Contribution of cyanobacterial alkane production to the ocean hydrocarbon cycle

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Cyanobacterial hydrocarbon production in oceans

Biological Sciences: Environmental Sciences

Abstract

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 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 to 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/mL/day, which translates into a global ocean yield of approximately 308-771 million tonnes 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.

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 are ubiquitous in the oceans, where natural seepage and human activities are estimated to release between 0.4 and 4.0 million tonnes 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, from 1 to 60 fg/mL, are associated with particulate matter >0.7 μm in diameter (4). Larger amounts may be associated with particulate matter <0.7 μm in diameter, since ocean concentrations are higher than the solubility of pentadecane and heptadecane, which is approximately 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). Since 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 $2.9\pm0.1\times10^{27}$ and $7.0\pm0.3\times10^{26}$ 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). Whilst 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

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up to 200 meters (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 hydrocarbons have a key, as yet unidentified function in cyanobacteria which 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 *Leptolyngbya* sp. PCC7376 and *Moorea producens* 3L, which encode Ols homologues, all of the other marine cyanobacteria including *Cyanobium*, *Acaryochloris*, *Crocosphaera*, *Trichodesmium*, *Lyngbya*, *Oscillatoria*, *Nodularia* and *Microcoleus* species encode FAR/FAD homologues (15, 19). Thus it is likely that alkanes are the predominant hydrocarbons released by cyanobacteria into the marine environment. Homologues 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 to 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, approximately 96% of the total, with the remainder consisting of heptadecane (Table 1, 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 to 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, Fig. S1). The remainder consisted of 8-heptadecene. The hydrocarbon composition of *Prochlorococcus* and marine *Synechococcus* species differs from 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 to 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 tonnes of alkanes in the oceans (Table 1). While 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 days (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 rate of alkanes by *Prochlorococcus* globally is estimated to be between 269 and 539 million tonnes per annum (Table 1). *Synechococcus* maximum growth rates are comparable or slighter faster than *Prochlorococcus* (25, 26), with similar

mechanisms of mortality (27, 28). However, estimates of *in situ* specific growth rates are broader, between 1 to 6 days (29, 30), due to the varying environments *Synechococcus* occupies. Therefore total annual production of hydrocarbons by *Synechococcus* could vary between 39 and 232 million tonnes per annum (Table 1).

Natural oil seepage from within marine sediments is estimated to be between 0.2-2 million tonnes 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 approximately 0.435 million tonnes of oil (7), can further increase anthropogenic hydrocarbon inputs into the marine environment. In contrast, the 308-771 million tonnes 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 fg/mL – pg/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 pg/mL/day (see 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 days 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 amongst the dominant bacteria found metabolizing crude oil during large spill events (34-38). Other bacteria, including *Marinobacter*, *Pseudomonas* and *Acinetobacter* species, which can utilise 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 non-obligate 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 *Alcanivorax borkumensis* SK2. *Alcanivorax borkumensis* SK2 has previously been demonstrated to utilize 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 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 10^1-5×10^3 cells/mL (35, 41). However, in most studies *Alcanivorax* cell numbers are below the detection limit (34), as assayed via qPCR (5, 6) or *in situ* hybridization (36), and their population dynamics in the oligotrophic ocean are unknown. In order to determine the yield of *Alcanivorax borkumensis* SK2 on cyanobacterial alkanes, cells were grown in 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 three (Fig. 2). The addition of heptadecane to this culture sustained cell numbers for a further three days, although a smaller increase in population size was observed compared to the initial growth period, suggesting

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that some other component of the medium became limiting. Based on these data, we find that the yield of *Alcanivorax borkumensis* SK2 is 0.1 pg dry cell weight/pg heptadecane (see methods) – lower than other yields previously reported for other hydrocarbon degrading bacteria, which range from 0.5-1.77 pg/pg hydrocarbon (42). Thus, we expect that a marine cyanobacterial hydrocarbon production rate of 2 - 540 pg/mL/day could support a population of 1.5×10^1 - 4.1×10^3 *Alcanivorax borkumensis* SK2 cells/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. While pyruvate is present in the oceans (43, 44), and *Alcanivorax borkumensis* SK2 can utilize 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 C19 saturated terpenoid alkane produced in significant quantities by some algal species (45), which *Alcanivorax borkumensis* SK2 can utilize (10), and a branched C21 alkane produced by some algae (45).

