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Supplementary information

Complex marine microbial communities partition metabolism of scarce resources over the diel cycle

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Supplementary Information

Complex Marine Microbial Communities Partition Metabolism of Scarce Resources Over the Diel Cycle

Supplementary Discussion

Community-Scale Synchronization of Phototrophy and Organic Carbon Catabolism.

Below we describe features of each of the four emergent clusters identified via a SOM approach by their associated peak timing - morning, afternoon, dusk, and night. Supplementary Table 3 contains lists of mentioned lipids and metabolites for each cluster and Supplementary Data 1 contains all pathway enrichments, whereas Supplementary Table 1 contains breakdowns of the percentage of different classes of biomolecules contained in each cluster. Complete records of all signals and their attributed cluster are present in Supplementary Data 6.

Morning (0600): Analysis of the morning cluster revealed significant enrichment of transcripts for both cyanobacteria and eukaryotes involved in the photosynthesis pathway (Fisher's exact test, BH-adjusted $p<0.1$, see Supplementary Data 1). Eukaryotes had additional significant enrichment for transcripts in the carotenoid synthesis, carbon fixation, and porphyrin/chlorophyll metabolism pathways. The chemical data in the morning cluster corroborate transcriptional enrichments, including 9 out of 11 diel pigments from the lipidome (chlorophylls and carotenoids such as lutein and zeaxanthin)[1,2] and the metabolite pantothenic acid (vitamin B_5), which is a precursor to coenzyme A[3] and critical to fatty acid synthesis (see Supplementary Table 3). In summary, we find evidence of early morning production of photosynthetic-associated machinery across diverse taxa and the accumulation of biomolecules central to photosynthesis.

Afternoon (1400): In the afternoon cluster, we see evidence consistent with afternoon synthesis of amino acids, nucleosides, and carbohydrates, carbon fixation, and energy metabolism-related transcriptional processes. Similar to the morning cluster, photosynthesis transcripts are enriched for both eukaryotes and cyanobacteria, and the porphyrin/chlorophyll metabolism pathway is enriched in eukaryotes (see Supplementary Data 1). This cluster includes diadinoxanthin, a photoprotective carotenoid utilized by both dinoflagellates and diatoms[1,2] (see Supplementary Table 3). Metabolites in the afternoon cluster

include serine and alanine, precursors in the formation of betaine lipids produced by phytoplankton, as well as precursors involved in the synthesis of glyceroglycolipids associated with photosynthetic carbon fixation, such as chitobiose and UDP-glucosamine (see Supplementary Table 3). This cluster also shows significant enrichments of the TCA cycle and oxidative phosphorylation pathways in both eukaryotes and heterotrophic bacteria, indicating that these groups ramp up organic carbon catabolism in response to the production of fixed carbon. Additionally, heterotrophic bacteria have enrichments for transcripts in the RNA degradation and cell cycle pathways (primarily proteases and molecular chaperones), potentially indicating cell replication (see Supplementary Data 1). Our findings that bacteria respond during the day to light-driven forcing is consistent with prior evidence of increased bacterial production during the daytime at Station ALOHA[4].

Dusk (1800): The dusk cluster shows transcriptional and chemical evidence of the cumulative effects of carbon fixation and transition to catabolism and replication with sunset. Over 90% of diel triacylglycerol lipids (TAGs), storage lipids used by eukaryotes[5], and over half of the diel metabolites fall into this cluster (see Supplementary Table 3). These metabolites include several osmolytes known to be produced by prokaryotic and eukaryotic phytoplankton[6,7], such as the saccharide trehalose and organosulfur molecules dimethylsulfoniopropionate (DMSP) and 2,3-dihydroxypropane-1-sulfonate (DHPS)[8] (see Supplementary Table 3). The dusk enrichment of tricarboxylic acid (TCA) cycle transcripts in both cyanobacterial and eukaryotic groups suggests the use of this accumulated organic carbon as an energy source (see Supplementary Data 1). We also find an enrichment in eukaryotic transcripts associated with fatty acid oxidation to acetyl-CoA, particularly in haptophytes and ochrophytes. This corresponds with the peak in concentrations of TAGs and the fatty acids arachidonic acid and eicosapentaenoic acid (EPA), the latter of which has been shown to be a major constituent of the fatty acid profiles of the betaine lipids of eukaryotic phytoplankton in subtropical gyres[9] (see Supplementary Table 3). Over 70% of the diel betaine lipids (85/112) and phospholipids (87/116) peak at dusk, following the peak of many of their precursor metabolites in the afternoon (See Supplementary Table 3). These major classes of polar

glycerolipids are important constituents of the cell membranes of eukaryotic phytoplankton[10], and biological membranes of all marine organisms, respectively. The dusk cluster encapsulates the peak in concentration of carbohydrates and other products of primary production in the particulate fraction, as well as transcripts suggesting the breakdown of these products across the microbial community for energy.

