# METATRANSCRIPTOME ANALYSIS OF PHYTOPLANKTON COMMUNITIES IN RESPONSE TO HURRICANE HARVEY

A Thesis

by

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## ABSTRACT

Hurricanes influence microplankton communities through many processes including mixing of the water column, changing community structure, and changing nutrient availability. In August of 2017, Hurricane Harvey inundated the Texas coastline with historic quantities of freshwater leading to drastic hydrographic changes. A month after the passage of the hurricane, two research cruises were conducted a week apart to examine the microplankton response using abundance and gene expression data.

Using metatranscriptome and abundance data it was determined that the two major groups of phytoplankton, diatoms and dinoflagellates, were subject to nitrogen limiting conditions and large changes in hydrography. In response to nutrient limitation, both groups utilized inorganic and organic forms of nitrogen from external sources and from internal processes. A characterization of the major metabolic functions of several diatom and dinoflagellate genera which changed markedly between responses cruises revealed a diversity of strategies contributing to their overall response.

# DEDICATION

To young Emily: from the moment you saw your first pteropod and became hooked on the world of plankton may that child-like curiosity and fascination continue.

To Emily at her lowest point: may this be a reminder of all that you can accomplish when it seems like everything is falling apart and no one understands.

To future Emily: may you look back at your time at Texas A&M and your time compiling this body of work as a transformative and positive experience in which you learned not only so much about your work but also yourself.

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### Contributors

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The data analyzed for this thesis was provided by Dr. Lisa Campbell and Dr. Darren Henrichs. The conversion of cell counts to abundance per unit of sample volume was provided by graduate student James Fiorendino. Hydrographic data and nutrient concentration during the study period was described in a previous work published in 2021 by James Fiorendino.

All other work conducted for the thesis was completed by the student independently.

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## NOMENCLATURE

| | |
|---|---|
| IFCB | Imaging FlowCytobot |
| CTD | Conductivity, Temperature, Depth sensor |
| RNA | Ribonucleic acid |
| DNA | Deoxyribonucleic acid |
| cpm | Counts Per Million |
| KEGG | Kyoto Encyclopedia of Genes & Genomes |
| KAAS | KEGG Automatic Annotation Server |
| ANOSIM | Analysis of similarity |

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## Introduction

Phytoplankton play vital roles in the marine ecosystem including mediation of biogeochemical cycles, being major contributors to primary production and forming the basis of food webs in the marine environment (Field et al. 1998). To survive in the dynamic and competitive marine environment, phytoplankton have adapted a wide array of metabolic and morphological adaptations leading to an astonishing quantity of diversity. One of the limitations of phytoplankton functionality and composition is nutrient availability. Nutrient availability has been demonstrated to limit the growth of coastal phytoplankton in regions around the world. The limiting nutrient predominantly affecting coastal regions is nitrogen as phosphorus is typically replete (Moore et al. 2013). Phytoplankton have adapted many strategies for dealing with nutrient stress such as the ornithine-urea cycle, utilizing amino acids as sources of nitrogen, and having various affinities for different organic and inorganic forms of nitrogen (Armbrust et al. 2004, Bronk et al. 2007, Solomon et al. 2010, Alexander et al. 2015). These responses are critical in determining the community composition and the resilience of various species.

Determining the composition of the phytoplankton community and the factors that drive many species to coexist using limited resources has been a complex problem that has been the subject of numerous studies. One of the proposed mechanisms explaining this paradox is the ability of some species to outcompete others for a limited quantity of resources. Two major phytoplankton groups: diatoms and dinoflagellates have adapted different mechanisms for competing for resources. Diatoms evolved the ornithine-urea cycle in which many byproducts are formed and used for biosilification, signaling purposes, or synthesis of amino acids (Armburst et al. 2004). Diatoms have also been noted to contain internal vacuoles capable of

nutrient storage which can compose 30-90 % of the total cell volume (Smayda 1970). Dinoflagellates have been found to contain large internal pools of ammonium and urea which they can reduce to use for amino acid synthesis and other cellular functions (Solomon & Glibert, 2008). Mixotrophy is also a common adaptation in dinoflagellates and can increase the array of sources of nutrients when limitations on autotrophy are present. Overall, diatoms have been shown to thrive in nutrient-rich environments where dinoflagellates thrive in nitrogen limited environments (Zhang et al. 2019).

Genus and species-specific responses to nutrient limitation can also vary due to several interrelated factors such as light, temperature, and the available nutrients. Many studies have examined differences in the affinity and utilization of various nitrogen sources at a genus, species, and strain level and determined that each have different preferences and abilities to utilize different forms of nitrogen under different conditions (Levasseur et al. 1993, Ferguson et al. 1976, Solomon & Glibert 2008, Sinclair et al. 2009). As these experiments were restricted to laboratory studies, the interaction effects of other members of the phytoplankton community and the constant flux of the natural environment are negated, and the paradigm of nitrogen utilization is oversimplified.

The development of high throughput sequencing has yielded the technique of metatranscriptomics to analyze the functional responses of community assemblages lessening the limitations of laboratory studies and allowing for minute examination up to the species level. Previous metatranscriptome analyses have focused on responses of phytoplankton communities to harmful algal blooms (Harke et al. 2017, Zhang et al. 2019, Metengier et al. 2020), along environmental gradients (Hewson et al. 2014, Gong et al. 2018), or along shifts in community composition from dominance of one group to another (Alexander et al. 2015, Zhang et al. 2019).

These studies have found that the response to nutrient limitation is largely characterized by an increase in internal cycling mechanisms for the limiting nutrient and/or an increase in transporters. In some cases, such as in response to harmful algal blooms, nutrient stress is not a strong driver of species succession and community response is instead driven by other processes such as cell mobility, cell signaling, antimicrobial response, or energy harvesting (Zhang et al. 2019, Metengier et al. 2020).

In recent years, the effects of extreme events, such as tropical cyclones, on phytoplankton communities have been extensively studied. Hurricanes can decrease seawater salinity near the coast due to increased precipitation and runoff, introduce new nutrients via upwelling, and transport offshore populations of phytoplankton to coastal regions (Paerl et al. 2006, Wetz & Paerl 2008). The extent of stratification within the water column can also change and has been demonstrated to limit or aid in the response of certain major groups (Fiorendino et al. 2021).

Studies on the early response and succession of the phytoplankton community to the influence of hurricanes have revealed that diatoms can bloom immediately following hurricanes due to increased ability to uptake new nutrients. A subsequent bloom of dinoflagellates or ciliates may then occur using nutrients that were released by the diatoms and/or directly consuming the diatoms. (Anglès et al. 2015). Another study of microplankton community response was conducted in an estuary shortly after Hurricane Harvey immediately before this study was conducted. In August of 2017, Hurricane Harvey made landfall and stalled for four days on the coast of Texas. The Houston area was inundated with 1.5 m of water over that period, eventually releasing an equivalent of $17 \pm 5$ $km^3$ (around three times the volume of Galveston Bay) of fresh water from Galveston Bay to the oceanic shelf (Thyng et al. 2020). Steichen et al. 2020 described the changes of the phytoplankton community within Galveston

Bay, a local estuary north of our study region, over a period of 24 days following the monumental release of freshwater from Hurricane Harvey and determined a shift from a ciliate dominated community pre-Hurricane Harvey to a diatom-dominated community following Hurricane Harvey and then transitioning to a dinoflagellate dominated community. Results of the Anglès and Steichen studies, provide a baseline of observations and justification for studying the response of the microplankton community along the Texas coast following the mass efflux of freshwater.

Metatranscriptomics and Imaging FlowCytobot (IFCB) abundance data were used to determine and quantify the cellular activities and the changes in abundance of the microplankton community in the month after Hurricane Harvey during two response cruises a week apart. Metatranscriptomics was used to characterize whole gene expression profiles for the phytoplankton community to determine responses of major metabolic functions and nitrogen metabolism. IFCB analysis was conducted to quantify and describe the phytoplankton community. Utilizing the two approaches together provides a comprehensive understanding of the response of the phytoplankton community to freshwater efflux by characterizing community assemblage and functional response on a level that has not been attempted previously.

Changes in hydrography and nutrient availability were determined to have a strong influence on microplankton community composition and biomass during the same time period as this study (Fiorendino et al. 2021). Hydrographic data revealed a persistence of a low salinity waters along the coast of Texas and an increase in the magnitude of down-shore current velocity (Fiorendino et al. 2021). As this water was transported downcoast, a breakdown of stratification was observed. At some stations, an onshore intrusion of offshore water occurred along the coast further altering community composition. Throughout the course of the response cruises, DIN:

DIP ratios suggested a nitrogen limited system. Nitrate was below detection at almost all stations, whereas urea and ammonium were present at all stations.

