Diel Partitioning in Microbial Phosphorus Acquisition in the Sargasso Sea

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Abstract

The daily cycle of photosynthetic primary production at the base of complex marine food webs is often limited by the availability and uptake of scarce nutrients. According to temporal niche partitioning theory, competition for scarce resources can be alleviated insofar as the intensity of nutrient uptake and assimilation activities are distributed heterogeneously across organisms over periodic input cycles. Recent analysis of community transcriptional dynamics in the nitrogen-limited suptropical North Pacific gyre revealed evidence of temporal partitioning of nitrogen uptake and assimilation between eukaryotic phytoplankton, cyanobacteria, and heterotrophic bacteria over day-night cycles. Here, we present results from a Lagrangian metatranscriptomic time series survey in the Sargasso Sea and demonstrate temporally partitioned phosphorus uptake in this phosphorus-limited environment. In the Sargasso, heterotrophic bacteria, eukaryotic phytoplankton, and cyanobacteria express genes for phosphorus assimilation during the morning, day, and dusk, respectively. These results support the generality of temporal niche partitioning as an emergent mechanism structuring uptake of limiting nutrients in open ocean microbial ecosystems.

Introduction

In the ocean, communities of microorganisms set ecological rhythms of primary production and respiration in response to the daily light cycle, fueling the base of the marine food web and modulating biogeochemical cycles [7, 13, 16, 20, 34]. Diel oscillations in carbon fixation among photosynthetic plankton cascade through community metabolism and gene expression, resulting in emergent ecosystem-level cycles in biomass and biomass molecular composition [2–4, 12, 27, 28]. Diel transcriptional and metabolic organization also expands beyond carbon metabolism. Field studies in nitrogen-starved ocean regions [36] have identified diel transcriptional cycles in nitrogen uptake [38] and nitrogen fixation [39]. Heterotrophic taxa from the same region have also been reported to express organic nutrient transporters on a diel

cycle [27–29]. Organisms taking turns in fulfilling specific ecological niches or taking up resources has been termed temporal niche partitioning in terrestrial ecology [18, 30]. Niche partitioning over diel cycles has been ascribed to predatory behavior in marine animals [19] and invoked in microbial community successional dynamics in aquatic environments [8, 27]. Diel cycles identified across diverse mechanisms for satisfying nitrogen requirements among multiple taxa in a nitrogen-limited system suggest that ecosystems facing limitation for other nutrients may have similar strategies.

The Sargasso Sea is an open ocean environment where plankton are considered to be persistently phosphorus-stressed at the surface [9, 34, 36]. In the subtropical northwestern Atlantic, concentrations of dissolved phosphate can be sub-nanomolar [40], sufficient to induce phosphate stress in primary producers [9, 36]. Diverse strategies have evolved to mitigate phosphorus limitation among marine microbes. For example, sulfono- and betaine lipids have been shown to replace phospholipids as a method of reducing cell phosphorus quotas [6, 26, 37]. Utilizing phosphonates, organic phosphorus-containing molecules, as an alternative phosphorus source has also been described in heterotrophic [33] and photosynthetic bacteria [32]. In the Sargasso Sea, dissolved organic phosphorus has been measured to average almost 95% of total dissolved phosphorus [23]. High copy numbers of the C-P lyase *phnJ* gene and strong responses in a proxy for C-P lyase activity have also been observed *in situ* [31]. This evidence reinforces the ecological relevance of phosphorus limitation in the Sargasso Sea, which we hypothesized subjects phosphorus uptake to similar pressures with respect to competition as in the case of nitrogen uptake, demonstrated previously within nitrogen-limited systems [27]. Indeed, a field study of *Trichodesmium* gene expression found evidence of diel phosphate and phosphonate utilization consistent with this hypothesis [10].

Here, we tested the hypothesis that a phosphorus-limited ocean region will partition phosphorus uptake over the diel cycle within a Lagrangian metatranscriptomic time series survey, sampling every 4 hours over the course of 5 days. Our community metatranscriptomic analyses demonstrate widespread temporal partitioning of diverse phosphorus uptake and incorporation genes, and suggests a diel cascade of phosphorus uptake from heterotrophic bacteria at sunrise, to eukaryotic phytoplankton during the day, to cyanobacteria at dusk. The results reinforce the generality of temporal niche partitioning as a possible mechanism of competition alleviation in open ocean microbial ecosystems.