The ocean hydrocarbon cycle. Catabolic degradation of hydrocarbons by marine bacteria produces significant amounts of CO_2, 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 C5-C40 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 utilize this new energy source.

Although cyanobacterial hydrocarbons are only a small proportion (0.00032%) of the estimated 662 billion tonnes 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 tonnes) is turned over within days (48). Cyanobacterial hydrocarbons, with an estimated pool of 2.12 million tonnes, 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 algae 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), *sll0208* (FAD)*,* and *Synechococcus* sp. PCC7002 Syn7002_A1173 (Ols) (WP_012306795) with the 115 completed cyanobacterial genomes listed in the NCBI database (http://www.ncbi.nlm.nih.gov/genome/browse/) and Biller et al, 2014 (20). *Synechococcus* sp. PCC7002 was not classified as a marine species since 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 μm 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 μmol photons m^{-2} s^{-1} for CCMP1986 and MIT9312; 10 – 20 μmol photons m^{-2} 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 μmol photons m^{-2} s^{-1} with shaking at 160 rpm. *Synechococcus* sp. WH7803, WH7805 and WH8102 were cultured in culture flasks at 24°C and 40 μmol photons m^{-2} s^{-1} without shaking. Cultures were routinely assessed for purity by plating aliquots on ASW solid medium containing 0.8% w/v yeast extract and 1.5% w/v 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 *Alcanivorax 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, washed twice with ASW medium before being cultured in 10 mL ASW medium with no carbon source or with either 1% (v/v) crude oil (Nigerian bonny light crude oil) or filter sterilized, analytical standard (≥99.5%) grade heptadecane (100 μL 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 (µ) were calculated during exponential phase (21-45 hours for *Alcanivorax borkumensis,* 21-71 hours for *Acinetobacter baylyi*, and 21-138 hours for *Marinobacter 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% w/v yeast extract and 1.5% w/v agar and on either BHI medium, *Alcanivorax borkumensis* medium 809 or *Marinobacter* medium 970 with 1.5% w/v agar for *Acinetobacter baylyi* ADP1, *Alcanivorax borkumensis* SK2 and *Marinobacter aquaolei* VT8 cultures, respectively.

For growth of *Alcanivorax borkumensis* SK2 in 40 µg/mL heptadecane, 4×10^7 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 µg/mL, equivalent to 0.5 µL 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 utilized by *Alcanivorax 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 2Z particle counter. Three biological replicates of each sample were measured.

The dry cell weight of *Alcanivorax borkumensis* SK2 was determined by culturing three biological replicates in ASW medium and heptadecane (100 µL in 10 mL) to late exponential phase. Samples were washed three times with water and cell counts per mL determined using a Beckman Coulter 2Z 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 prior to measurement. Dry cell weights were recorded using an Adam PW 214 analytical balance. A mass of 0.39 ± 0.042 pg per *Alcanivorax borkumensis* SK2 cell was calculated via dividing the dry cell weight by the cell count. In order to calculate the growth yield the increase in cell population of 1.01×10^7 observed in the first two days in media containing 40 µg/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/pg of heptadecane.

Cell counting and sample preparation for GC-MS analysis. *Prochlorococcus* cultures were harvested in exponential phase by centrifugation at 15,000 x *g* for 15 minutes 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 hours. 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 µL of sample per minute through the FACS device until 100,000 particles were counted. The background particle count from the media (~200 counts per second) was subtracted from the total count. Cell counts were performed in triplicate for each sample. 15 mL of each sample was centrifuged at 5000 rpm, 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 minutes, then centrifuged (GeneVac EZ-2, SP Scientific, Ipswich, UK) for 10 minutes (2150 rpm) to pellet any remaining material. The supernatant was transferred to a glass GC sample vial and stored at -80ºC. 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 (Sigma C8-C20 Alkane mix) 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 Quadruple EI MS, A1-1310 Auto sampler) with a Thermo TG-SQC GC column (15 m x 0.25 mm, 0.25 µm film thickness). The injection volume was 1 µL 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 min^{-1}. The following gradient was used: initial oven temperature 30 °C, 2 min; 150 °C at 15 °C min^{-1}; 230 °C at 3.4 °C min^{-1}. A transfer line temperature of 240 °C was used. The mass spectrometry conditions in the