As nitrogen was limited and there were strong hydrographic changes observed between the two response cruises, the objectives for this study were to determine differences in nitrogen metabolism of diatoms and dinoflagellates and to assess those differences at genus and species levels among stations that were influenced by the offshore intrusion and variable downcoast current. Our hypotheses were: (1) Changes to nitrogen metabolism would dictate changes in diatom and dinoflagellate abundance. (2) that diatoms would use inorganic forms of nitrogen and dinoflagellates would use organic forms of nitrogen. Initial differential expression analysis was performed at the group level to assess the differences in nitrogen utilization response between diatoms and dinoflagellates. Subsequent differential expression analysis was performed at the genus level for diatoms and dinoflagellates that showed a notable change in abundance between the two response cruises. Furthermore, differential expression analyses were performed at the species level for *Karenia*, a prominent harmful algal species in the Gulf of Mexico which was observed to increase in two orders of magnitude.

## Methods

### Response cruises

Two research cruises were conducted a month following Hurricane Harvey. On the first response cruise (Leg 1, September 23-25) and the second response cruise (Leg 3, September 29-October 1), samples were collected at six stations from Galveston Island to Port Aransas (Figure 1). The Leg 2 cruise track was limited to the mouth of Galveston Bay and no biological samples were collected. Metabarcoding and Imaging FlowCytobot (IFCB) data were used to determine the quantity and the composition of the phytoplankton community**.** Metatranscriptomics data

were used to determine the response of the phytoplankton community by providing taxonomic and functional annotations. Depth profiles of temperature, salinity, and chlorophyll were taken at each station using a SBE25 CTD (SeaBird Scientific, USA). Nutrient samples for ammonium, nitrate, nitrite, urea, phosphate, and silicate were collected around 3m using a CTD rosette, filtered through Whatman 25 mm GF/F filters, and stored at -20°C until analysis using standard autoanalyzer methods (Gordon et al. 1994).

**Imaging FlowCytobot (IFCB)**

Microplankton abundance was characterized at each station using image data collected by an Imaging FlowCytobot (IFCB, McLane Research Laboratories, Inc. USA). The IFCB is an instrument used to obtain high resolution images of cells containing chlorophyll by subjecting a small water sample through flow cytometry and video imaging (Olson and Sosik, 2007). To analyze the microplankton community triplicate 5 mL samples were collected at around 3m from a CTD rosette and analyzed immediately onboard. Samples were filtered to exclude objects greater than 153 µm. A custom convolutional neural network composed of previous categorized images was used to analyze images into one of 112 groups (Henrichs et al. 2021). 101 categories were manually inspected to verify classification. All data was uploaded and made available at https://toast.tamu.edu/timeline?dataset=HRR_cruise. For each category, the abundance (cells/mL) was calculated by dividing the total number of cells by the total number of milliliters of seawater analyzed by the IFCB.

**RNA Sequencing and Assembly**

Triplicate samples for RNA were collected at the surface at each station and filtered onto a 47 mm, 5 µM Supor filter (Millipore) using a vacuum pump. 1 L was filtered at each station

during the first response cruise and 1.4 - 3 L were filtered at each station during the second response cruise. Filters were folded and immersed in RNALater (manufacturer) and left to sit for 10 min. before being placed into a -20°C freezer aboard the ship. Filters were transported to the Campbell Lab at Texas A&M College Station on dry ice and placed into a -80°C freezer until extraction. Filters were cut into strips before lysis buffer was added and RNA was extracted using the Qiagen AllPrep DNA/RNA Micro Kit following the manufacturer's protocol (Qiagen). RNA extracted from samples was sent to the University of Delaware Sequencing and Genotyping Center for Illumina sequencing (HiSeq2500 2x150bp paired end reads). 90 samples collected within a few meters of the surface and at increasing depth were sent for sequencing with the goal of 25 million pairs of reads per sample.

FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) was used to quality trim the reads with a minimum phred score of 35. Normalized reads were assembled using Trinity (Grabherr et al. 2011), Velvet (Zerbino and Birnney 2008) and Oases (Shultz et al. 2012). Reads from all six samples at a station (three at the surface and three at depth) were combined for assembly. The assembled transcripts for all assemblies were merged into one large assembly using cd-hit-est (CD-HIT, Li et al. 2006) with clustering of 90% similarity cutoff. 1,331,131 transcripts were assembled and quantified for read abundance.

**Assignment of Transcript Taxonomy**

Taxonomic annotation was performed against the Gordon and Betty Moore Foundation's Marine Microeukaryote Transcriptome Project (MMETSP) (Keeling et al. 2014) and additional transcriptomes of *Karenia mikimotoi, K. papilionacea* (Ryan & Campbell 2016), and *Prorocentrum texanum* from the Campbell lab at Texas A&M University using BlastN with a minimum e-value cutoff of 1e-20.

A reference transcriptome of *K. selliformis* was also obtained for this study by sequencing a *K. selliformis* (ARC356) culture isolated from Delaware. BBNorm was used to trim reads for adapters and based on quality, filter by length, and filter reads by quality (v38.90: Bushnell 2014). The same Trinity, Velvet and Oases approach used to assemble reads as described above for the Hurricane Harvey dataset.

**Functional Annotation**

The reference databases PFAM, TIGRFAM, SUPERFAMILY, BLAST, and Kyoto Encyclopedia of Genes and Genomes (KEGG) database were used as reference databases for which functional annotation was determined. Pfam, TIGRFAM, and Superfamily annotations were collected using Interproscan (v5**;** Jones et al. 2014). BLAST annotations were determined using a cutoff value of 1e-20. KEGG annotations were performed using the KAAS server (Moriya et al. 2007). Construction of major metabolic figures were grouped based on KEGG orthology module hierarchies.

**Analysis of Similarity (ANOSIM)**

An analysis of similarity was performed to determine if significant differences existed between pre-determined groupings. ANOSIM is a non-parametric statistical test which uses a dissimilarity matrix calculated from normalized read counts. ANOSIM was performed using the vegan package on R (v2.5-7; Clarke K.R. 1993) using log(x+1) normalized reads of nitrogen metabolism for the genera: *Chaetoceros, Skeletonema, Thalassiosira, Ditylum, Pseudo-nitzschia, Cylindrotheca, Karenia, Karlodinium, Scrippsiella,* and *Prorocentrum* to determine if there were significant differences in nitrogen metabolism among several categories. These ten genera were chosen based on abundance data from the IFCB that classified them as increasing, decreasing,

constant presence, or irregular between response cruises. The categories used to determine significant differences were between response cruises, between locations North, South, and station S16 within each response cruise, and across locations North and South within each response cruise. For locations grouped by North, South and S16, the north stations were stations GI, SS, and S21 and the south stations were S11 and S06 (Figure 1). For locations grouped by North and South, station S16 was grouped into South. These categories were determined based on offshore intrusions observed at station S16 and an increase in downcoast current velocity at southern stations S16, S11, and S06 between response cruises. Analyses were performed using a Bray-Curtis dissimilarity matrix with 9999 permutations. For each analysis an ANOSIM statistic R and a p-value was given. R-statistics greater than 0.7 with p-values less than 0.05 were considered significant.

**Differential Expression Analyses**

Differential expression analysis was performed to determine if there were significant differences between response cruises and across stations for genes expressed within nitrogen metabolism and what those changes were. Differential expression analysis was performed using edgeR (v3.32.0; Robinson et al. 2010) on RStudio (v 4.0.3; RStudio Team 2020). Count files for individual genera: *Chaetoceros, Skeletonema, Thalassiosira, Ditylum, Pseudo-nitzschia, Cylindrotheca, Karenia, Karlodinium, Scrippsiella,* and *Prorocentrum* were supplied as input for differential expression analysis. Analysis was performed to compare differences in expression of each station using the first response cruise as the baseline for comparison against the second response cruise. Additional analyses were performed across all stations and both legs for each genus to examine shifts in response spanning across all parameters. Transcripts that were greater than 100 counts per million in at least two of the replicates were retained for further analysis to

ensure the most abundant transcripts were used for analysis. TMM normalization was applied to the remaining reads to account for variation in library size for each sample. GLM common dispersion was applied and differentially expressed genes were determined as those that had a p-value of less than 0.05 as determined by the Benjamini-Hochberg multiple testing correction (Benjamini & Hochberg, 1995).

