Daily changes in phytoplankton lipidomes reveal mechanisms of energy storage in the open ocean

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Sunlight is the dominant control on phytoplankton biosynthetic activity, and darkness deprives them of their primary external energy source. Changes in the biochemical composition of phytoplankton communities over diel light cycles and attendant consequences for carbon and energy flux in environments remain poorly elucidated. Here we use lipidomic data from the North Pacific subtropical gyre to show that biosynthesis of energy-rich triacylglycerols (TAGs) by eukaryotic nanophytoplankton during the day and their subsequent consumption at night drives a large and previously uncharacterized daily carbon cycle. Diel oscillations in TAG concentration comprise 23 ± 11% of primary production by eukaryotic nanophytoplankton representing a global flux of about 2.4 Pg C yr⁻¹. Metatranscriptomic analyses of genes required for TAG biosynthesis indicate that haptophytes and dinoflagellates are active members in TAG production. Estimates suggest that these organisms could contain as much as 40% more calories at sunset than at sunrise due to TAG production.
iel cycles in sunlight represent an important source of environmental variability for plankton communities in the oligotrophic subtropical gyres of the world’s ocean. This is particularly true for phytoplankton, which depend on sunlight to drive the biosynthesis of macromolecules essential for growth. These organisms possess numerous metabolic capabilities that allow them to survive in darkness, when they are effectively deprived of their primary energy source. For example, diel cycles in phytoplankton gene expression patterns reveal widespread coordination of metabolic pathways to optimize internal biochemical energy allocation. However, the attendant diel cycles in the biochemical composition of phytoplankton communities and the biogeochemical consequences of these changes for the ocean carbon cycle remain poorly understood. As subtropical gyres of the oligotrophic ocean are the world’s largest biomes, account for about 40% of the Earth’s surface area, and contribute significantly to global productivity and carbon export, diel cycles in phytoplankton metabolism are expected to be engrained in the roles these environments play in the global carbon cycle.

A common strategy for energy allocation in phytoplankton centers on the daytime accumulation of energy storage molecules that can then be used to drive metabolism and respiratory activity during the night. Laboratory cultures of phytoplankton display clear diel cycles in storage lipid accumulation, but whether this metabolic behavior is manifested in the ocean is an open question. While cyanobacteria store energy in the form of carbohydrates (mainly glycogen), eukaryotic phytoplankton predominantly accumulate neutral lipids, mainly in the form of triacylglycerols (TAGs). TAGs are more effective energy stores than carbohydrates, because they contain more chemical energy per mole of carbon and larger quantities can be stored inside the cell as they are non-polar and anhydrous (i.e., lacking a hydration shell). Thus, large phytoplankton, such as diatoms and dinoflagellates, have a greater capacity for energy storage when compared with small cyanobacterial phytoplankton, such as Prochlorococcus and Synechococcus. The accumulation of energy storage molecules in the form of glycogen and/or TAGs is apparently also a strategy for phytoplankton to deal with unfavorable growth conditions. In the oligotrophic surface waters of the subtropical gyres, nitrogen is persistently scarce; this scarcity has been linked in cultures of many eukaryotic phytoplankton to enhanced TAG accumulation. Thus, in subtropical gyres, we hypothesize that TAGs could play a number of roles in eukaryotic phytoplankton metabolism, contribute to the ability of eukaryotic phytoplankton to compete with cyanobacteria, and thereby play a previously uncharacterized role in the carbon cycle of these environments.

Oscillations in phytoplankton storage lipids have the potential to play important roles in biogeochemical carbon cycling and energy flow through the broader microbial community. For example, the biochemical composition of phytoplankton defines their nutritional quality, which has implications for the population dynamics of the zooplankton that graze on them. Yet, field studies on diel periodicity of phytoplankton biochemical composition are rare. To this end, we investigated the lipid composition of the planktonic community (i.e., the metaplankton) at high temporal resolution over multiple day/night cycles during two research cruises to the North Pacific Subtropical Gyre (NPSG). Complementary analyses of the plankton community metatranscriptome were conducted to help constrain the sources of the lipids we observed. Comparison to measurements of primary productivity showed that diel synthesis and consumption of TAGs account for a large fraction of the flux of carbon and energy through the ecosystem.