positive mode were: ion source, 250 ºC; mass range 45-650 Da; scan time of 0.35 seconds. 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 µg/mL), 8-heptadecene was quantified using peak areas derived from heptadecane standards (0.06 - 31 µg/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 3×10^3 to 1×10^6 cells/mL and 3×10^3 to 5×10^4 cells/mL, respectively (14), at the sea surface, by the average amount of hydrocarbons per cell (Table 1). This equates to 1.5-509 pg/mL of hydrocarbons produced every 1-2 days by *Prochlorococcus* and 1.9-31.7 pg/mL of hydrocarbons produced every 1-6 days by *Synechococcus*.

Acknowledgements

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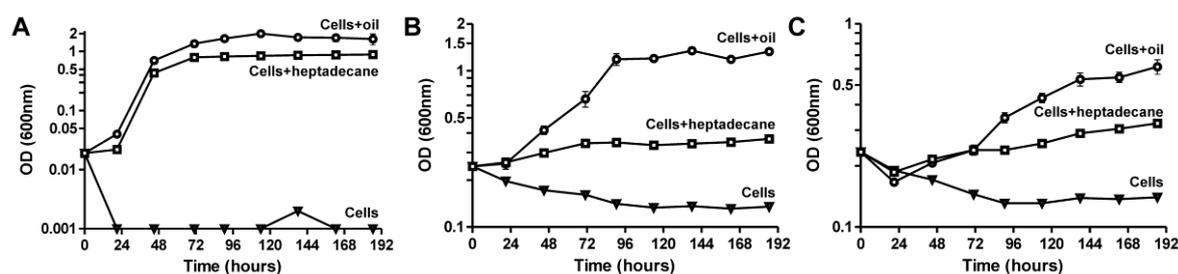
Figures

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 (open triangles), ASW medium with heptadecane (7.77 mg/mL) (open squares), and ASW medium with oil (open circles). Values represent the mean and SD from three biological replicates. The growth rate constants (μ) on oil and heptadecane were, respectively, 0.12 and 0.12 hr^{-1} for *Alcanivorax*, 0.019 and 0.006 hr^{-1} for *Acinetobacter*, and 0.010 and 0.004 hr^{-1} for *Marinobacter*.

Fig. 2: Marine heptadecane concentrations support the growth of *Alcanivorax borkumensis* SK2. Growth of *Alcanivorax borkumensis* SK2 was quantified in ASW medium (open triangles) or ASW medium supplemented with 40 µg/mL heptadecane (open squares, open circles). The cultures containing heptadecane were split and an additional 40 µg/mL heptadecane added to one set at day 3 (open 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.
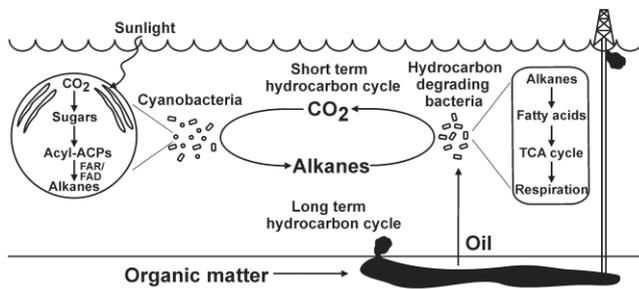
Fig. 3: The hydrocarbon cycle. A schematic representation of the 'short term hydrocarbon cycle', which occurs over days, and the 'long term hydrocarbon cycle' which takes place over thousands to millions of years. A simplified process showing the conversion of CO_2 to alkanes using energy derived from 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.