Results

We analyzed 58 metatranscriptomes collected during the Lagrangian sampling expedition on the R/V *Atlantic Explorer* (AE1926) from October 12-17, 2019 (Figure 1a) in the vicinity of the Bermuda Atlantic Time Series (BATS) study location in the Sargasso Sea to examine diel changes in microbial community-level gene expression (SI Data S1). Using a nonparametric method with multiple testing correction set at a 10% false discovery rate [35], we evaluated whether gene transcripts had 24-hour periodicity in their abundance patterns (see Methods). In all, we evaluated 97,829 different genes from eukaryotic phyla, cyanobacterial genera *Prochlorococcus* and *Synechococcus*, and heterotrophic bacterial orders for diel periodicity. Our analysis found 11,194 significantly diel genes (SI Data S2). Diel genes spanned 21 phyla of eukaryotic phytoplankton (9.33% of total genes), the cyanobacterial groups *Prochlorococcus* and *Synechococcus* (37.4% of total genes), and heterotrophic bacteria representing 6 major classes comprised of 44 orders (13.2% of total genes). A similar analysis conducted on data from a cruise in the North Pacific Subtropical Gyre examined 64,011 genes [27], and found a similar proportion (8.94% vs 11.4% reported

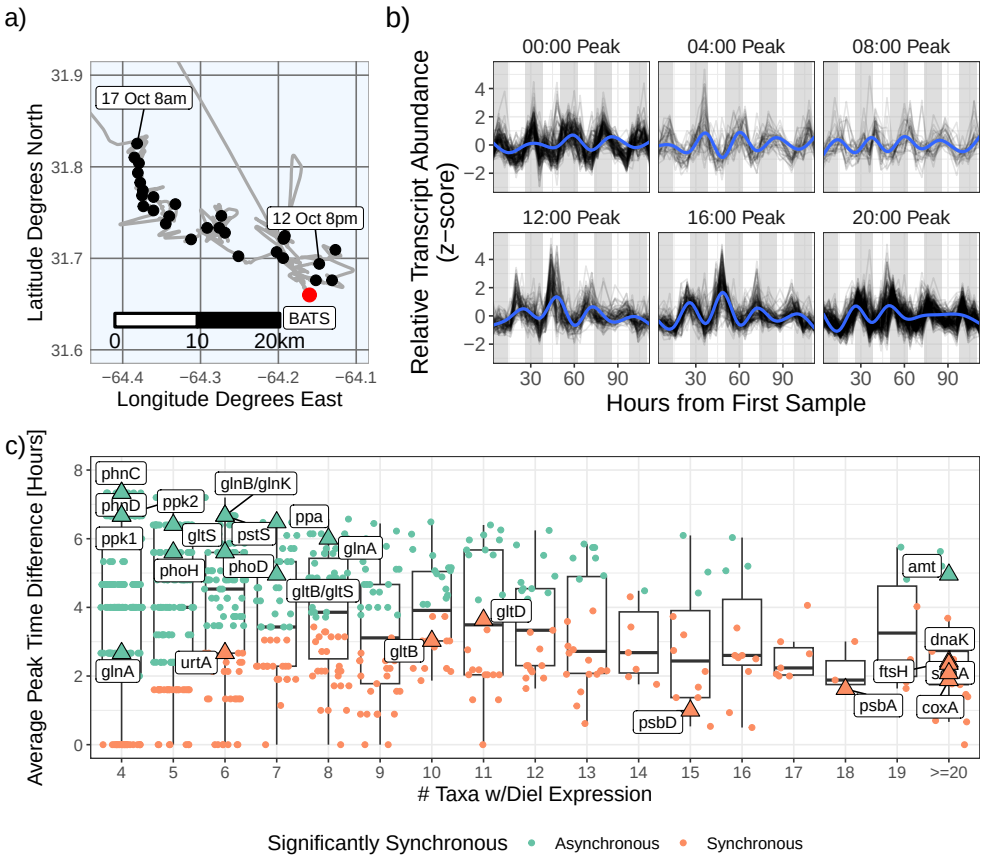

Figure 1. Analysis of community-level diel gene expression. a) Lagrangian cruise track with BATS station annotated in red and sampling locations annotated in black. Initial and final samples are labelled with local date and time. b) Example gene transcript abundance time series for diel genes belonging to the transporter KEGG pathway. Individual black lines represent the transcript abundance of one gene, the blue line represents a generalized additive model smoothing among all genes. Panels indicate the classification of genes by peak time as determined by the peak time analysis described in Methods. Shaded vertical bars indicate hours after sunset and before sunrise (18:00-06:00 local time). c) Mean pairwise differences in peak timing for genes with diel expression across at least 4 distinct taxa. Key genes in nitrogen assimilation and phosphorus assimilation are indicated. Orange point color indicates a gene with significantly (FDR=10%) synchronized transcription. Green points indicate a gene without synchronized transcription, labeled 'asynchronous'.

here) of genes with diel transcript abundances.

We estimated the peak time of day for each gene determined to have diel periodicity. Eukaryotic phytoplankton, cyanobacteria, and heterotrophic bacteria had diel expression patterns of different genes occurr throughout all times of day, with eukaryotic phytoplankton expressing the greatest number of diel genes at dusk, heterotrophs during the day, and cyanobacteria divided between dawn and dusk (SI Data S3). Example gene expression time series for diel genes with annotations as ABC-type transporters are shown in Figure 1b.

Next we assessed the extent to which the diel timing was synchronized among genes with similar putative function. Genes were functionally annotated using the Kyoto

Encyclopedia of Genes and Genomes (KEGG) orthology assignments [1]. The diel genes spanned 3,647 ortholgues. We further analyzed the 849 KEGG orthologues with diel expression in at least four different taxa (eukaryotic phyla/cyanobacterial genera/heterotrophic bacterial orders). For each of these orthologues, we calculated the mean difference in peak time between all pairs of taxa with diel expression of that orthologue. We used an empirical hypothesis testing framework to assess whether that difference was significantly smaller than would be expected by chance given the data set (see Methods). At a false discovery rate of 10%, we identified 324 out of 849 orthologues with significantly synchronized timing across taxa. Significantly synchronized genes included cell division and replication related functions (*ftsH*,*dnaK*), photosynthesis-related genes (*psbD*,*psbA*), and carbon metabolism genes related to the tricarboxylic acid (TCA) cycle and glycine metabolism (*sdhA*,*coxA*) (Figure 1c, SI Data S4).