**Results**

Diel patterns of phytoplankton lipids. During a cruise in the NPSG to Station ALOHA (22°45’N, 158°W) in summer of 2015, we collected samples within sunlit surface waters (15 m) every 4 h over the course of 8 days. We then conducted downstream lipidomic analyses to determine the concentrations of several major lipid classes (Fig. 1). A survey, conducted at a coarser time resolution over 3 days in oligotrophic North Atlantic, suggested that small fluctuations in intact polar membrane lipids were possible in oligotrophic ocean environments. The betaine lipid diacetylglycerylcarboxy-N-hydroxymethyl-choline (DGCC) is an intact polar membrane lipid that is associated with eukaryotic phytoplankton in samples from the oligotrophic regions. The time-of-day average (Fig. 1b) of DGCC was highest at dusk (~18:00 h local time) and lowest at dawn (~06:00 h), but the 1.27 ± 0.2-fold difference was not significant (t-test, t(df = 8) = −1.947, p = 0.0874). Furthermore, diel (24-h) periodicity was not detected for concentrations of this lipid class (p > 0.05) using the RAIN (Rhythmicity Analysis Incorporating Non-parametric methods) algorithm. DGCC is expected to scale nearly linearly with the number of cells, and thus the relatively stable concentrations of DGCC at Station ALOHA indicates that the number of cells was also similarly invariant throughout the diel cycle of sunlight, i.e. production of cells was balanced by grazing and other mortality factors.

In contrast, TAG concentrations showed significant diel periodicity (RAIN algorithm, p = 0.004) with dramatic oscillations over the diel cycle (2.3 ± 0.4-fold mean ± SD; t-test, t(df = 8) = −6.200; p = 0.0002). Highest abundances in TAGs occurred during the day near dusk, and lowest abundances at dawn. The significantly greater oscillations in TAG concentrations relative to DGCCs (and relative to particulate organic carbon (Supplementary Figure 1)) indicates that the TAG signal is not simply a reflection of cell size or abundance, but is instead consistent with the synthesis and accumulation of TAGs by cells during daylight hours and depletion at night. Eukaryotic phytoplankton have been proposed as the main source of TAGs in the ocean and the distribution of individual molecular species of TAGs is consistent with other reports from eukaryotic phytoplankton from the Pacific with a dominance of TAGs containing 48, 50, and 52 carbon atoms and one unsaturation. (Supplementary Figure 2). To confirm that eukaryotic phytoplankton dominate the TAG signal in the NPSG, we screened several strains of Prochlorococcus and Pelagibacter, the numerically dominant genera of cyanobacteria and heterotrophic bacteria, respectively, in oligotrophic gyres. These two groups account for more than half of the biomass in these regions. No TAGs were detected in any of the strains, consistent with the lack of TAG biosynthesis genes in both groups. Other bacteria (e.g., from the genera Mycobacterium, Streptomyces, and Rhodococcus) known to produce TAGs are not abundant in surface waters of the NPSG. By contrast, the eukaryotic phytoplankton in the oligotrophic gyres are dominated by haptophytes, dinoflagellates, diatoms, and prasinophytes, and all of these groups have the ability to synthesize TAGs. The relative magnitude of TAG increase we observed is in the range of what has been observed in culture experiments. In the haptophyte Isochrysis sp. grown under N-limitation, neutral lipid concentrations, which were likely mostly TAGs, increased by ~1.7-fold between the beginning and end of the photoperiod. Similarly, in the dinoflagellate coral symbiont Symbiodinium, the quantity of TAGs in lipid bodies increase by approximately twofold. Thus, we concluded that the marked diel cycles in TAG concentrations we observed in the NPSG were most likely driven by eukaryotic phytoplankton.
Another class of lipids, the ubiquinones (UQs), also displayed a diel periodicity in concentration (RAIN algorithm, \(p = 0.002\)), but the phase was opposite of TAGs with a pre-dawn maximum followed by a precipitous drop to an early-morning minimum (0.3 ± 0.1-fold mean ± SD; \(t\)-test, \(t(df = 8) = 3.616, p = 0.0068\)) (Fig. 1a and b). UQs are essential components of the electron transport chain used for cellular respiration by most aerobic organisms\(^{30}\). In the NPSG, these lipids are likely derived primarily from eukaryotic microbes as cyanobacteria do not synthesize UQs – cyanobacteria use plastoquinones for photosynthetic electron transport and respiration\(^{31}\). Furthermore, we observed that the distribution of different UQ molecules in the...
NPSG differs from that of *Pelagibacter* (Supplementary Figure 3). Respiratory quinone concentrations in cultures and environmental samples have been suggested to reflect respiratory activity of their producers34,35 and the observed diel oscillations of UQs may indicate enhanced cellular respiration at night, which is consistent with the consumption of TAGs by phytoplankton. In addition, it has recently been proposed, based on genomic analysis, that increased ubiquinone levels might be required in *Escherichia coli* cells when grown on long-chain fatty acids36. Ubiquinones exhibit antioxidant properties and could mitigate elevated levels of reactive oxygen species generated by fatty acid degradation. That this strategy occurs in eukaryotic phytoplankton remains to be shown, but it could explain our observation of higher ubiquinone concentrations at night when TAGs are degraded.