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

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

Cellular hydrocarbon amounts were quantified by GC-MS. Measurements are from three biological replicates. S.d. is indicated. n.d.- not detected. 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.
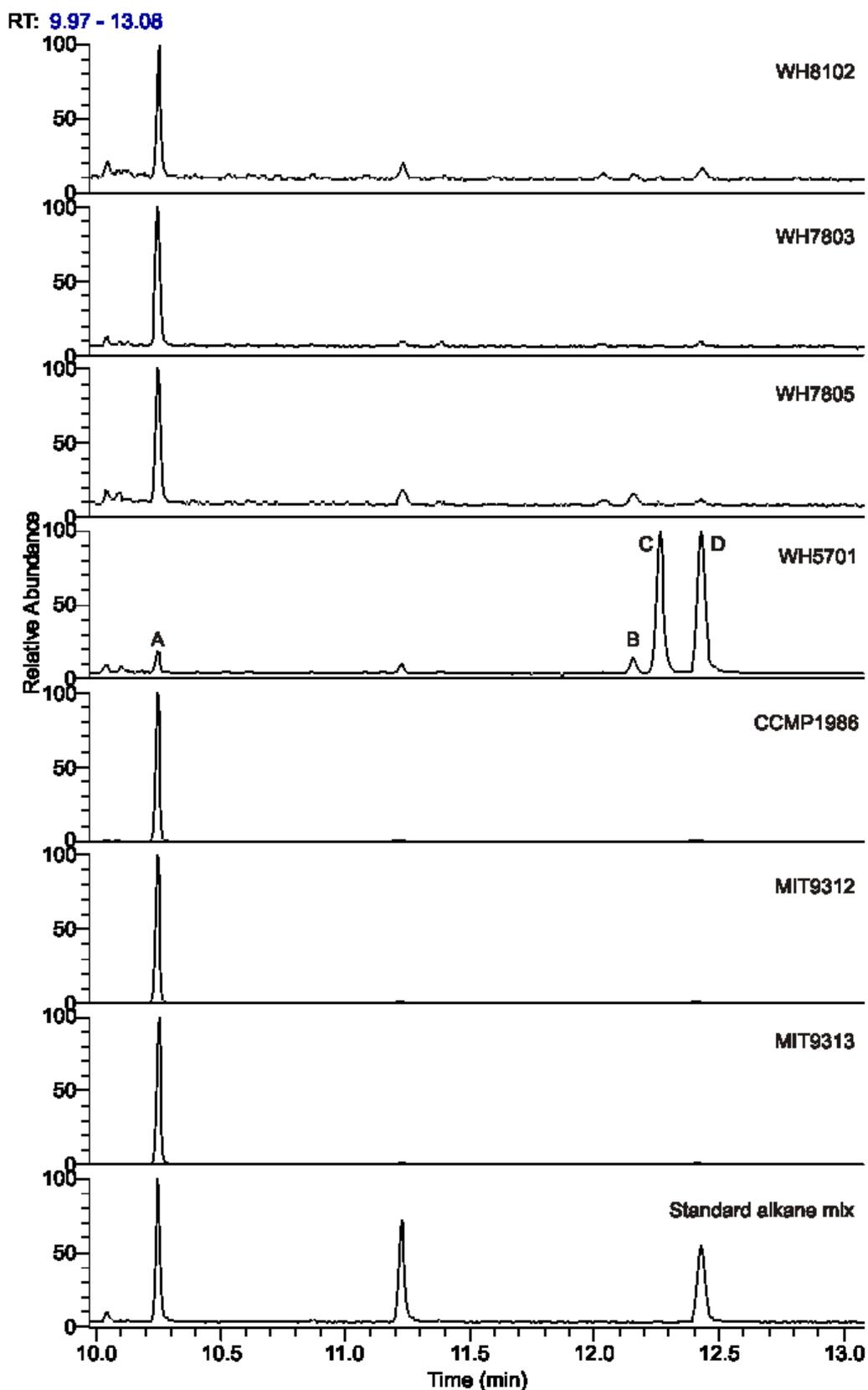
Fig. S1. Chromatograms showing separation of hydrocarbons extracted from *Synechococcus* species (WH8103, WH7803, WH7805, WH5701) and *Prochlorococcus* species (CCMP1986,