A pattern also emerged from genes with high degrees of asynchrony across taxa. In particular, genes encoding the uptake and assimilation of nutrients had high asynchrony. We found asynchronous expression of some nitrogen assimilation-related genes, such as components of the GS-GOGAT system and its regulatory proteins as well as the ammonium *amt* uptake receptor (Figure 1c). Asynchronized expression of these genes is possibly related to temporal niche partitioning, which may help to reduce the severity of competition for ammonium [27]. These results may suggest that this form of nitrogen partitioning is common to the oligotrophic ocean. However, we find an even higher degree of asynchrony in genes involved in the uptake and incorporation of phosphorus. The phosphonate transporter components *phnC* and *phnD*, inorganic phosphorus transporter *pstS*, phosphate stress regulatory genes *phoH* and *phoD*, inorganic pyrophosphatase *ppa*, and polyphosphate kinases *ppk1* and *ppk2* all had among the highest degrees of asynchrony in the data set (Figure 1c). A higher degree of asynchrony for phosphorus uptake functions may be further indication of the relevance of diel temporal partitioning as an emergent ecological strategy to overcome limitation of shared scarce resources.

We then examined the taxon-specific transcriptional dynamics of genes involved in the uptake of inorganic and organic phosphorus species to look for specific evidence of uptake partitioning among different functional groups of organisms. We extracted KEGG orthologues specifically annotated as either inorganic phosphate transporters (*pstABC,pstS* - KEGG orthologues K02036,K02037,K02038,K02040) or organic phosphonate transporters (*phnCDE,phnK,phnS* - KEGG orthologues K02041, K02042, K02044, K05781, K11081) and analyzed the relative peak timings of different taxonomic groups. Among these transporters, *pstS*, *phnC*, and *phnK* had significantly diel transcript expression in at least 4 different taxa, and none of the transporters had synchronized expression in our synchronicity analysis. Assembling the gene expression time series of all phosphorus-uptake related transporters among all taxa show different timings in uptake function among cyanobacteria, eukaryotic phytoplankton, and heterotrophic bacteria (see Figure 2). The inorganic phosphorus transporter gene *pstS* had diel transcription in Rhodospiralles and another alphaproteobacterial order peaking at 08:00 (roughly corresponding to sunrise) along with transcripts assigned to the phylum Dinophyceae, while transcripts assigned to diatoms peaked at 12:00 (noon) and both *Prochlorococcus* and *Synechococcus* had peak expression at 20:00 (roughly corresponding to dusk) (Figure 2). Meanwhile, organic phosphonate transporters are widely transcribed by heterotrophic bacteria, primarily alphaproteobacteria, throughout the day. No eukaryotic organisms had significantly diel transcription of *phn* phosphonate transporters. *Prochlorococcus* and *Synechococcus* had diel expression of the phosphonate transporter subunits *phnC* and *phnD* (*Prochlorococcus* only), but