## Results and Discussion

### Overall community response to Hurricane Harvey

For both metatranscriptome and Imaging FlowCytobot (IFCB) abundance results, diatoms were the dominant group in the first response cruise while dinoflagellates dominated in the second response cruise (Figure 2). Combined, diatoms and dinoflagellates comprised between 40-65 % of the total measured community all stations. Diatoms ranged between 8-30% of the total cell count with abundances increasing at stations GI, S16, S11, and S06 and decreasing at stations SS and S21 between response cruises. Dinoflagellates composed 14-40 % of the total cell count increasing at stations SS and S21 and decreasing at stations GI, S16, S11, and S06 between legs.

Metatranscriptome read abundances showed a larger shift between the first response cruise and the second response cruise for diatoms and dinoflagellates in comparison to abundance data from the IFCB. Overall, the combined percentages of diatoms and dinoflagellates ranged from 60-75% of the total metatranscriptome reads (Figure 2). Diatom contribution to metatranscriptome reads ranged from 1-15% of the total reads and decreased at all stations except S06 between response cruises. Dinoflagellates composed 25-55% of the total metatranscriptome reads. At the second response cruise, stations SS and S21, the proportion of dinoflagellate reads increased from 26.9% to 88.6% and 54.3% to 83.6 % respectively, while

stations GI, S16, and S11 only increased 20% between response cruises. The dominance of dinoflagellate reads at stations SS and S21 was consistent with IFCB data showing a 'bloom' of dinoflagellates at these stations between response cruises (Figure 2).

**Nitrogen metabolism response of diatoms and dinoflagellates**

The ANOSIM analysis of diatom and dinoflagellate nitrogen metabolism were used to determine if there were significant differences between different location categories. Based upon results from previous studies observing community response to hurricanes and changes in nutrient availability, it was hypothesized that significant differences in nitrogen metabolism response could be attributed to changes in hydrographic conditions observed during the two response cruises. Diatoms exhibited significant differences in nitrogen metabolism between stations during both response cruises independent from one another, with R values of 0.9667 and 0.8922 respectively (Table 1). Diatoms did show significant differences in nitrogen metabolism between categories, north and south, and north, south and station S16 but at a lesser correlation value than between stations (Table 1). This would indicate that while diatom nitrogen gene expression was impacted by the offshore intrusion present at station S16 during the second response cruise, there could potentially be another factor causing the changes to diatom nitrogen gene expression such as competition with dinoflagellates to obtain and utilize limited supplies of nitrogen. Dinoflagellates expressed significant differences between stations for both response cruises independently and between categories, north and south, and north, south, and station S16, during the second response cruise (Table 1). The significant differences between the two location categories could indicate that changes to dinoflagellate nitrogen metabolism could be driven by the offshore intrusion that persisted at northern stations and by the increase in downcoast current velocity at station S16 which served as a barrier between the northern

stations. Northern stations during the second response cruise displayed a dinoflagellate 'bloom' which could have occurred due to the offshore intrusion bringing waters high in dinoflagellate abundance to shore. An increase of downcoast current velocity seen at stations S16, S11, and S06 is indicative of downwelling as water is pushed alongshore and then due to the prevailing Coriolis force in the northern hemisphere 'piles up' onto the shore. It has been documented that dinoflagellates can thrive under these conditions in comparison to other members of the microplankton community as they can swim and undergo diel vertical migrations (Hetland & Campbell 2007). Station S16 was under the influences of both the offshore intrusion and the increase in downcoast current velocity which could explain the difference in dinoflagellate nitrogen gene expression observed.

**Major metabolic functions of diatoms and dinoflagellates**

ANOSIM results corroborated the motivation for categorizing stations by northern stations (GI, SS, S21), station S16, and southern stations (S11 and S06) for figures depicting major metabolic functions of diatoms and dinoflagellates and the various diatom and dinoflagellate genera. Analysis of the major metabolic functions of diatoms and dinoflagellates revealed distinct differences between groups. Diatoms expressed a higher proportion of genes involved in lipid metabolism, catabolic activity, and photosynthesis than dinoflagellates at all stations (Figure 3). Dinoflagellates expressed higher levels of genes involved in cofactor and vitamin metabolism, amino acid metabolism, cell division, and other metabolism (Figure 3). For both groups, genes involved in nitrogen metabolism were consistently a large proportion of the total reads for each station; However, expression within groups indicates that diatom nitrogen metabolism increased between response cruises and dinoflagellate nitrogen metabolism slightly decreased or remained the same proportion (Figure 3).

**Differential expression analysis of the major groups: diatoms**

An examination of major metabolic functions revealed that nitrogen metabolism gene expression increased between response cruises for north, south, and station S16 for diatoms. The responses of diatom nitrogen metabolism suggested two distinct strategies used during each response cruise. During the first response cruise, internal cycling of ammonium, nitrate, nitrite, amino acids, and urea was dominant (Figure 4). During the second response cruise, diatom expression of nitrogen metabolism genes included increased recycling of glutamate and nitrate. Diatoms could be utilizing nitrate and nitrite preceding and/or during the first response cruise as nitrate and nitrite were almost completely drawn down. This in turn could have lead to their dominance during the first response cruise. Subsequently, during the second response cruise, diatoms could be depending on internal recycling of glutamate and nitrate as they are being outcompeted by the dominating dinoflagellates. Additionally, diatoms could be utilizing their ability to store and later utilize nitrate during nitrogen limited conditions, which could have occurred between the first and second response cruise (Lomas & Glibert 2000). Nitrate/nitrite transporters, nitronate monooxygenase, urea symporters, and amino acid transporters were all more expressed at the first response cruise suggesting an important role in diversifying nutritional strategies (Figure 4). Nitronate monooxygenase has been recently determined to contribute to nitrite production at night by diatoms (Smith et al. 2016). Additionally, nitronate monooxygenase has been cited in fungi and bacteria to detoxify the highly toxic compound 3-nitropropionate, which is an inactivator of succinate dehydrogenase, a prominent enzyme in the citric acid cycle (Altson et al. 1977, Francis et al. 2012). The response of glutamine synthetase and glutamate dehydrogenase relative to glutamate synthase and the ornithine-urea cycle highlighted the importance of intracellular cycling of nitrogen from previously accumulated

carbon sources to direct use to support nitrogen metabolism (Levitan et al. 2015). Diatoms utilized both internal and external sources of inorganic and organic nitrogen and demonstrated distinct strategies between response cruises in response to changes in nutrient availability.

**Differential expression analysis of the major groups: dinoflagellates**

Dinoflagellates increased expression of nitrogen metabolism genes during the second response cruise at all stations. The one gene that was more expressed during the first response cruise was glutamate dehydrogenase. Glutamate dehydrogenase converts glutamate to ammonium and may play an important role in maintaining ammonium stocks during the first response cruise where dinoflagellates were less abundant and ammonium utilization was more prominent within diatoms (Figure 4). At stations where dinoflagellate abundance increased, a suite of transporters and internal cycling mechanisms were significantly expressed (Figure 4). Nitrate, nitrite, ammonium, amino acids, and urea were transported into the cell via various active transporters and symporters, and nitrate and nitroalkane were reduced to nitrite. Additionally, carbamoyl phosphate synthase, glutamine synthetase and glutamate synthase were upregulated, suggesting dinoflagellates were utilizing ammonium for primary amino acid synthesis and ornithine-urea cycle activity. At stations with increased abundance between response cruises, expression of ammonium transporters and urea symporters were upregulated which could corroborate reports of dinoflagellate ability to maintain large internal pools of urea and ammonium (Lomas & Glibert 2000, Solomon & Glibert 2008). Urease was significantly upregulated during the second response cruise suggesting that some of the urea that was taken into the cell via urea symporters was hydrolyzed to form ammonium (Figure 4). Dinoflagellates also exhibited a significant upregulation of nitrate reductase and nitroalkane monooxygenase during the second response cruise which could be indicative of nitrate utilization as nitrate is

drawn down completely by the second response cruise. Analysis of gene expression of dinoflagellate nitrogen metabolism during both response cruises revealed an ability to utilize exterior and interior sources of inorganic and organic nitrogen to support nitrogen metabolism and higher functions like primary amino acid synthesis and ornithine-urea activity.