MIT9312, MIT9313) by gas chromatography – mass spectrometry (Thermo Scientific Trace GC 1310 – ISQ LT Single Quadruple EI MS, A1-1310 Autosampler) using a Thermo TG-SQC GC column (15 m x 0.25 mm, 0.25 µm film thickness). Peaks were identified as A (10.25 minutes) pentadecane; B, (12.16 minutes) 8-heptadecene; C, (12.26 minutes) 8-heptadecene and D, (12.45 minutes) heptadecane. Samples are shown from a single replicate. The chemical identity of the peak at 11.22 minutes in the *Synechococcus* species could not be resolved by mass spectrometry so was excluded from further analysis.

Table S1. Hydrocarbon pathways in sequenced cyanobacterial strains. The *Synechocystis* sp. PCC 6803 Fad/Far and *Synechococcus* sp. PCC 7002 Ols genes were subjected to BLAST analysis against 115 sequenced cyanobacterial genomes. The positive values of these BLAST results are listed. Only matches greater than 30% identity over the length of the query sequence are shown.

Strain	*fad*	*far*	*ols*
Acaryochloris marina MBIC11017	292/337(86%)	191/230(83%)	
Anabaena cylindrica PCC7122	301/338(89%)	197/223(88%)	
Anabaena sp. 90	297/338(87%)	196/223(87%)	
Anabaena variabilis ATCC29413	305/338(90%)	204/230(88%)	
Arthrospira maxima CS-328	295/338(87%)	206/231(89%)	
Arthrospira platensis NIES-39	295/338(87%)	206/231(89%)	
Calothrix sp. PCC6303	298/338(88%)	202/228(88%)	
Calothrix sp. PCC7507	299/338(88%)	202/228(88%)	
Candidatus atelocyanobacterium thalassa	296/338(87%)	198/228(86%)	
Chamaesiphon minutus PCC6605	299/340(87%)	193/230(83%)	
Chroococcidiopsis thermalis PCC7203	301/339(88%)	200/228(87%)	
Coleofasciculus chthonoplastes PCC7420	298/338(88%)	199/230(86%)	
Crinalium epipsammum PCC9333	299/339(88%)	190/225(84%)	
Crocosphaera watsonii WH8501	291/339(85%)	206/231(89%)	
Cyanobacterium aponinum PCC10605	290/340(85%)	193/225(85%)	
Cyanobacterium stanieri PCC7202			1472/2442(60%)
Cyanobium gracile PCC6307	256/337(75%)	181/220(82%)	
Cyanobium sp. PCC7001	261/334(78%)	182/220(82%)	
Cyanothece sp. ATCC51142	294/339(86%)	206/228(90%)	
Cyanothece sp. CCY0110	301/338(89%)	207/230(90%)	
Cyanothece sp. PCC7424			1862/2796(66%)
Cyanothece sp. PCC7425	290/338(85%)	196/230(85%)	
Cyanothece sp. PCC7822			1871/2796(66%)
Cyanothece sp. PCC8801	306/339(90%)	205/230(89%)	