Night (0200): The night cluster contains transcriptional patterns that indicate a shift in community-wide metabolic processes away from the synthesis of lipids and secondary metabolites, e.g., including only 7.8% of diel lipids and 4% of diel metabolites (see Supplementary Table 1). Instead, both eukaryotes and cyanobacteria show significant enrichment for ribosomal subunit transcripts, suggesting an increase in protein synthesis (See Supplementary Data 1). This shift is corroborated by the nighttime peak in total hydrolysable amino acids and total hydrolysable nitrogenous bases (see Extended Data Figure 3). In the night cluster we also find transcripts involved in nitrogen fixation in the cyanobacterium *Crocosphaera* (corroborating recent analysis[11]), including nitrogenase and accessory proteins such as hydrogenase, ferrous iron transporters, and superoxide dismutase. This cluster also contains *Crocosphaera* transcripts for the biosynthesis of nitrogen-rich molecules such as pseudocobalamin (see Supplemental Discussion – *Cobalamin Dynamics*) and chlorophyll, which is reflected in the presence of pigments in the morning cluster. Diel expression for the biosynthesis of nitrogen-rich secondary metabolites also extends to nonribosomal peptide synthetases in eukaryotes. Similarly, heterotrophic bacterial transcripts related to the nitrogen-demanding porphyrin/chlorophyll metabolism and photosynthesis antenna protein pathways were significantly enriched at night. Further inspection revealed overnight diel enrichment of the complete biosynthetic pathway from protoporphyrin IX to bacteriochlorophyllide a in the Roseobacter group of Alphaproteobacteria and the OM60/NOR5 group of Gammaproteobacteria, both of which synthesize this pigment[12,13]. In summary, we find evidence of nighttime production of proteins associated

with cellular division, nitrogen rich compound production, and the preparation of photoheterotrophic machinery before sunrise.

Diel fluctuations and biomass

The main text describes direct evidence of pervasive oscillations in transcripts, lipids, primary and secondary metabolites, THAA and THNB over diurnal timescales in the NPSG. Diel oscillations in transcript abundance could potentially arise from multiple mechanisms. We posit two alternative hypotheses regarding the basis for diel oscillations of transcript abundances. First, the biomass-specific transcript abundance could remain constant while the biomass oscillates. Second, gene transcription levels could oscillate, leading to diel variation in transcript abundance in the absence of counter-balancing oscillations in biomass. A combination of both effects could also generate diel oscillations.

We have multiple lines of evidence that gene transcription is changing on diel time-scales, and that cells are not transcriptionally static, even in cases where taxa-specific biomass oscillates over a diel period. First, for some prominent taxa within the NPSG microbial community in the >0.2 μm component, we have direct evidence contrary to the first hypothesis. The biomass of *Prochlorococcus* peaks at dusk[8]. However, we identified more than 300 *Prochlorococcus*-associated genes whose transcript abundance was significantly diel; these 300 genes spanned all four SOM archetypes, the majority of which included transcripts were assigned to the morning (109/307) or afternoon (125/307) clusters. Similarly, *Crocosphaera* biomass peaked in the afternoon[8], yet the majority of *Crocosphaera* transcripts peaked in the morning (115/445), at dusk (88/445), or at night (225/445); this divergence in peak timing is inconsistent with a signal driven by oscillating biomass alone. Second, our analysis of characteristic diel profiles per taxa revealed a common feature: divergent peak timing (Figure 4). We found no examples of taxa with at least 4 diel transcripts in which all transcripts belonged to a single cluster (or even at a pair of clusters with adjacent peak times). These two lines of evidence are consistent with prior culture work[14,15] and existing community standards to interpret diel transcript oscillations as arising primarily due to physiological changes within taxa[16-18].

Cobalamin Dynamics

While the oligotrophic NPSG is nitrogen limited[19], micronutrients such as vitamin B_{12} (cobalamin) are a valuable resource to a wide array of organisms, like eukaryotic phytoplankton and heterotrophic bacteria, that need to acquire the compound exogenously[20-22]. In eukaryotic phytoplankton and other higher organisms experiencing cobalamin limitation, S-adenosyl homocysteine (SAH) is elevated and SAH's precursor and near-universal methyl donor S-adenosyl methionine (SAM) decreases[23,24]. We find SAH in the afternoon cluster while SAM peaks at dusk with POC (Supplementary Data 6) This daytime increase in the SAH/SAM ratio potentially indicates a temporary bottleneck in the methionine cycle that could be due to cobalamin availability. Cobalamin-like compounds photodegrade quickly in the ocean and the turnover time has been estimated to be on the order of hours to days[25]. We therefore looked for more evidence of diel cobalamin dynamics.