**Diatom genera abundance responses**

Six diatom genera were chosen for analysis of major metabolic function and differential expression analysis of nitrogen metabolism. The six genera were chosen and categorized based on observed changes in abundance between response cruises. The four categories for the genera were constant, irregular, decreased, and increased between response cruises. *Chaetoceros* and *Thalassiosira* displayed a constant abundance at all stations for both response cruises (Figure 6). Abundance of *Skeletonema* decreased cumulatively across all stations between response cruises (Figure 6). *Ditylum* had irregular abundance, appearing at only northern station SS for both response cruises and southern station S11 during the second response cruise (Figure 6). Some *Ditylum* species can be larger than 150 μm (the maximum cell size for IFCB detection), so abundance could be underestimated for the genus. *Pseudo-nitzschia* and *Cylindrotheca* increased at all stations between response cruises (Figure 6).

**Diatom genera response of major metabolic functions**

Major metabolic functions of the six different diatom genera observed were analyzed to determine if distinct changes to major functions were indicative of changes in abundance in response to Hurricane Harvey. Initial observation of major metabolic functions for all stations determined there were not large differences between stations, so stations were grouped by north stations (GI, SS, S21), station S16, and south stations (S11, S06). Nitrogen metabolism was consistently expressed across all stations and timepoints for all genera (Figure 6). *Chaetoceros,*

*Skeletonema,* and *Thalassiosira* displayed nearly identical breakdowns of major metabolic functions between response cruises and across different stations (Figure 6). *Ditylum* major metabolic functions displayed a greater proportion of cell signaling and catabolic activity than the five other diatom genera observed (Figure 6). The largest contributing metabolic function within cell signaling was the ubiquitin system, which has been documented in diatoms to mediate stress response (Muhseen et al. 2015). This observation is not surprising as *Ditylum* was present in such low abundances and has been demonstrated to respond at slower rates to osmotic stress which could have been brought about the large amounts of freshwater discharge that lowered salinity (Rijstenbil et al. 1989). *Pseudo-nitzschia* and *Cylindrotheca* both increased in abundance between legs and displayed prominent differences between one another and the other four diatom genera (Figure 6). *Pseudo-nitzschia* expressed the largest proportion of cell division and amino acid metabolism and the smallest proportion of catabolic activity and lipid metabolism out of the six diatom genera observed indicating that their increase in abundance could be contributed to active cell growth and utilization of stored nutrients in the form of lipids. *Cylindrotheca* employed a different strategy by expressing higher proportions of other metabolic pathways, including glycan metabolism, and biosynthesis of secondary metabolites (Figure 6). Glycan metabolism has previously been documented in marine microbial communities to recycle particulate carbon exuded from phytoplankton and could indicate possible nutrient exchanges occurring between the surrounding microbial community and *Cylindrotheca* (Becker et al. 2020). The exploitation of glycan metabolisms by *Cylindrotheca* could indicate a relationship with marine microbes that commonly utilize these strategies to access previously unavailable resources and could indicate a reason for its increase in abundance between response cruises. These findings support the assumption that although nitrogen was the limiting nutrient within the

system, nitrogen metabolism was not the solely responsible for changes in abundance observed in numerous diatom genera.

**Diatom genera response of differentially expressed genes of the nitrogen metabolism**

Closer examination of the nitrogen metabolisms of the six genera of diatoms was performed as the environment was consistently nitrogen limiting. Overall, the six genera of diatoms displayed significant utilization of both inorganic and organic forms of nitrogen between response and across stations thus not supporting the initial hypothesis that they would primarily utilize inorganic forms of nitrogen. Each genus utilized a combination of various transporters and internal cycling mechanisms to utilize the various forms of nitrogen available (Figure 7). The ornithine-urea cycle has been previously documented in diatoms as an important link between nitrogen and carbon metabolism and responsive to changes in nitrogen availability (Allen et al. 2011); however, none of these six diatom genera expressed significant differences in the entirety of the genes within ornithine-urea cycle between response cruises.

Nitrate/nitrite transporters, glutamine synthetase, glutamate synthase, urea symporters, and hydroxylamine reductase all displayed significant differences in expression between response cruises for all genera (Figure 7). *Ditylum* did not express consistent significant changes to amino acid transporters or urea symporters along all stations, and ammonium transporters were not found in our dataset. Glutamate dehydrogenase of *Ditylum* was consistently upregulated during the second response cruise suggesting its importance in contributing to ammonium stocks, whereas alternative sources like ammonium transporters and nitrite reductase were not found in our dataset which could be due to low abundance or a high cpm threshold used during differential expression analysis (Figure 7). Although ammonium was available during both response cruises, *Ditylum* could have either been outcompeted for external sources of ammonium

by other diatom genera or the low abundance of the genus didn't allow for a s or the initial threshold for differential expression was set too high and eliminated some of the transcripts. *Skeletonema* and *Thalassiosira* were the only two genera that displayed significant changes in expression of nitrite reductase between response cruises indicating its importance in maintaining internal pools of ammonium for those genera which could have been done to counter low concentration of ammonium within the cell as ammonium transporters were either not significantly expressed (*Skeletonema*) or were significantly expressed at low quantities (*Thalassiosira*) (Figure 7). *Skeletonema* was the only genus for which cyanate lyase and nitronate monooxygenase were differentially expressed between response cruises. Cyanate lyase was upregulated during the first response cruise at stations, GI and SS, contributing to the generation of ammonium. Subsequently, at southern stations, S16, S11, and S06, during the second response cruise, nitronate monooxygenase was upregulated supporting available quantities of nitrite internally (Figure 7). The exploitation of nitronate monooxygenase and cyanate lyase by *Skeletonema* were a unique response amongst the observed diatom genera and could reveal a tactic used to maintain abundance in a complex environment limited by resources. Glutamine synthetase and glutamate synthase were partitioned across the different legs by *Thalassiosira* with glutamate synthase being more significantly expressed during the first response cruise and glutamine synthetase being more expressed during the second response cruise (Figure 7). Glutamine synthetase and glutamate synthase activity yield glutamine and glutamate respectively, both of which are essential for amino acid metabolism and act as nitrogen acceptors and donators to balance redox potential within the cell (Dagenais-Bellefeuille & Morse, 2013). A higher activity of glutamate synthase has been suggested to indicate a more robust connection to the citric acid cycle due to the production of 2-oxogluterate, a key

component in the TCA cycle (Flynn et al. 1989, Hoch et al. 2006). Higher expression of glutamate synthase was observed during the first response cruise which could indicate a higher dependence on the TCA cycle for energy generation. The activity of glutamine synthetase requires ATP, glutamate, and ammonium to form glutamine, thus requiring a drawdown on available pools of those compounds that are essential energy currency, amino acids, and nutrients. Higher expression of glutamine synthetase would characterize *Thalassiosira* in the second response cruise as driving production of glutamine in a bid to regenerate available amino acids for future growth. The partitioning of the responses of glutamate synthase and glutamine synthetase could provide a potential explanation for how the genus managed to maintain abundance between response cruises. When examining the expression of nitrogen metabolism genes by *Pseudo-nitzschia* and *Cylindrotheca,* the two diatom genera that increased between response cruises, there were no unique differences in comparison to other diatom genera that could account for their increase in abundance between response cruises. This could suggest that changes in the proportion of amino acid metabolism, cell division, catabolic activity, and other metabolism could account more for their increase between response cruises (Figure 6). A closer examination of the nitrogen metabolism genes of select diatom genera revealed some unique responses (*Skeletonema* exploitation of cyanate lyase and nitronate monooxygenase) and partitioning of responses between legs that when coupled with changes in major metabolic functions may explain the shifts in abundance observed.

**Dinoflagellate genera abundance response**

Four genera of dinoflagellates were chosen for further analysis of major metabolic functions and differential expression of nitrogen metabolism based on changes in abundance between response cruises. *Karenia* increased between response cruises at almost all stations

(Figure 6). *Scrippsiella* exhibited a similar pattern of abundance to *Karenia* but on a smaller scale (Figure 6). *Karlodinium* had an irregular presence at some stations (Figure 6). *Prorocentrum* maintained a constant presence at all stations between response cruises (Figure 5).