Prochlorococcus had peak transcript abundance of these genes during the day and at dusk, concomitant with inorganic phosphorus transporters, while *Synechococcus* expressed *phnC* at 00:00 (midnight), after transcript abundance of *pstS* began to decline. Rhodospiralles had peak transcript abundance of both *pstS* and *phnD* at the same time (08:00), while the alphaproteobacterial order Rhodobacterales did not have diel expression of any inorganic phosphate transporters, but did have diel expression of *phnC* and *phnK* during at 16:00 (Figure 2). Notably, the taxa that did have diel expression of phosphorus transporter systems, regardless of inorganic or organic species, tended to have peak transcript abundance of all phosphorus transport genes at around the same time of day, with the exception of *Synechococcus*, which appeared to exhibit a temporal cascade from primarily inorganic phosphorus transporter transcripts at dusk to phosphonate transporter transcripts at midnight. Therefore, apart from *Synechococcus*, timing of diel expression for phosphorus uptake transporters is not influenced by phosphorus being in organic versus inorganic forms. These results suggest that diel partitioning of phosphorus uptake is not entirely due to extrinsic diel cycles in phosphorus speciation that may be induced by e.g., photolysis of phosphorus-containing dissolved organic matter.

Discussion

Here, we tested the hypothesis that complex microbes would temporally partition phosphorous uptake and utilization gene expression in a phosphorus-stressed open ocean oligotrophic ecosystem in the North Atlantic, paralleling previous findings on nitrogen uptake from a primarily nitrogen-limited ecosystem in the North Pacific [27]. Using community metatranscriptome data from a Lagrangian field campaign in the Sargasso Sea, we identified diel gene transcription of phosphorus uptake and assimilation genes across diverse microorganisms. We found diel gene expression was not synchronized across the community, and was in fact even less synchronized than genes with diel expression across diverse taxa related to nitrogen uptake and assimilation. Studying the dynamics of phosphate and phosphonate transporters in more detail, we identified a diel cascade in phosphate transporter expression starting with heterotrophic bacteria in the morning, eukaryotic phytoplankton during the day, and cyanobacteria at dusk. Likewise, we found diel gene transcript abundances for phosphonate transporters with the heterotrophic bacterial order Rhodospiralles expressing phosphonate transporters in the morning (coinciding with phosphate transporters), Rhodobacter during the day, *Prochlorococcus* at dusk (coinciding with its phosphate transporters), and *Synechococcus* overnight (following its phosphate transporters). Our results suggest temporal partitioning of uptake for both phosphorus species, and coinciding timing of phosphate and phosphonate uptake on a taxon-by-taxon basis further suggests that substrate-level specialization is not necessarily sufficient to avoid competition for this scarce resource [25], and further suggests diel cycles in transporter gene transcription are not directly tied to changes in the relative pools of different phosphorus species. Complementary evidence for diel temporal niche partitioning for distinct nutrients (N in the North Subtropical Pacific [27] and P in the Sargasso as shown here) may be indicative of complex, community-level metabolic plasticity across ocean basins. Diel temporal niche partitioning to mitigate competition for limiting resources in oligotrophic environments represents an additional mechanism to resolve the paradox of the plankton and the maintenance of complex, marine microbial communities [15].