**Metatranscriptome analysis of the plankton community.** Within the NPSG, the phytoplankton community is dominated by cyanobacterial and eukaryotic picophytoplankton cells less than ~2–3 µm and eukaryotic nanophytoplankton in the 2–20 µm size range36. To identify which eukaryotic phytoplankton were the likely sources of TAGs in the NPSG, we analyzed metatranscriptome expression patterns of two genes that encode characterized diacylglycerol acyltransferase isoforms (DGAT1 and DGAT2), both of which commit the final step in TAG biosynthesis37. Thus, a transcriptional signal of these genes is expected for all cells producing TAGs regardless of how the observed diel cycle is regulated. In most eukaryotic algae, DGAT1 is encoded by a single-copy gene, whereas DGAT2 is often encoded by multiple gene copies38. DGAT1 is widely distributed across marine phytoplankton, with distinct clades of sequences that largely reflect taxonomic groupings. Notably, however, cultured isolates of the smallest and most abundant eukaryotic picophytoplankton, the prasinophytes *Ostreococcus* and *Micromonas*, lack DGAT1 and instead rely on multi-copy DGAT2 for TAG biosynthesis38. In the NPSG, haptophyte and dinoflagellate taxa dominate the DGAT1 transcriptome signal (~6.4 × 10^5 counts L^-1), together accounting for almost 70% of the detected DGAT1 transcripts (Table 1; Supplementary Figure 4), regardless of time of day. Despite the distinct diel pattern in TAG abundance, transcript abundance for the DGAT1 gene remained relatively constant throughout the four days of transcript sampling (Fig. 1d), suggesting that TAG abundance in the NPSG might be regulated through intracellular consumption instead of production. Dinoflagellates, which accounted for the majority of DGAT1 transcripts, are known for their lack of transcriptional regulation39 and an absence of transcriptional rhythmicity within the Haptophyta clade was also expected given that in culture, the haptophyte *Chrysochromulina tobin*, displays no diel oscillations in DGAT140. We also examined transcript abundance associated with DGAT2 (Fig. 1e) to determine the role of prasinophytes in TAG biosynthesis. As with the DGAT1 gene, transcript abundance (~9.1 × 10^6 counts L^-1) for the DGAT2 gene across the different clades remained constant over the 4 days, although potential diel cycles for specific copies could be obscured by this analysis; culture studies with *C. tobin* indicate that only one of the nine DGAT2 gene copies displays a diel cycle in transcript abundance40. The DGAT2 transcript signal was also dominated by dinoflagellate taxa with few (average <1%) transcripts detected for prasinophytes (Fig. 1e). A number of genes involved in the production of the precursors to TAGs, phosphatic acids (e.g., acyltransferases) and in the consumption of TAGs (e.g., lipases) were also examined, and none showed regular diel variations, with the exception of a single, relatively scarce acyltransferase and a single acyl-CoA binding protein from haptophytes (Supplementary Table 1). Together the metatranscriptome data suggest that eukaryotic nanophytoplankton, particularly the haptophytes and dinoflagellates, dominate TAG production in the NPSG.