**Dinoflagellate genera major metabolic functions**

Overall, the major metabolic functions of dinoflagellate genera were more consistent amongst one another than the six diatom genera observed. All genera displayed transcripts for amino acid metabolism and carbohydrate metabolism consistently and displayed lower proportions of transcripts of lipid and other metabolism pathways in comparison to diatom genera (Figure 8). *Karenia* displayed the most consistent response across response cruises with large proportions of major metabolic activity dedicated to cell division and nitrogen metabolism. While *Scrippsiella* displayed a similar shift in abundance to *Karenia, Scrippsiella* displayed a very different metabolic response. Cell signaling composed a large proportion of metabolic expression and decreased between response at every station, while photosynthetic activity and catabolic activity increased (Figure 8). The differences displayed by the two genera could indicate distinct coping strategies utilized by the two genera to both increase in abundance. *Karenia* directly increased abundance between response cruises by increasing the amount of cell division while Scrippsiella decreased stress related processes within cell signaling and increased energy obtaining measures via photosynthesis and the catabolism of internal structures between response cruises. *Karlodinium* displayed the greatest proportion of amino acid metabolism, carbohydrate metabolism, and catabolic activity among the four dinoflagellate genera and the lowest proportion of photosynthetic activity and cell signaling (Figure 8). *Karlodinium* has been documented as a mixotrophic dinoflagellate and could be utilizing heterotrophy to supplement nutritional needs in place of autotrophy. *Prorocentrum* expression of nitrogen metabolism

displayed the largest changes of all the dinoflagellate genera observed (Figure 8). Additionally, carbohydrate metabolism either remained the same or decreased in proportion (Figure 8). This could suggest that shared resources for both carbon metabolism and nitrogen metabolism were being disproportionately allocated to nitrogen metabolism as this genus was more impacted by the nitrogen limiting environment and the competition for limited resources by other genera. Major metabolic functions of dinoflagellate genera revealed unique responses that attributed to their shifts in abundance between response cruises.

**Dinoflagellate genera overall nitrogen metabolism response**

A close examination of the nitrogen metabolism of the four dinoflagellate genera found distinct differences in responses to nitrogen limitation. ANOSIM results of nitrogen metabolism for each genus revealed that all genera displayed significant differences of nitrogen metabolism between stations of the second response cruise (Table 1). *Karlodinium*, a mixotrophic dinoflagellate, displayed more similar nitrogen metabolism responses across stations during the second response cruise in comparison to the other four genera. Only *Karenia* and *Prorocentrum* displayed differences in nitrogen metabolism across locations (north stations, south stations, and station S16) and locations (north stations and south stations, with station S16 being included in south stations) (Table 1). These results indicate that the nitrogen metabolism of both genera were potentially influenced by the persistent onshore current from offshore waters either due to their increase in abundance and/or changes to the available nutrients. Between stations S21 and S16 a decrease of down shore currents at station S21 and an increase in down shore currents at southern stations indicates higher levels of downwelling which could lead to changes in nutrient availability and location of the two genera.

**Dinoflagellate genera response of differentially expressed genes of the nitrogen metabolism**

Overall, *Karenia* had the most diverse response of the analyzed dinoflagellate genera (Figure 9). *Karenia* displayed higher gene expression of genes used to recycle ammonium and urea internally via ornithine carbamoytransferase, arginosuccinate synthase, and urease and took up ammonium via ammonium transporters during the first response cruise. During the second response cruise, a greater diversity of nitrate/nitrite transporters, amino acid transporters, and urea symporters were employed, as well as nitrate and nitrite reductases, glutamine synthetase, glutamate synthase, glutamate dehydrogenase, and arginase (Figure 9). The greater diversity of response during the second cruise could support the increases in abundance. Killberg-Thoreson et al. 2014 detailed the nitrogen uptake kinetics of *Karenia brevis* exposed to various sources of nitrogen and found a distinct cascade of substance preferences in order by ammonium, nitrate, urea, humic compounds, and amino acids. While our data is limited and cannot be used to make direct comparisons with uptake rates, the magnitude of significant changes of stations between legs suggested that ammonium was not the preferred nitrogen source and rather nitrate and nitrite were. Bronk et al. 2014 proposed that nitrate was the most important nitrogen source during the initiation phase of a bloom whereas ammonium was the most important nitrogen source during bloom maintenance and stationary phases. Considering this background knowledge, our sample could be potentially classified as in the initiation stage of a bloom; however, due to lack of uptake rates and further observations this cannot be concluded for certain. While *Scrippsiella* displayed a similar pattern of abundance to *Karenia,* changes the diversity of nitrogen metabolism response was not the same. The glutamine synthetase, glutamate synthase, and glutamate dehydrogenase utilization was not as significant as in *Karenia* (Figure 9). *Scrippsiella*

displayed a partitioning of urea generation and utilization between response cruises. During the first response cruise, urea was generated via arginase and then during the second response cruise the generated urea along with urea transported into the cell were reduced to ammonium. The generation of urea internally during the first response cruise is notable as urea concentrations were higher during the first response cruise and subsequently decreased during the second response cruise. The upregulation of urea symporters and the hydrolysis of urea to ammonium during the second response cruise could indicate that ammonium limitation was more limiting than the available quantities of urea as ammonium concentration decreased between the first and second response cruise as well. At the genus level for dinoflagellates, nitrogen metabolism revealed partitioning of responses for *Karenia* and *Scrippsiella* which could contribute to their abundance changes.

**An examination of *Karenia* species response**

A closer examination at the species level for *Karenia* was performed as *Karenia* 'bloomed' by two orders of magnitude at stations SS and S21. The overall number of transcripts expressed, and the number of nitrogen metabolism reads were dominated by *K. selliformis* (Figure 10A). This observation was consistent with IFCB results which showed *K. selliformis* to be dominant, and *K. brevis* and *K. papilionacea* were observed at ten times lower quantities than *K. selliformis (*Figure 10A, B).

`Differential expression analysis of nitrogen metabolism genes was performed for all *Karenia* species; however, results from *K. brevis* and *K. papilionacea* only contained significant differences for hydroxylamine reductase, glutamine synthetase, and glutamate synthase (Figure 10E). *K. brevis* and *K. papilionacea* both displayed higher quantities of glutamine synthetase during the first response cruise indicating generation of the amino acid glutamate whereas *K.*

*selliformis* displayed higher quantities of the same gene during the second response cruise (Figure 10E). The marked difference in expression between the three species could partially explain *K. selliformis* dominance during the second response cruise. K. *papilionacea* expressed higher quantities of hydroxylamine reductase, driving production of ammonium during the first response cruise whereas *K. selliformis* expressed higher quantities of hydroxylamine reductase during the second response cruise at stations where the bloom occurred which could explain the increase observed. Many nitrogen metabolism genes for *K. selliformis* were upregulated during the second response cruise at stations where there was an increase in *Karenia.* An analysis of differential expression of *K. selliformis* revealed increased utilization of the nitrate/nitrite transport and reduction to ammonium, reduction of hydroxylamine to ammonium, and uptake of urea during the second response cruise (Figure 10E). These activities are suggestive of generation of ammonium which was then utilized by both glutamine synthetase and glutamate synthase to generate glutamine and glutamate respectively during the second response cruise. Ammonium decreased in concentration between response cruises, so the observed changes in expression could be an attempt to maintain ammonium for utilization via glutamate synthase and glutamine synthetase. This is the first study for which partitioning of nitrogen metabolism genes by different species of *Karenia* has been observed.

A comparison of the nitrogen metabolism of the three *Karenia* species revealed novel partitioning of a limited resource. Overall, our sample was dominated by *K, selliformis* in terms of significant nitrogen metabolism response, abundance of nitrogen metabolism genes, and transcript quantity however there were variations observed between *K. selliformis. K. brevis*, and *K. papilionacea* the major HAB-forming species in the Gulf of Mexico.

