\text{min}^{-1}$ . The following gradient was used: initial oven temperature 30 °C, 2 min; 150 °C at 15 °C $\cdot\text{min}^{-1}$ ; 230 °C at 3.4 °C $\cdot\text{min}^{-1}$ . A transfer line temperature of 240 °C was used. The MS conditions in the positive mode were as follows: ion source, 250 °C; mass range 45–650 Da; scan time of 0.35 s. Pentadecane and heptadecane were identified by co-retention with standards and NIST mass spectral search libraries (National Institute of Standards and Technology NIST v2.0); two peaks were identified as 8-heptadecene using the NIST library alone. Pentadecane and heptadecane were quantified using standard curves derived from peak areas of pentadecane and heptadecane alkane standards (0.06–31  $\mu\text{g/mL}$ ) and 8-heptadecene was quantified using peak areas derived from heptadecane standards (0.06–31  $\mu\text{g/mL}$ ). Any background signal in the extraction blank was subtracted when determining hydrocarbon amounts.

**Calculations.** Amounts of hydrocarbons produced by *Prochlorococcus* and *Synechococcus* per mL of seawater were calculated by multiplying the numbers in the ocean, which range from  $3 \times 10^3$  to  $1 \times 10^6$  cells per mL and  $3 \times 10^3$  to  $5 \times 10^4$  cells per mL, respectively (14), at the sea surface, by the average amount of hydrocarbons per cell (Table 1). This equates to 1.5–509  $\mu\text{g/mL}$  of hydrocarbons produced every 1–2 d by *Prochlorococcus* and 1.9–31.7  $\mu\text{g/mL}$  of hydrocarbons produced every 1–6 d by *Synechococcus*.

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