**Table 1 Relative abundance of DGAT1 transcripts in the NPSG**

| Taxonomic group               | Relative abundance (%) |
|-------------------------------|------------------------|
| Opisthokonts, glaucophytes, cryptophytes | 6.4 |
| Oikopleura dioica            | 1.1 |
| Lepeophtheirus salmonis      | 2.3 |
| Alveolates                    | 4.4 |
| Strombidium inclinatum        | 3.5 |
| Dinophyceae (dinoflagellates) | 42.5 |
| Ceratium fusus               | 8.6 |
| Gymnodinium catenatum         | 9.6 |
| Noculca scintillans           | 2.4 |
| Scrippsiella trochoidea       | 1.4 |
| Bolidomonas sp                | 4.4 |
| Heterokonts                  | 12.1 |
| Viridiplanae (green algae)    | 1.4 |
| Paraphysomonas imperforata    | 1.0 |
| Haptophytes                   | 25.3 |
| Chrysochromulina polypleis   | 1.3 |
| Chrysochromulina brevifilum  | 11.8 |
| Bacillariophyta (diatoms)     | 3.2 |

1 Taxonomic groups represent the best read placements in the maximum-likelihood phylogenetic tree constructed from publicly available marine genomes and transcriptomes (see Supplementary Figure 4).
2 Relative abundances for species within clades are given when recruited reads were >1% of total reads.
3 Not including Dinophyceae.
4 Not including Bacillariophyta.

**TAG production rates throughout the photic zone.** In culture experiments, nutrient limitation can be the major stimulus for TAG accumulation in phytoplankton13 and the growth rate of eukaryotic phytoplankton in the NPSG has been shown to be limited by nutrients40,41. An archetypal microbial response to nutrient stress, particularly phosphorus stress, is the substitution of non-phosphorus membrane lipids for phospholipids; this
response has been observed in nearly all of the major classes of plankton in the NPSG, including *Prochlorococcus*, *Synechococcus*, *Pelagibacter*, diatoms, heterokonts, and haptophytes\(^2\),\(^3\),\(^4\),\(^5\). Furthermore, we have previously shown that differences in the ratios of non-phosphorus lipids to phospholipids between the oligotrophic ocean ecosystems, including the NPSG, reflect differences in ambient phosphate concentrations and turnover rates\(^6\). These ratios of membrane lipids showed no significant diel variations over the investigated time interval (Fig. 1c), suggesting the absence of diel fluctuations in nutrient stress. In haptophytes, TAGs, along with diagnostic viral glycolipids, have also been shown to accumulate in response to viral infection\(^7\), but these viral glycolipids were not detected in any of the NPSG samples we analyzed. Thus, the diel TAG cycle in the NPSG was not likely a reflection of daily fluctuations in nutrient stress or viral mortality.