## Conclusion

Observations of the metatranscriptome response of the microplankton community were conducted a month after the passage of Hurricane Harvey and displayed significant changes over just one week. In response to nitrogen limitation, diatoms and dinoflagellates depended on internal recycling and external sources of both inorganic and organic sources of nitrogen during both response cruises. Overall metabolic functions revealed that nitrogen metabolism was not the only responsive metabolic function to the large amounts of freshwater discharge and offshore intrusion and that changes to the overall metabolic functions could potentially contribute more to the observed changes in abundance. This was evident in an observation at the major group level (diatoms and dinoflagellates) via the higher expression of lipid metabolism and catabolic activity in diatoms suggesting a generation of internal storage vacuoles for nutrients and a salvaging of nutrients from internal organelles. Diatoms were outcompeted during the second response cruise by dinoflagellates who in turn increased expression of cell division, other metabolism, and amino acid metabolism leading to an increase in abundance. At the genus level for both diatoms and dinoflagellates, changes in abundance of major genera could be attributed to changes in metabolic profile and/or changes to nitrogen metabolism. While this dataset is missing critical uptake rates for various nitrogen forms and internal measurements of nutrient concentrations, analysis of the microplankton community in response to Hurricane Harvey reveals diverse strategies by partitioning of response over different timepoints (*Thalassiosira* partitioning of glutamate synthase and glutamine synthetase between response cruises and *Karenia* and *Scrippsiella* partitioning of urea symporters, arginase, and urease between response cruises), exploiting unique sources of nitrogen (*Skeletonema* differential expression of cyanate lyase and

nitronate monooxygenase during the first and second response cruise), and remodeling of major metabolic functions (*Cylindrotheca,* expressed higher proportions of glycan metabolism to support increase of abundance between response cruises, *Prorocentrum* allocated shared resources from carbohydrate metabolism to nitrogen metabolism under a decrease in nitrogen availability between response cruises,). These results are consistent with a community under numerous stresses including nitrogen limitation, decrease in salinity, and introduction of offshore waters and offshore species. This work displays the wide array of responses phytoplankton have within their arsenal to survive.

## Tables

| Group | Level | Comparison | R-squared | p-value |
|---|---|---|---|---|
| **Diatom** | Leg 1 | Station | 0.9667 | 1e-04 |
| **Diatom** | Leg 3 | Station | 0.8922 | 1e-04 |
| **Diatom** | Leg 3 | Location (N, S16,S) | 0.5746 | 1e-04 |
| **Dinoflagellate** | Leg 1 | Station | 0.7417 | 1e-04 |
| **Dinoflagellate** | Leg 3 | Location (N,S16,S) | 0.8668 | 1e-04 |
| **Dinoflagellate** | Leg 3 | Location- only N,S | 0.8066 | 1e-04 |
| **Chaetoceros** | Leg 1 | Station | 0.9417 | 4e-04 |
| **Thalassiosira** | Leg 1 | Station | 0.9187 | 1e-04 |
| **Thalassiosira** | Leg 3 | Station | 0.8436 | 1e-04 |
| **Cylindrotheca** | Leg 1 | Station | 0.7344 | 1e-04 |
| Cylindrotheca | Leg 3 | Station | 0.7737 | 1e-04 |
| **Ditylum** | Leg 1 | Station | 0.8708 | 1e-04 |
| **Ditylum** | Leg 3 | Station | 0.8049 | 1e-04 |
| **Ditylum** | Leg 3 | Location (N,S16,S) | 0.8204 | 1e-04 |
| **Pseudo-nitzschia** | Leg 1 | Station | 0.901 | 1e-04 |
| **Pseudo-nitzschia** | Leg 3 | Station | 0.8189 | 1e-04 |
| **Skeletonema** | Leg 1 | Station | 0.8896 | 1e-04 |
| **Skeletonema** | Leg 3 | Station | 0.814 | 1e-04 |
| **Karenia** | Leg 3 | Location (N,S16,S) | 0.9312 | 1e-04 |
| **Karenia** | Leg 3 | Location- only N,S | 0.9897 | 3e-04 |
| **Scrippsiella** | Leg 3 | Station | 0.7942 | 1e-04 |
| **Karlodinium** | Leg 3 | Station | 0.7835 | 1e-04 |
| **Prorocentrum** | Leg 3 | Station | 0.7967 | 1e-04 |
| **Prorocentrum** | Leg 3 | Location (N,S16,S) | 0.9495 | 1e-04 |
| **Prorocentrum** | Leg 3 | Location – only N,S | 0.7967 | 1e-04 |

Table 1: Analysis of similarity results (ANOSIM) of nitrogen metabolism genes from group comparisons (diatoms and dinoflagellates) and genera (*Chaetoceros, Skeletonema, Thalassiosira, Ditylum, Pseudo-nitzschia, Cylindrotheca, Karenia, Scrippsiella, Karlodinium, Prorocentrum*) for different spatial and temporal categories. All results listed have a p-value <0.05 and an R-squared value 0.7. The levels of comparison are between the first and second response cruise and amongst the two response cruises along distinct geographic categories. The distinct geographic categories were location (N,S16,S),location (N,S) and station. In the category location (N, S16, S), the north stations were GI, SS, and S21 and the south stations were S11 and S06. In the category location (N, S) station S16 was grouped into the southern stations category. The station category treated each station independent of one another for a either response cruise.

## Figures

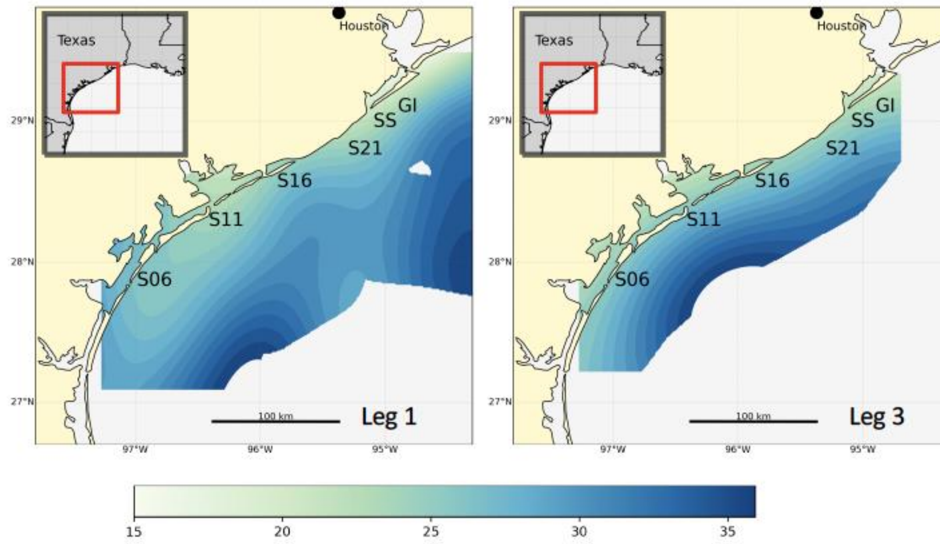



Figure 1: Interpolated salinity data from the ship's flow-through system for the first response cruise (Leg 1, left) and the second response cruise (Leg 3, right). The six different stations for each cruise are labeled.

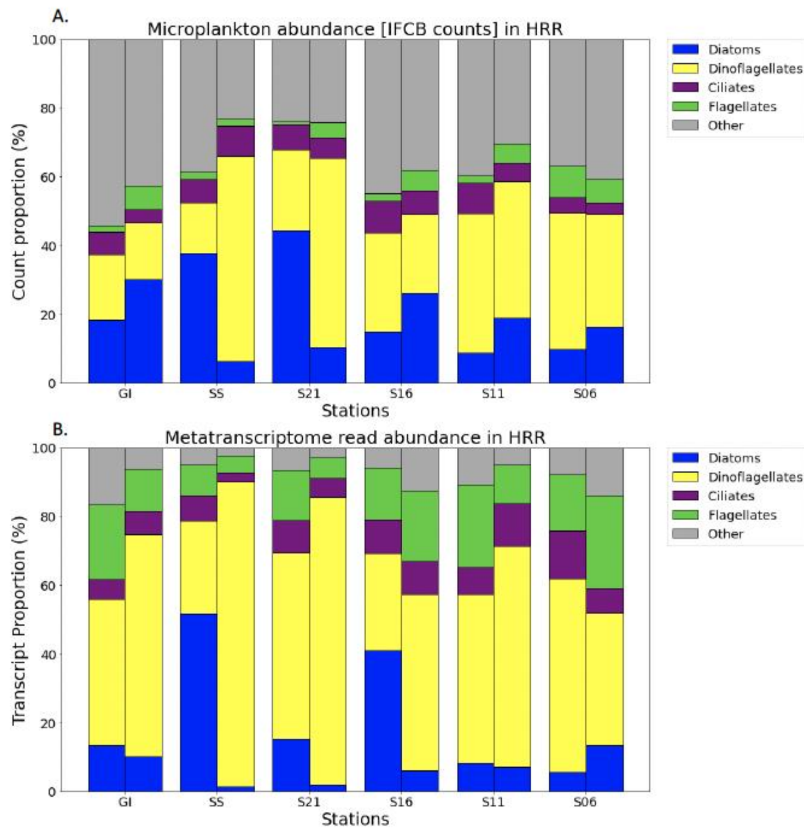



Figure 2: Microplankton abundance from IFCB counts (A) and metatranscriptome read abundance (B) from Hurricane Harvey recovery cruise for each station with the first response cruise (left bar) & the second response cruise (right bar). Groups include: diatoms (blue), dinoflagellates (yellow), ciliates (purple), flagellates (green), other (grey).