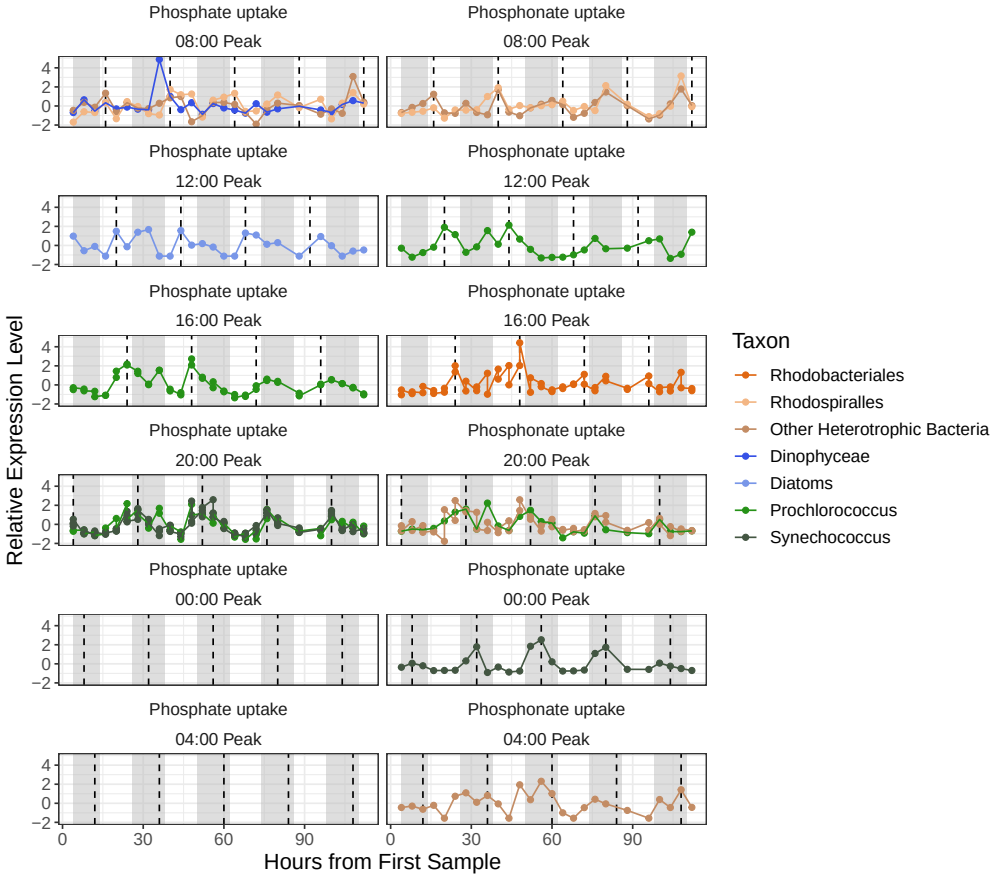

Figure 2. Temporal dynamics of phosphorus acquisition gene transcription. Each line plot shows the gene transcript abundance time series of a specific phosphorus-uptake related gene for a specific eukaryotic phylum or bacterial order (or cyanobacterial genus). Line color indicates the taxa expressing the transcript. Panels are divided horizontally by time of peak expression as calculated in methods, with 08:00 representing 8am local time, 12:00 noon local time, and so on. Shaded bars indicate hours between sunset and sunrise (18:00-06:00 local). Dashed lines are added to correspond to the peak time of genes in each panel. Panels are divided vertically by the species the gene is involved in transporting. The phosphate uptake category includes phosphate transporter subunits *pstABC,pstS* (KEGG K02036,K02037,K02038,K02040). The phosphonate uptake category includes the phosphonate transporter subunits *phnCDE* and putative phosphatase uptake subunits *phnK,phnS* (KEGG K02041, K02042, K02044, K05781, K11081).

Materials and Methods

Sample Collection and Processing

Fieldwork was conducted from 12 October to 19 October, 2019, in the Sargasso Sea near the location of the BATS station. A drogue was deployed before the first cast to facilitate a Langrangian sampling stratey. Water column sampling took place every 4 hours for a period of approximately 5 days sampling 5 m depth. See Supporting Information for details on RNA collection and extraction, library preparation and metatranscriptome processing.

Gene Expression Diel Periodicity and Synchronization Analysis

Please see Supporting Information for more details on timeseries analysis. Briefly, variance-stabilized RNA read counts were subjected to RAIN nonparametric diel analysis as described in [27]. Peak rank time calculations were performed and synchronicity analysis was conducted as described in [27].