We posit instead that the magnitude of the diel signal in biosynthesis of TAGs is driven directly by sunlight availability. This is evidenced by depth profiles of net TAG concentrations collected at dusk and dawn in March 2016 (Fig. 2a), showing that the magnitude of diel periodicity observed in surface waters is dampened with depth and disappears below 100 m, the depth where net photosynthetic carbon assimilation approaches zero (Fig. 2b). Accordingly, daily TAG production rates derived from these concentrations were highest in the surface waters (0.42 ± 0.15 mg TAG C m\(^{-3}\) d\(^{-1}\); \(n = 6\)) and decreased with water depth (Fig. 2b). The nitricline at Station ALOHA is typically at around 125 m\(^6\), and thus decreasing nutrient stress with depth cannot explain the observed decrease in TAG production rate. The invariant molecular distribution of TAGs across different water depths (Fig. 2c) further suggests a similar biological source of TAGs throughout the euphotic zone, which is consistent with the major nanophytoplankton groups, diatoms, haptophytes, and dinoflagellates, being found throughout these waters in similar abundances\(^8\). Accordingly, the molecular distribution of DGCCs was highly similar at different water depths and different times of the day (Supplementary Figures 2 and 5).

**TAG contribution to primary production.** To estimate the contribution of TAGs to daily biomass production, we calculated net TAG production rates based on the difference in TAG concentrations between dawn and dusk, and compared these rates with net primary production rates determined during the same time interval (Table 2). In cells of some eukaryotic phytoplankton in culture, including dinoflagellates and haptophytes, TAGs can account for up to 50% of the cellular dry weight\(^9\), illustrating the potential for a large investment of photosynthetic energy into TAG synthesis. The \(^{14}\)C-based primary productivity (\(^{14}\)C-PP) of

![Fig. 2](image-url)
the total NPSG community at 15 m in summer 2015 ranged between 14.7 ± 2.4 and 17.6 ± 0.4 mg C m$^{-3}$ day$^{-1}$, whereas primary production by nanophytoplankton (2–20 µm particle size fraction) was between 2.9 ± 0.9 and 5.4 ± 2 mg C m$^{-3}$ day$^{-1}$, which is as expected in the NPSG where Prochlorococcus is numerically dominant. The TAG production rate in surface waters (15 m) was 1.0 ± 0.2 mg C m$^{-3}$ day$^{-1}$ (mean ± SD; n = 4), which represented 6.4 ± 1.7% of total daytime net primary production and 28 ± 10% of primary production by nanophytoplankton. Similar results were found in the spring of 2016, when TAG synthesis averaged 4.1 ± 0.01 mg C m$^{-3}$ day$^{-1}$ (n = 3) and was 7.5 ± 0.5% of total daytime net primary production and 14 ± 2% of nanoeukaryotic primary production. An estimate of the global significance of TAGs by assuming that the TAG production rates from the spring are representative of mean rates throughout the year at Station ALOHA, integrating them through the photic zone, and extending them to the total area of subtropical gyres (see Supplementary Methods for calculation), indicates that the daily accumulation and depletion of TAGs could drive a carbon flux achieving 2.4 Pg C yr$^{-1}$. This is a low estimate of global TAG synthesis because this phenomenon likely also occurs outside the subtropical gyres in high latitude, equatorial, or coastal regions where primary production rates are higher and eukaryotic phytoplankton are more abundant. Thus, the total global percentage contribution of TAGs to primary production could be substantial, although light may limit carbon fixation in some of these other environments$^{47,48}$.

### Discussion

The diel cycle in TAG concentrations we observed in the NPSG is evidence that the biochemical composition and caloric content of eukaryotic phytoplankton changes profoundly throughout the course of a day. The availability of light energy cycles between extremes of darkness and abundance on a diel cycle, and phytoplankton buffer these variations by transforming this resource into stored chemical energy. Lipid molecules such as TAGs contain more chemical energy per unit mass than other classes of biochemicals, such as starch or carbohydrates$^{31}$, and the caloric content of phytoplankton is strongly correlated to their lipid content$^{19}$. TAG molecules contain only atoms of hydrogen, carbon, and oxygen, elements that are readily available from water and dissolved carbon dioxide; consequently, TAG synthesis by phytoplankton does not incur a stoichiometric requirement for other elements, such as nitrogen, that are scarce in their environments. Thus, TAGs are an efficient molecular vehicle for storing chemical energy that is suited to the environmental conditions experienced by phytoplankton in the NPSG.