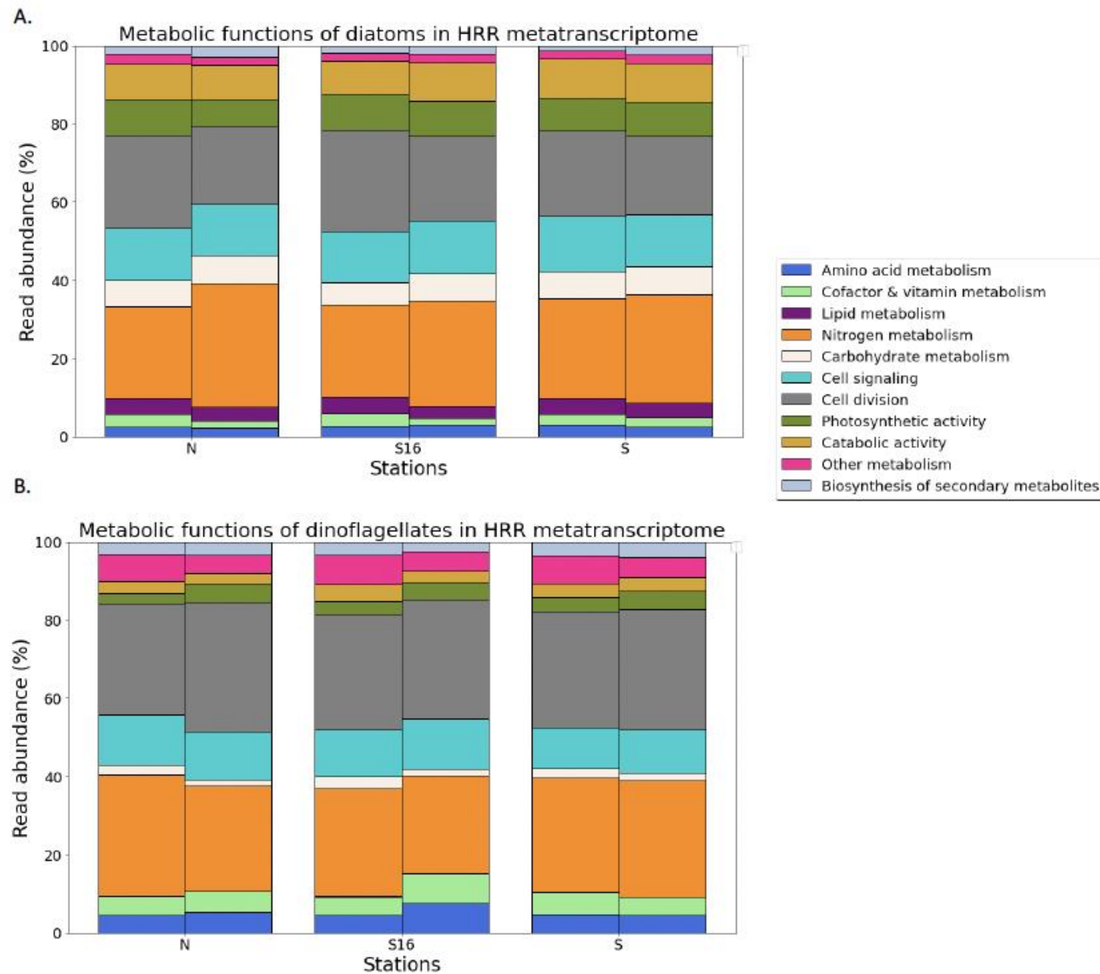



Figure 3: Overall metabolic functions of diatoms (A) and dinoflagellates (B) from post-Hurricane Harvey response cruise metatranscriptome data from each station with the first response cruise (left bar) & the second response cruise (right bar). Stations were binned into north (N), S16 (station S16), and south (S) as results from ANOSIM indicated differences in nitrogen metabolism across those levels for the second response cruise stations. North stations were GI, SS, and S21 and south stations were S11 and S06. Functions were clustered based on the KEGG pathway database hierarchy. Functions with a cumulative percentage of greater than 10 % across all stations and timepoints were included.

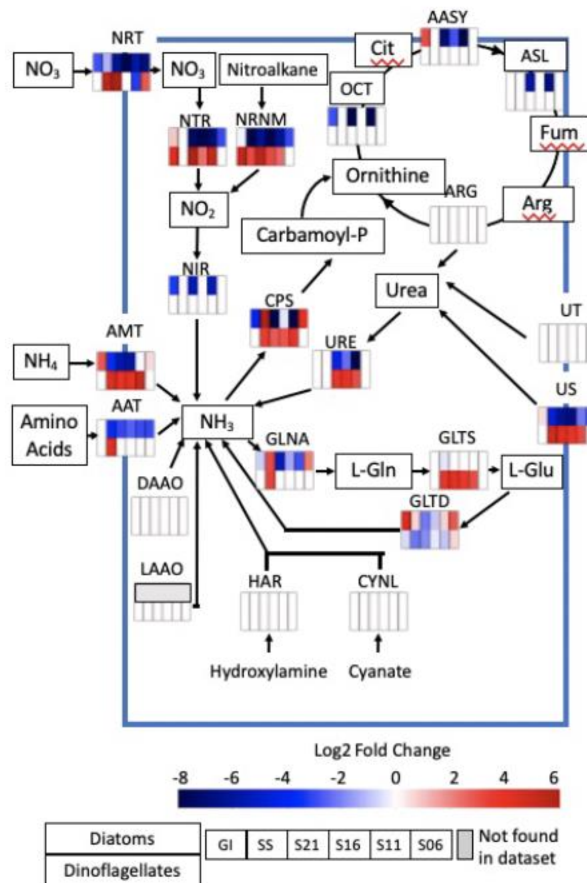
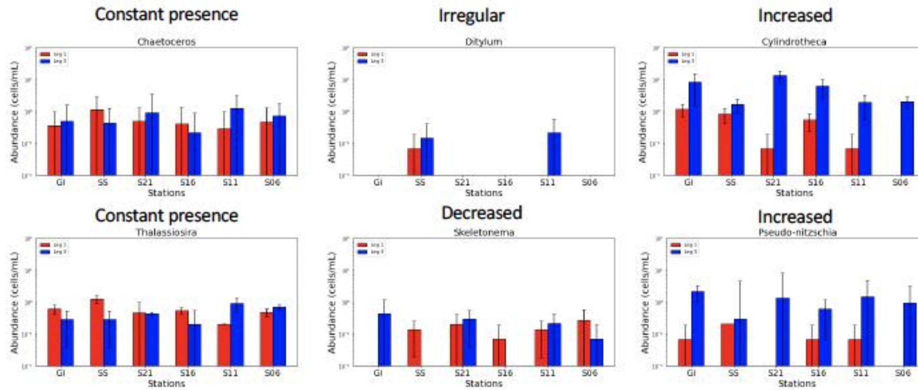



Figure 4: Log-fold change of nitrogen metabolism genes between the first response cruise and the second response cruise for diatoms (top bar) & dinoflagellates (bottom bar) at each station. Blue values represent genes more highly expressed in the first response cruise and red values represent genes more highly expressed in the second response cruise. Bars shaded in light grey represent genes that were not found within the dataset. NRT: nitrate/nitrite transporter, NTR: nitrate reductase, NRNM: nitronate monooxygenase, NIR: nitrite reductase, AMT: ammonium transporter, AAT: amino acid transporter, DAAO: D-amino acid oxidase, LAAO: L-amino acid oxidase, HAR: hydroxylamine reductase, CYNL: cyanate lyase, GLNA: glutamine synthetase, L-Gln: L-glutamine, GLTS: glutamate synthase, L-Glu: L-glutamate, GLTD: glutamate dehydrogenase, US: urea symporter, UT: urea transporter, URE: urease, CPS: carbamoyl phosphate synthase, Carbamoyl P:

carbamoyl phosphate, OCT: ornithine carbamoyltransferase, Cit: citruline, AASY: arginosuccinate synthase, ASL: arginosuccinate lyase, Fum: fumerate ARG: arginase.