Cobalamin is produced by some bacteria and archaea, and pseudocobalamin, a cobalamin analogue with a different lower ligand, is produced by cyanobacteria[26]. These chemical variants of Vitamin B_{12} require enzymatic remodeling in order to complete vitamin cross feeding between these groups[26,27]. A previous study of transcripts in the NPSG suggests aspects of this cross feeding are modulated over day-night transitions[28]. Here we found evidence of diel cyanobacterial pseudocobalamin production (Supplementary Data 6). In addition, we identify *Crocosphaera* cobalt-uptake receptors (cbiMN) and cobalamin-synthesis transcripts (cobW and cobSV) in the dusk cluster, followed by a putative cobalamin transport membrane protein in the night cluster (bacA[29]). *Crocosphaera* has been shown to produce and excrete a cobalamin-like compound (likely pseudocobalamin) at a high rate in culture[30]. Notably, in this study, *Crocosphaera* transcripts involved in pseudocobalamin synthesis peak during *Crocosphaera*'s nighttime nitrogen-fixing metabolic phase. This coupling is consistent with the high nitrogen content of cobalamin-like compounds and the likelihood for light-driven degradation. Moreover, the nighttime peak of pseudocobalamin synthesis also adds evidence supporting a mechanistic linkage between intracellular nitrogen availability and the production of cobalamin-like compounds proposed by earlier work[31]. Prior work found that N-

replete cultures of *Synechococcus* (a cyanobacterium which does not fix nitrogen) produce more cobalamin-like compounds than N-limited cultures[30].

Concurrent with the nighttime *Crocosphaera bacA* transporter, we find diel cobalamin transporters (*bacA* and *btuB*) in 3 different eukaryotic phyla (Supplementary Data 6). There is additional diel expression in the haptophytes specifically, with both parts of the cobalamin-dependent methionine synthesis gene (methyltetrahydrofolate-homocysteine methyltransferase *metF* and *metH*) landing in the afternoon cluster (Supplementary Data 6). Though it is not significantly diel, *CobS* required for lower-ligand remodeling of pseudocobalamin to cobalamin is expressed in six eukaryotic taxa. These lines of evidence point towards active production, transport, and remodeling, and indicate that cobalamin-like compounds have diel dynamics across broad taxa in the surface ocean.

Iron Ligand Transporter Dynamics

Trace metals, and particularly iron, more broadly comprise another class of micronutrients which are present in the NPSG at very low levels[32-34]. Iron is a critical metal cofactor to a wide array of enzymes[35], including those involved in photosynthesis and nitrogen fixation[14]. Dissolved trace metal concentrations measured during the same cruise used for sampling in this study show no obvious diel fluctuation in trace metal concentrations and stronger day-to-day variability than dawn/dusk variability in dissolved iron concentrations[36]. Beyond the concentration of the dissolved iron pool, however, microbial iron cycling is also mediated by cellular iron demand and the secretion, uptake, and exchange of organic high-affinity iron-binding ligands (e.g. siderophores). Some siderophores produced by marine heterotrophic bacteria exhibit photoreactivity and generate photoproducts that are more weakly iron-binding[37-39]. Field observations in the NPSG have found siderophores with structural similarity to aquachelin and vibrioferrin, two siderophores with known photoreactivity[33,34,39]. Photoreactivity potentially indicates diel changes in the speciation of the available iron pool for microbial uptake, and has been implicated in potential metabolic exchange between heterotrophic bacteria and eukaryotic phytoplankton[38]. Therefore,

the demand and uptake dynamics of iron may be impacted by diel shifts in metabolic activities amongst the NPSG microbial community.