Due to the significant daily changes in the TAG content of eukaryotic phytoplankton, the timing of their mortality could have a large impact on ocean carbon cycling in the NPSG. Using the same approach as Platt and Irwin$^{49}$, we calculate that phytoplankton in the NPSG could contain as much as 40% more calories at dusk than at dawn (see Supplementary Methods for calculations). Thus, we hypothesize that eukaryotic phytoplankton would be more favorable prey for zooplankton at dusk than at dawn. Vertical mesozooplankton distributions in the NPSG and throughout the oceans show strong diel changes, with mesozooplankton ascending from the mesopelagic zone to the surface at dusk and descending at dawn. This daily migration, the largest coordinated movement of animals on the planet$^{50}$, balances two competing objectives: rapid growth through efficient feeding in phytoplankton-rich surface waters, and minimization of risk of mortality from visual predators. Traditionally, the cue for diel vertical migration of mesozooplankton was thought to be a relative change in visible light$^{51}$. However, diel vertical migration was also observed in deep sea plankton and it was hypothesized that a precise biochemical clock could maintain the solar diurnal rhythms in deep sea plankton motions$^{52,53}$. We hypothesize, based on the evidence in this study, that the caloric enrichment of phytoplankton at night could provide a strong evolutionary reinforcement for diel vertical migration by mesozooplankton. Life cycles of some mesozooplankton species depend on the nutritional quality of phytoplankton, as defined by lipid content$^{54}$.

In addition to potentially impacting the timing of zooplankton grazing, the diel fluctuations in TAG content of eukaryotic phytoplankton are likely tightly coupled to the fate of organic matter within the NPSG. If TAGs are respired entirely by phytoplankton, then their synthesis would not contribute to net primary production even though TAG production is a substantial fraction of gross production. Using a simple model, we estimate that around 70% of the TAGs produced during the day are consumed by eukaryotic phytoplankton themselves, whereas 30% is consumed by mortality processes (see Supplementary Methods, Supplementary Figure 11 and Supplementary Table 2 for model details). Grazing mortality diverts the energy in TAGs to higher trophic levels, whereas mortality via autolysis or viral lysis of TAG-rich eukaryotic phytoplankton results in the release of relatively hydrophobic, caloric-rich, and nutrient-poor molecules to the pool of dissolved organic matter that fuels heterotrophic bacteria. The consequences of these fluctuations for carbon export, dissolved organic matter composition, nutrient cycling, and ecosystem function are unconstrained. TAGs accumulate in distinct intracellular lipid droplets, the fate of which upon release from lytic cells is also unknown. Understanding the timing and the amount of organic matter transferred from TAG-producing phytoplankton to higher trophic levels and/or the dissolved organic carbon pool will elucidate the consequences of TAG cycling for the marine food web and carbon cycling.
Methods
Sample collection and lipid analysis by HPLC-MS. Seawater samples were collected during R/V Kilo Maru cruises KM1513 (July/August 2015) and KM1605 (March 2016) near Station ALOHA (22°45′N, 158°00′W) in the oligotrophic North Pacific Subtropical Gyre using standard Niskin-type bottles. Triplicate samples were obtained from the bottles during the July cruise and single samples during the March cruise. Samples (~2 L) were filtered using vacuum filtration (ca. ~200 mm Hg) onto 47 mm diameter 0.2 μm hydrophilic Durapore filters (Millipore) and immediately flash-frozen and stored at −196 °C until further processing. Filters were extracted using the TOTALLY RNA Kit (Invitrogen) with some modifications. Briefly, frozen filters were added to 50 mL Falcon tubes containing 5 mL of denaturation solution and extraction beads (125 μl 100 μm zirconia beads, 125 μL 500 μm zirconia beads, and 250 μL 425–600 μm silica glass beads). In addition, to account for the addition of 14 internal mRNA standards were also added to the extraction buffer; these standards were synthesized according to Satinski et al. with the exception that eight standards were synthesized with poly(A) tails mimicking eukaryotic mRNAs. Total extracted RNA was treated with DNase I (Ambion, New York, USA) and purified with DNase inactivation reagent (Ambion). Purified total RNA was used to construct TruSeq cDNA libraries according to the Illumina TruSeq® RNA Sample Preparation v2 Guide. Briefly, RNAs were poly(A)-selected, converted to cDNAs, and sheared to ~225 bp fragments which were used to prepare cDNA libraries for paired-end (2 × 150) sequencing using the Illumina NextSeq 500 sequencing platform (Illumina Inc., San Diego, CA). Raw Illumina data were quality controlled with trimmomatic v0.36 as the parameters MAXINFO:1350:5, LEADING:3, TRAILING:3, MINLEN:60, and AVGQUAL:20, and matching read pairs were merged using flash v1.2.11 with parameters -t 150 -f 250 -s 25. FASTQ files were converted to FASTA and translated with seqnt and transp vEMBOSS:6.6.0.0 using Standard Genetic Code, keeping only potential ORFs with length ≥40 amino acids.