Cylindrospermopsis raciborskii CS-505	301/338(89%)	191/222(86%)	
Cylindrospermum stagnale PCC7417	299/338(88%)	199/231(86%)	
Dactylococcopsis salina PCC8305	282/338(83%)	199/231(86%)	
Geitlerinema sp. PCC7407	292/339(86%)	204/231(88%)	
Gloeobacter violaceus PCC7421	266/338(78%)	183/221(82%)	
Gloeocapsa sp. PCC7428	299/338(88%)	193/226(85%)	
Halothece sp. PCC7418	287/338(84%)	202/228(88%)	
Leptolyngbya sp. PCC7376			2151/2724(78%)
Lyngbya sp. PCC8106	296/338(87%)	196/229(85%)	
Microcoleus sp. PCC7113	301/340(88%)	201/230(87%)	
Microcoleus vaginatus FGP-2	297/338(87%)	194/230(84%)	
Microcystis aeruginosa NIES-843	299/338(88%)	207/231(89%)	
Moorea producens 3L			1300/2202(59%)
Nodularia spumigena CCY9414	302/338(89%)	199/230(86%)	
Nostoc azollae' 0708	299/338(88%)	197/223(88%)	
Nostoc punctiforme PCC73102	298/338(88%)	196/222(88%)	
Nostoc sp. PCC7107	305/338(90%)	195/230(84%)	
Nostoc sp. PCC7120	305/338(90%)	204/230(88%)	
Nostoc sp. PCC7524	303/338(89%)	202/230(87%)	
Oscillatoria acuminata PCC6304	294/338(86%)	202/231(87%)	
Oscillatoria nigro-viridis PCC7112	297/338(87%)	194/230(84%)	
Oscillatoria sp. PCC6506	301/338(89%)	196/230(85%)	
Pleurocapsa sp. PCC7327			1890/2861(66%)
Prochlorococcus marinus str. AS9601	262/337(77%)	173/214(80%)	
Prochlorococcus marinus str. CCMP1375	263/337(78%)	169/218(77%)	
Prochlorococcus marinus str. CCMP1986	261/337(77%)	173/214(80%)	
Prochlorococcus EQPAC1	261/337(77%)	173/214(81%)	
Prochlorococcus GP2	262/337(78%)	173/214(81%)	
Prochlorococcus LG	263/337(78%)	169/218(78%)	

Prochlorococcus marinus str. MIT9107	258/337(77%)	171/214(80%)
Prochlorococcus marinus str. MIT9116	258/337(77%)	171/214(80%)
Prochlorococcus marinus str. MIT9123	258/337(77%)	171/214(80%)
Prochlorococcus marinus str. MIT9201	261/337(77%)	172/214(80%)
Prochlorococcus marinus str. MIT9202	262/337(77%)	148/186(80%)
Prochlorococcus marinus str. MIT9211	257/334(76%)	171/219(78%)
Prochlorococcus marinus str. MIT9215	262/337(77%)	171/214(79%)
Prochlorococcus marinus str. MIT9301	262/337(77%)	173/214(80%)
Prochlorococcus marinus str. MIT9302	262/337(78%)	173/214(81%)
Prochlorococcus marinus str. MIT9303	237/303(78%)	183/236(77%)
Prochlorococcus marinus str. MIT9311	260/337(77%)	174/214(81%)
Prochlorococcus marinus str. MIT9312	260/337(77%)	174/214(81%)
Prochlorococcus marinus str. MIT9313	265/337(78%)	178/218(81%)
Prochlorococcus marinus str. MIT9314	261/337(77%)	172/214(80%)
Prochlorococcus marinus str. MIT9515	263/337(78%)	169/214(78%)
Prochlorococcus marinus str. MIT9321	262/337(78%)	173/214(81%)
Prochlorococcus marinus str. MIT9322	262/337(78%)	173/214(81%)
Prochlorococcus marinus str. MIT9401	262/337(78%)	173/214(81%)
Prochlorococcus marinus str. MIT9515	263/337(78%)	169/214(79%)
Prochlorococcus marinus str. MIT0601	260/337(77%)	172/214(80%)
Prochlorococcus marinus str. MIT0602	259/338(77%)	171/218(78%)
Prochlorococcus marinus str. MIT0603	259/338(77%)	171/218(78%)
Prochlorococcus marinus str. MIT0604	262/337(78%)	172/214(80%)
Prochlorococcus marinus str. MIT0701	267/337(80%)	177/220(80%)
Prochlorococcus marinus str. MIT0702	268/337(80%)	177/220(80%)
Prochlorococcus marinus str. MIT0703	268/337(80%)	177/220(80%)
Prochlorococcus marinus str. MIT0801	259/334(78%)	174/222(78%)
Prochlorococcus marinus str. NATL1A	258/334(77%)	174/222(78%)
Prochlorococcus marinus str. NATL2A	257/334(76%)	174/222(78%)