Across all analyzed taxa, metal and metal-ligand transporters make up 21% (35/168) of diel transporters. Heterotrophic bacteria (primarily alpha- and gammaproteobacteria) account for 8 of these transporters, 6 of which transport either ferric iron or iron-ligands, as well as two TonB receptors, which canonically are associated with siderophore active transport (Supplementary Data 6). Interestingly, all of these heterotrophic iron transporters belong to either the morning or night clusters, and the *fhuA* (ferrichrome outer membrane uptake receptor) orthologue was identified as significantly synchronous in our peak timing analysis (Supplementary Data 8). Ferrioxamines, a class of hydroxamate siderophores similar to ferrichrome, were also measured at the time of sampling and are shown to be the most abundant class of siderophores at the surface[34]. Ferrioxamines have also been isolated from marine gammaproteobacteria[34]. If we only consider the direct effects of light-forcing, the widespread diel expression of iron uptake amongst heterotrophic bacteria is unexpected because, unlike photosystem proteins of photoautotrophs, the bacteriochlorophyll *a* pigment requires a magnesium cofactor[40] while proteorhodopsin uses a retinal chromophore[41,42]. Therefore, we might not expect morning iron requirements to be related directly to photoheterotrophy. However, the morning cluster is significantly enriched for ribosomal subunits in heterotrophs and therefore morning may be an important proteinogenic time. Thus, diel iron uptake may be in response to demand for iron as an enzyme cofactor. For example, the afternoon cluster and morning cluster contain *Roseobacter* and SAR116 transcripts for the iron-manganese containing form of superoxide dismutase, which may be an important enzymatic accessory to photoheterotrophy[43] and protection from UV-induced reactive oxygen species during cellular replication.

We can contrast diel transcript analysis associated with heterotrophic bacteria with that associated with cyanobacteria. For instance, in *Prochlorococcus*, we identified diel transcription of a ferric iron uptake receptor with peak timing in the afternoon, coinciding with maximum light incidence and diel transcriptional peaks of high-iron machinery such as photosystem II and ferredoxin[44]. In *Crocosphaera*, we find ferrous iron transporters in the dusk cluster when iron demand is high for synthesizing nitrogen

fixation machinery, accompanying iron storage protein bacterioferritin and FeS cluster assembly protein *sufC*[14]. Together, we find evidence for partitioned iron demand and uptake across bacteria throughout the diel cycle. Heterotrophs express siderophore uptake receptors in the morning, a non-diazotrophic cyanobacteria expresses ferric iron uptake receptors in the afternoon, and a diazotrophic cyanobacteria expresses ferrous iron transporters (potentially indicating intracellular iron recycling) at dusk. These three timings correspond to the expression of iron-containing enzymes for each respective group – superoxide dismutases for heterotrophic bacteria, photosynthesis accessory proteins for *Prochlorococcus*, and nitrogen fixation machinery for *Crocosphaera*.

Supplementary Table 1.
Summary of Cluster Distribution Across Datasets. Rows indicate the original high-throughput datasets analyzed via RAIN for diel periodicity detection (transcriptomes in both size fractions, lipids, and metabolites). The total number of diel signals detected is listed, and then the membership across clusters is indicated by the column. Percentages refer to percent total of the row (i.e., in the first row, the value 464 in the morning total column represents 27% of the 1739 diel >0.2 micron transcripts).

Data Type	Total Diel	Morning Total	Afternoon Total	Dusk Total	Night Total
>0.2um	1739	464 (27%)	565 (32%)	263 (15%)	447 (26%)
>5um	3983	929 (23%)	531(13%)	1571 (39%)	952 (24%)
Lipid	501	49 (10%)	17 (3%)	399 (80%)	36 (7%)
Metabolite	50	2 (4%)	20 (40%)	26 (52%)	2 (4%)
Total	6273	1444 (23%)	1133 (18%)	2259 (36%)	1437 (23%)

Supplementary Table 2.
Summary of synchronicity analysis. Numbers in cells indicate the number of KEGG orthologues that were identified as 'narrowly diel', 'asynchronous', or 'synchronous' for each number of taxa. Each KEGG Orthologue with diel transcript abundance in at least one taxon was considered separately, the number of taxa that were found to have diel expression of that particular KO.

Number of Taxa	Total # KOs	Narrowly Diel	Asynchronous	Synchronous ($p<0.05$)
3 or fewer	2898	2898	NA	NA
4	104	NA	95	9
5	77	NA	65	12
6	40	NA	26	14
7	28	NA	15	13
8	17	NA	7	10
9	10	NA	3	7
10	6	NA	3	3
11	4	NA	1	3
12	2	NA	0	2
13	2	NA	0	2
14	4	NA	1	3
15	0	NA	0	0
16	1	NA	0	1
17	0	NA	0	0
18	1	NA	0	1

Supplementary Table 3. Cluster Assignment Overview for Data Mentioned in Supplementary Information Section 'Community Scale Synchronization of Photosynthesis and Organic Carbon Catabolism'.