Metatranscriptome Preparation, Sequencing, and Processing

Seawater was filtered through a 0.2 micron pore size Sterivex filtration unit and immediately transferred to a -80 C freezer. RNA was extracted using a publicly available phenol-chloroform based protocol [11] and DNA was reduced using the Turbo DNA-free kit (Ambion). Metatranscriptome libraries were prepared by reducing ribosomal RNA using the QIAGEN's FastSelect kit (Bacteria) and sequenced (2 x 151 nt) using the low-input protocol for total RNA on the Illumina Novaseq S4 platform under the DOE Joint Genome Institute (JGI) Community Sequence Proposal ID 505733. For samples with <10 ng of RNA total, the ultra-low input protocol was used. Raw read filtering and trimming was done using BBDuk v38.67 and BBMap v38.84 from the BBtools packages [5]. Trimmed filtered reads were concatenated across samples and assembled using MEGAHIT v1.2.9 [21]. MetaGeneMark v3.38 was used to call open reading frames (ORFs). For the cellular community, trimmed filtered reads were mapped to the combined assembly using BBMap v38.84 [5] using default parameters and tabulated using featureCounts [22]. The ORF protein sequences were annotated using eggNOG-mapper v2.1.4 (with DIAMOND blastp alignment [14]) for functional annotation, and aligned to the PhyloDB database (https://github.com/allenlab/PhyloDB) using the software package EUKulele [17] for taxonomic annotation.

Diel Transcript Abundance Analysis

Prior to timeseries analysis, all metatranscriptome read mappings were transformed using the DESeq2 variance stabilizing transformation [24]. Methods for periodicity determination and peak time estimation are described in detail in [27]. Briefly, for each time series a linear trend was removed to account for moving averages. Then, the rain [35] method was applied and an FDR control at 10% were set to assess significance. For significantly diel time series, peak time was estimated by calculating the mean rank of each time of day across all samples and selecting the largest.

Transcript Synchrony Analysis

Methods are described in detail in [27]. Briefly, only KEGG orthologues with diel transcription in at least 4 different taxa were analyzed. For each orthologue, the pairwise difference in estimated peak time was calculated for each pair of taxa with diel expression of that orthologue. The mean of this value across all pairs was used as a test statistic and significance was assessed using a monte carlo simulated null ensemble of all peak times from the dataset. Significance was set at an FDR=10% threshold.

Data and code availability

Sequence data are publicly available under the DOE JGI Community Sequencing Proposal ID 505733 (`https://genome.jgi.doe.gov/portal/Infvirtimeseries/Infvirtimeseries.info.html`)

Code and processed sequence data used for analyses are available at `https://github.com/d-muratore/phosphorus_partitioning`.

Supplemental files available for this manuscript are described below:

Supplemental Data 1: Ship navigation data with sampling locations and sample environmental data for all metatranscriptome samples.

Supplemental Data 2: Results of RAIN diel periodicity analysis. Columns include KEGG orthologue tested, taxonomic assignment of KEGG orthologue, RAIN test p-value, and a reject/fail to reject decision on the null hypothesis that the transcript does not have a 24-hour diel oscillation.

Supplemental Data 3: Peak time and functional and taxnomic annotation for genes with diel periodicity.

Supplemental Data 4: Results of KEGG orthologue synchronicity analysis. Columns show the KEGG Orthologue number of the gene tested, the number of taxa in the dataset with diel expression of this KO, the pathway assignment of the KO, the average difference in peak rank time between all organisms with diel expression of the KO, the associated empirical p-value (10,000 Monte Carlo simulations) and the reject/fail to reject decision of the null hypothesis that the transcript is not synchronized.