Full-length DGAT1 and DGAT2 protein sequences from Arabidopsis thaliana (NP_179553; DGAT1) (NP_566952; DGAT2), Saccharomyces cerevisiae (NP_014888; DGAT2), Phaeodactylum tricornutum (43469; DGAT2), and Ostreococcus tauri (np_2000475; DGAT1) (np_2001223; DGAT1) were used as queries to identify orthologs in marine eukaryotic genomes and transcriptome assemblies available through Joint Genome Institute (JGI) and the Marine Microbial Eukaryote Transcriptome Sequence Project (MMETSP)77, respectively, using BLASTp. These included both experimentally verified and putative DGAT1/2 orthologs previously described38,44,78. DGAT1/2 orthologs in each query were identified by an HMM similarity search using DGGT (DGAT1) and aligned using MAFFT (Version 7) with the E-INS-i algorithm88. The alignment was trimmed using trimAl v1.2.rev using the -gt .05 --seqlen 0.5 --seqgap 50 options, and any sequences that did not contain the highly conserved DGAT1 histidine residue were removed88. The trimmed alignment file was used to search the Pfam database (v31.0)79 for the protein families Acyltransferase (PF01553), Triglyceride lipase (PF00887). Outgroups included several related sterol O-acyltransferase protein sequences. A bootstrapped (n = 100) best-scoring (n = 10) maximum-likelihood tree was built using RAxML (Version 8.2.10). To recruit environmental metatranscriptomic reads to the DGAT2/1 reference alignments, an hmm profile was constructed from each reference alignment using hmmBuild followed by transcript identification and alignment using hmmsearch (parameters: -i 35 –inc 35) and hmmscan, respectively, using the HMMPFAM package v3.1b273. Taxonomy was assigned to each environmental sequence using placcr v1.1.970 based on the read placement with the best maximum likelihood score to the reference tree (parameters: -keep-at-most 1). Read counts for each edge were normalized by recovery of internal mRNA standards to estimate natural transcript abundance per liter of seawater88. To assess transcript expression of additional protein families, we obtained homologs for each of the protein families Acyltransferase (PF01553), Triglyceride lipase (PF01764), Lipase maturation factor (PF06762), and Acyl-CoA binding protein (PF08087). These hmm profiles were then used with hmmscan using the same parameters listed above. Statistical significance for diel periodicity of transcripts was tested using the RANIN package in R89.