Cluster distributions (and presence/absence in the case of single mentioned metabolites/gene transcripts) are presented for data cited in Supplementary Information section 'Community-Scale Synchronization of Photosynthesis and Organic Carbon Catabolism'. Pathway enrichments discussed are indicated in bold. Transcripts are either labeled by direct taxonomic affiliation or using the abbreviations E – eukaryotic taxa, BA – bacterial photoautotrophs, BH – bacterial heterotrophs as designated in the pathway enrichment analysis (see Figure 4 for example taxa from each group). An 'X' is used to indicate the cluster assignment of a single metabolite/KO transcript. ** For more detailed discussion of pseudocobalamin synthesis and putative metabolic exchange, please refer to the Supplementary Discussion.

Description	Analyte	Morning	Afternoon	Dusk	Night
Betaine lipids	Lipid	14	5	**80**	13
Phospholipids	Lipid	13	2	**87**	14
Pigments	Lipid	9	1	0	1
Triacylglycerols (TAGs)	Lipid	5	6	**157**	2
Total Hydrolysable Amino Acids (THAA)	Macromolecule				**X**
Total Hydrolysable Nitrogenous Bases (THNB)	Macromolecule				**X**
2,3-dihydroxypropane-1-sulfonate (DHPS)	Metabolite			**X**	
Alanine	Metabolite		**X**		
Arachidonic acid	Metabolite			**X**	
Chitobiose	Metabolite		**X**		
Dimethylsulfoniopropionate (DMSP)	Metabolite			**X**	
Eicosapentaenoic acid (EPA)	Metabolite			**X**	
Pantothenic acid (Vitamin B5)	Metabolite	**X**			
Serine	Metabolite		**X**		
Trehalose	Metabolite			**X**	
UDP-glucosamine	Metabolite		**X**		
Ammonium transmembrane transporter *amt* (K03320)	Transcript (E)	0	1	**3**	1
Ammonium transmembrane transporter *amt* (K03320)	Transcript (BA)	0	0	**2**	0
Ammonium transmembrane transporter *amt* (K03320)	Transcript (BH)	1	1	0	1
Carbon Fixation Pathway	Transcript (E)	**10**	0	0	4
Carbon Fixation Pathway	Transcript (BA)	5	0	0	6
Carbon Fixation Pathway	Transcript (BH)	1	0	0	0
Carotenoid Synthesis Pathway	Transcript (E)	**12**	0	1	0
Carotenoid Synthesis Pathway	Transcript (BA)	0	4	0	2
Carotenoid Synthesis Pathway	Transcript (BH)	1	0	0	1
Cell Cycle Pathway	Transcript (E)	3	5	4	1
Cell Cycle Pathway	Transcript (BA)	3	5	4	3
Cell Cycle Pathway	Transcript (BH)	0	**30**	4	2
Nitrogen fixation *sodN, nifD, nifW, nifE, nifB, nifT, nifN* (K00518,K02586,K02595,K02587,K02585,K02593,K02592)	Transcript (*Crocosphaera*)				**X**
Oxidative Phosphorylation Pathway	Transcript (E)	20	**18**	25	6
Oxidative Phosphorylation Pathway	Transcript (BA)	11	10	9	9
Oxidative Phosphorylation Pathway	Transcript (BH)	4	**72**	12	1
Photosynthesis Pathway	Transcript (E)	**44**	**35**	0	4
Photosynthesis Pathway	Transcript (BA)	**30**	**21**	1	3
Photosynthesis Pathway	Transcript (BH)	1	2	0	0
Porphyrin/Chlorophyll Metabolism Pathway	Transcript (E)	27	19	2	1
Porphyrin/Chlorophyll Metabolism Pathway	Transcript (BA)	3	6	6	17
Porphyrin/Chlorophyll Metabolism Pathway	Transcript (BH)	0	1	1	**11**
Pseudocobalamin biosynthesis**	Transcript			**X**	**X**
Ribosome Pathway	Transcript (E)	19	0	34	**44**
Ribosome Pathway	Transcript (BA)	27	3	2	**57**
Ribosome Pathway	Transcript (BH)	28	3	4	13
RNA Degradation Pathway	Transcript (E)	1	6	7	5
RNA Degradation Pathway	Transcript (BA)	2	7	1	8
RNA Degradation Pathway	Transcript (BH)	6	**45**	2	5
TCA Cycle Pathway	Transcript (E)	1	**17**	**34**	0
TCA Cycle Pathway	Transcript (BA)	2	0	**5**	0
TCA Cycle Pathway	Transcript (BH)	2	**27**	5	1
Urea uptake transporter *urtA* (K11959)	Transcript (*Prochlorococcus*)				**X**

Urease *URE, ureC, ureA, ureE, ureF, ureD/H, ureAB* (K01427, K01428, K01430, K03187, K03188, K03190, K14048)	Transcript (Chlorophyta, Haptophyta, Rhodophyta)	0	0	9	**6**

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