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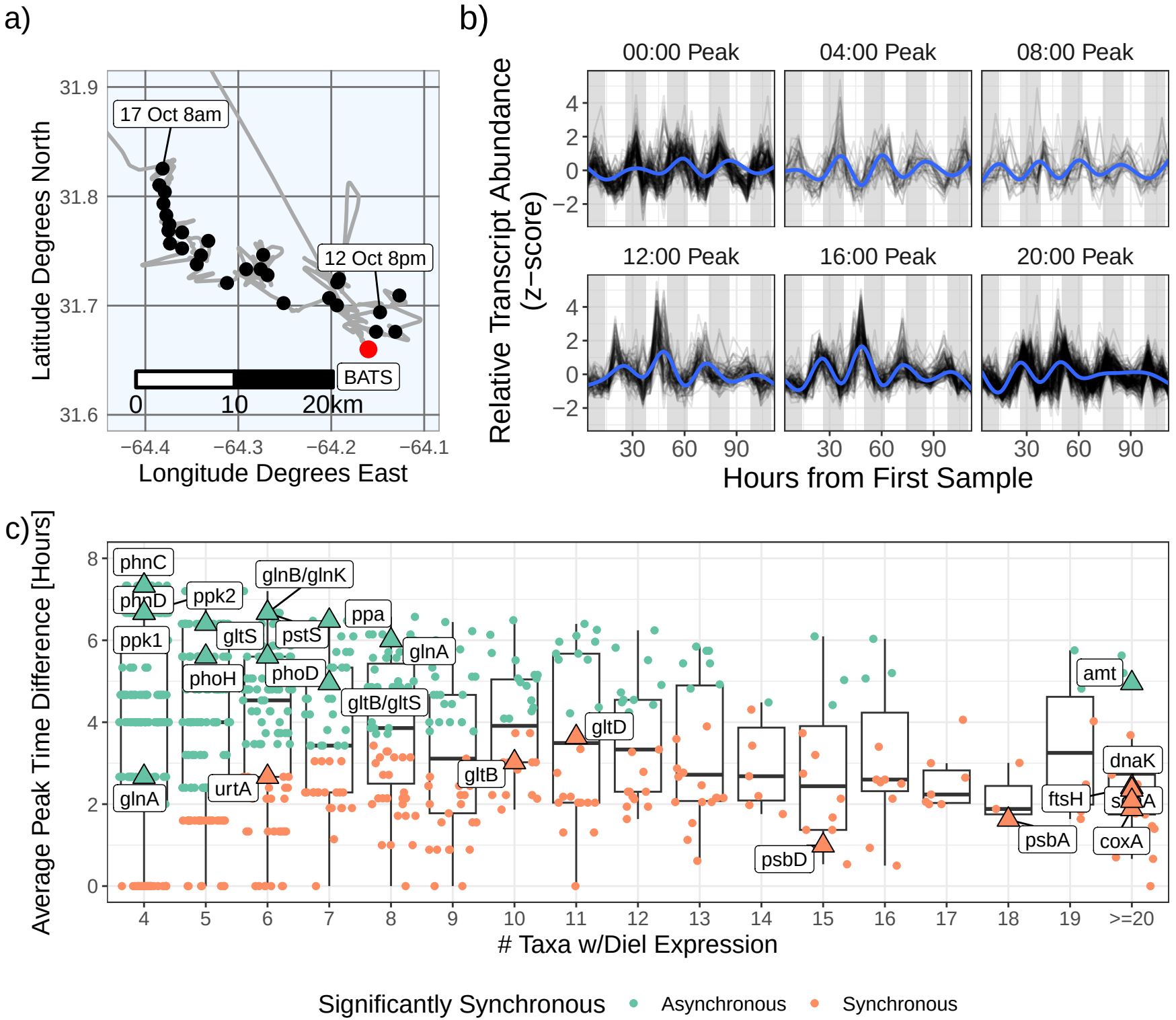
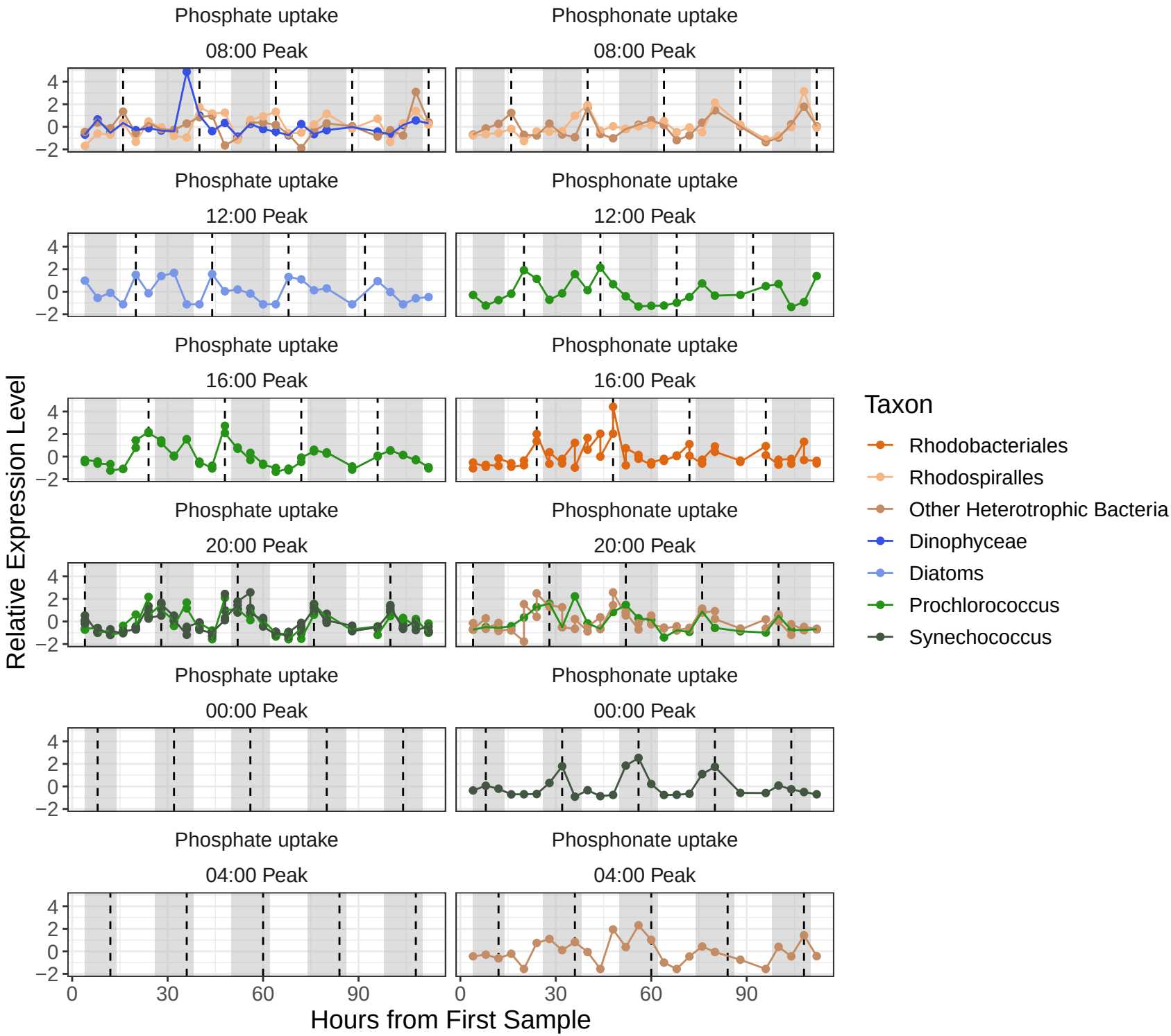