Duplicate samples were sequentially filtered through a 100 μm nylon mesh pre-filter followed by a 142 mm 0.2 μm polycarbonate filter using a peristaltic pump. Filters were flash-frozen in liquid nitrogen and subsequently stored at −80 °C until further processing. Filters were extracted using the TOTALLY RNA Kit (Invitrogen) with some modifications. Briefly, frozen filters were added to 50 mL Falcon tubes containing 5 mL of denaturation solution and extraction beads (125 μL 100 μm zirconia beads, 125 μL 500 μm zirconia beads, and 250 μL 425–600 μm silica glass beads). In addition, to account for the addition of 14 internal mRNA standards were also added to the extraction buffer; these standards were synthesized according to Satinski et al. with the exception that eight standards were synthesized with poly(A) tails mimicking eukaryotic mRNAs. Total extracted RNA was treated with DNase I (Ambion, New York, USA) and purified with DNase inactivation reagent (Ambion). Purified total RNA was used to construct TruSeq cDNA libraries according to the Illumina TruSeq® RNA Sample Preparation v2 Guide. Briefly, RNAs were poly(A)-selected, converted to cDNAs, and sheared to ~225 bp fragments which were used to prepare cDNA libraries for paired-end (2 × 150) sequencing using the Illumina NextSeq 500 sequencing platform (Illumina Inc., San Diego, CA). Raw Illumina data were quality controlled with trimmomatic v0.36 as the parameters MAXINFO:1350:5, LEADING:3, TRAILING:3, MINLEN:60, and AVGQUAL:20, and matching read pairs were merged using flash v1.2.11 with parameters -t 150 -f 250 -s 25. FASTQ files were converted to FASTA and translated with seqnt and transp vEMBOSS:6.6.0.0 using Standard Genetic Code, keeping only potential ORFs with length ≥40 amino acids.

Primary production (PP) measurements. Standard Hawaii Ocean Time-series protocols were used to measure in vitro dust to deep water PP via the 14C radioracter method89. Water samples were collected from 25 m before dawn and incubated on deck. Local dawn to dusk PP was calculated from irradiance and plumed with flow through surface seawater for temperature regulation. Rate measurements in light bottles were corrected to remove the rate measured in parallel dark bottles, which were on average 4 ± 1% (n = 14) of light values. To determine night-time respiration, samples were incubated for 12 h in the light with 14C-labeled inorganic carbon and then incubated for a further 12 h in the dark as described in Marra and Barber74. The potential contributions of the 2–20 μm size class to overall PP was calculated using an optical approach to measure particle size distributions (PSD) as described in White et al. Briefly, an in situ laser diffraction meter (LISSST-x100, Laser Micrometry Scatterometer/Transmissometer) was used, Sequences were identified, here simply LISSST, was used to make continuous underway measurements of the PSD. Water was supplied from the ship’s uncontaminated seawater, which drew seawater from ~7 m below the waterline of the forward hull. For the first 10 min of every hour incoming seawater was passed through a 0.2 μm filter. This dissolved blank was considered to be direct water. An optical signature of 100% was collected in 10 min increments from each hour. Particle volume estimated via a spherical inversion of forward scatter in 32 log-scaled bins was converted to carbon using the allometric scaling of...
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**Acknowledgements**

We are grateful to the officer and crew of the R/V Kilo Moana cruises KM1513 and KM1605. We further thank Allison Coe in the Chisholm Lab at MIT for providing *Prochlorococcus* biomass. Thanks to four anonymous reviewers whose comments greatly improved this manuscript. This work was supported by a grant from the Simons Foundation, and is a contribution of the Simons Collaboration on Ocean Processes and Ecology (SCOPE award # 329108, B.A.S.V.M.). K.W.B. was further supported by the Postdoctoral Scholarship Program at Woods Hole Oceanographic Institution & U.S. Geological Survey.

**Author contributions**

K.W.B., lipidomics, data analysis, and paper writing; B.A.S.V.M, study design, paper writing; J.R.C., lipidomics and data analysis; B.P.D., R.D.G. and E.V.A., transcriptomics and data analysis. A.E.W., primary productivity measurements. H.F.F., lipidomics and data analysis; D.J.R. and J.E.O., onboard sampling; P.C., Pelagibacter biomass provision; All authors provided editorial comments on the manuscript.

**Additional information**

Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-018-07346-z.

**Competing interests:** The authors declare no competing interests.

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