Prochlorococcus marinus str. PAC1	257/334(77%)	174/222(78%)	
Prochlorococcus marinus str. SB	260/337(77%)	173/214(81%)	
Prochlorococcus marinus str. SS2	263/337(78%)	169/218(78%)	
Prochlorococcus marinus str. SS35	263/337(78%)	169/218(78%)	
Prochlorococcus marinus str. SS51	263/337(78%)	169/218(78%)	
Prochlorococcus marinus str. SS52	263/337(78%)	169/218(78%)	
Prochlorococcus marinus str. UH18301	260/337(77%)	172/214(80%)	
Pseudanabaena sp. PCC 7367	285/339(84%)	201/226(88%)	
Raphidiopsis brookii D9	299/338(88%)	194/221(87%)	
Rivularia sp. PCC 7116	294/338(86%)	194/228(85%)	
Stanieria cyanosphaera PCC7437			1897/2788(68%)
Synechococcus elongatus	279/337(82%)	195/231(84%)	
Synechococcus sp. BL107	259/337(76%)	172/210(81%)	
Synechococcus sp. CC9311	265/339(78%)	181/219(82%)	
Synechococcus sp. CC9605	266/337(78%)	171/210(81%)	
Synechococcus sp. CC9902	259/337(76%)	172/210(81%)	
Synechococcus sp. JA-2-3B'a(2-13)	268/338(79%)	179/221(80%)	
Synechococcus sp. JA-3-3Ab	268/338(79%)	180/221(81%)	
Synechococcus sp. PCC6312	294/338(86%)	188/221(85%)	
Synechococcus sp. PCC7002			2720/2720(100%)
Synechococcus sp. PCC7335	285/339(84%)	196/231(84%)	
Synechococcus sp. PCC7502	283/339(83%)	188/222(84%)	
Synechococcus sp. RCC307	265/337(78%)	182/220(82%)	
Synechococcus sp. RS9916	263/337(78%)	173/210(82%)	
Synechococcus sp. RS9917	262/337(77%)	176/210(83%)	
Synechococcus sp. WH5701	264/334(79%)	182/220(82%)	
Synechococcus sp. WH7803	264/337(78%)	180/219(82%)	
Synechococcus sp. WH7805	265/337(78%)	175/210(83%)	
Synechococcus sp. WH8102	263/337(78%)	174/210(82%)	

Synechococcus sp. WH8109	265/337(78%)	174/210(82%)
Synechocystis sp. PC6803	340/340(100%)	231/231(100%)
Thermosynechococcus elongatus BP-1	290/338(85%)	186/221(84%)
Trichodesmium erythraeum IMS101	297/338(87%)	192/220(87%)

Table S2. Hydrocarbon amounts from gas-chromatography-mass spectrometry analysis for samples. Also shown are dry cell weights and cell counts for each sample. Values are from three biological replicates.

Species	Pentadecane per pellet (μgs)	Heptadecane per pellet (μgs)	8-Heptadecene per pellet (μgs)	Total hydrocarbons per pellet (μgs)	Dry cell weight per pellet (mgs)	Cells per pellet (x10^9)
Prochlorococcus marinus str. CMP1986	29.042 ± 13.354	1.147 ± 0.522	n.d.	30.187 ± 13.876	7.933 ± 1.745	65.383 ± 13.415
Prochlorococcus marinus str. MIT9312	15.509 ± 14.795	0.595 ± 0.508	n.d.	16.104 ± 15.303	9.452 ± 1.899	48.57 ± 4.045
Prochlorococcus marinus str. MIT9313	10.339 ± 6.404	0.403 ± 0.162	n.d.	10.742 ± 6.566	7.082 ± 0.811	16.51 ± 2.923
Synechococcus sp. WH5701	0.152 ± 0.036	1.846 ± 0.381	2.073 ± 0.409	4.071 ± 0.826	2.967 ± 0.681	1.573 ± 0.084
Synechococcus sp. WH7803	0.434 ± 0.148	n.d.	0.039 ± 0.002	0.473 ± 0.150	1.433 ± 0.115	1.098 ± 0.032
Synechococcus sp. WH7805	0.394 ± 0.094	n.d.	0.104 ± 0.015	0.498 ± 0.109	2.1 ± 0.1	1.598 ± 0.042
Synechococcus sp. WH8102	0.293 ± 0.052	n.d.	0.048 ± 0.004	0.341 ± 0.056	1.533 ± 0.153	1.123 ± 0.038