a)
17 Oct 8am
12 Oct 8pm
BATS
0
10
20km
Latitude Degrees North
Longitude Degrees East
31.9
31.8
31.7
31.6
−64.4
−64.3
−64.2
−64.1
b)
00:00 Peak
04:00 Peak
08:00 Peak
12:00 Peak
16:00 Peak
20:00 Peak
Relative Transcript Abundance (z−score)
Hours from First Sample
c)
Average Peak Time Difference [Hours]
Taxa w/Diel Expression
phnC
phnD
ppk1
ppk2
glnB/glnK
gltS
pstS
ppa
glnA
phoH
phoD
gltB/gltS
gltD
gltB
urtA
psbD
amt
dnaK
ftsH
psbA
coxA
Significantly Synchronous
Asynchronous
Synchronous


Phosphate uptake
08:00 Peak
Phosphonate uptake
08:00 Peak
Phosphate uptake
12:00 Peak
Phosphonate uptake
12:00 Peak
Phosphate uptake
16:00 Peak
Phosphonate uptake
16:00 Peak
Phosphate uptake
20:00 Peak
Phosphonate uptake
20:00 Peak
Phosphate uptake
00:00 Peak
Phosphonate uptake
00:00 Peak
Phosphate uptake
04:00 Peak
Phosphonate uptake
04:00 Peak
Relative Expression Level
Hours from First Sample
Taxon
Rhodobacteriales
Rhodospiralles
Other Heterotrophic Bacteria
Dinophyceae
Diatoms
Prochlorococcus
Synechococcus