## Diatoms




## Dinoflagellates

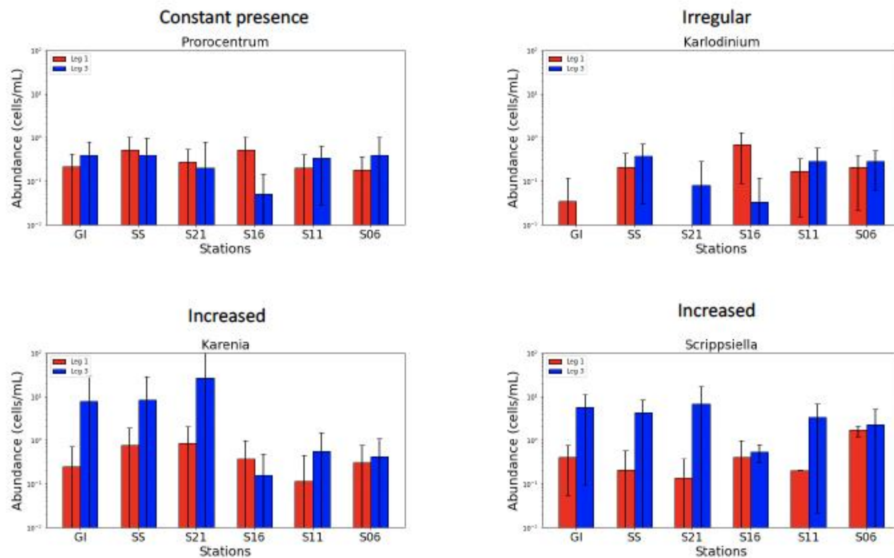
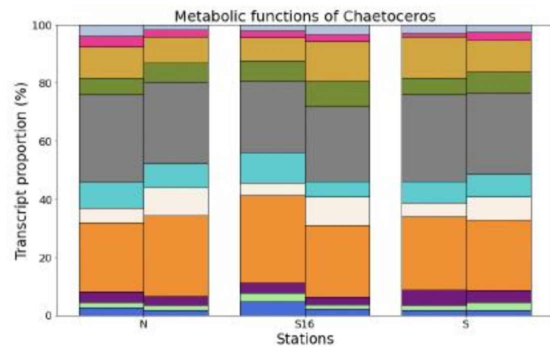
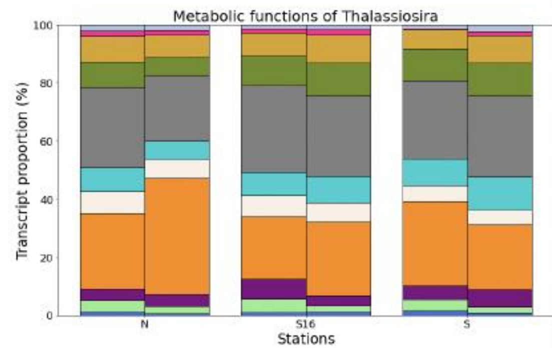
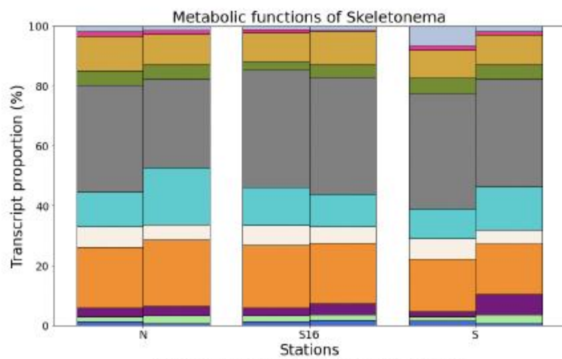
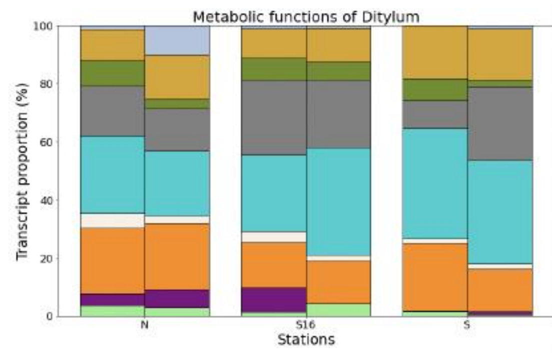


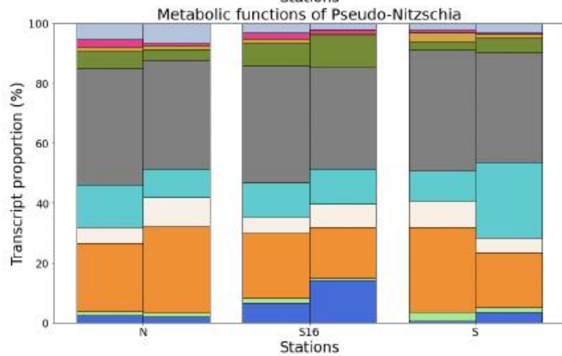

Figure 5: Diatom and dinoflagellate abundance from IFCB data for post-Hurricane Harvey IFCB abundance plots for the first response cruise (red bar) and the second response cruise (blue bar) stations for *Chaetoceros, Skeletonema, Thalassiosira, Ditylum, Pseudo-nitzschia, Cylindrotheca, Karenia, Scrippsiella, Karlodinium,* and *Prorocentrum* with standard deviation. Each genus was grouped under one of four categories to classify their changes of abundance between response cruises. The four categories were constant presence, irregular, decrease, and increased. The y-axis is in log scale. Y-axes range from 0.01 to 100.

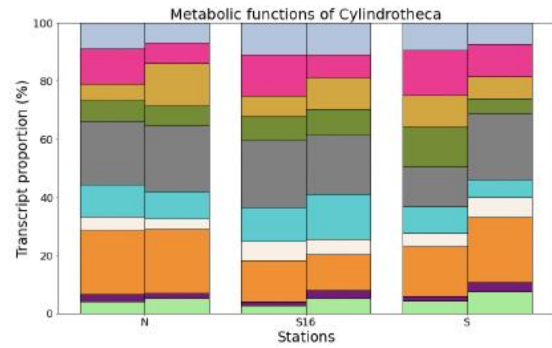

Metabolic functions of Chaetoceros
Transcript proportion (%)
Stations
N
S16
S


Metabolic functions of Thalassiosira
Transcript proportion (%)
Stations
N
S16
S


Metabolic functions of Skeletonema
Transcript proportion (%)
Stations
N
S16
S


Metabolic functions of Ditylum
Transcript proportion (%)
Stations
N
S16
S


Metabolic functions of Pseudo-Nitzschia
Transcript proportion (%)
Stations
N
S16
S


Metabolic functions of Cylindrotheca
Transcript proportion (%)
Stations
N
S16
S

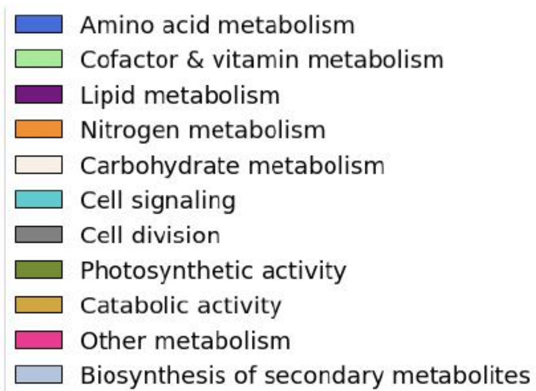

Amino acid metabolism
Cofactor & vitamin metabolism
Lipid metabolism
Nitrogen metabolism
Carbohydrate metabolism
Cell signaling
Cell division
Photosynthetic activity
Catabolic activity
Other metabolism
Biosynthesis of secondary metabolites

Figure 6: Overall metabolic functions of *Chaetoceros, Skeletonema, Thalassiosira, Ditylum, Pseudo-nitzschia, Cylindrotheca* post-Hurricane Harvey recovery metatranscriptome data from each station with the first response cruise (left bar) & the second response cruise (right bar). Stations were binned into north (N), S16 (station S16), and south (S) as results from ANOSIM indicated differences in nitrogen metabolism across those levels for the second response cruise stations. North stations were GI, SS, and S21 and south stations were S11 and S06. Functions were clustered based on the KEGG pathway database hierarchy. Functions with a cumulative percentage of greater than 10 % across all stations and timepoints were included.

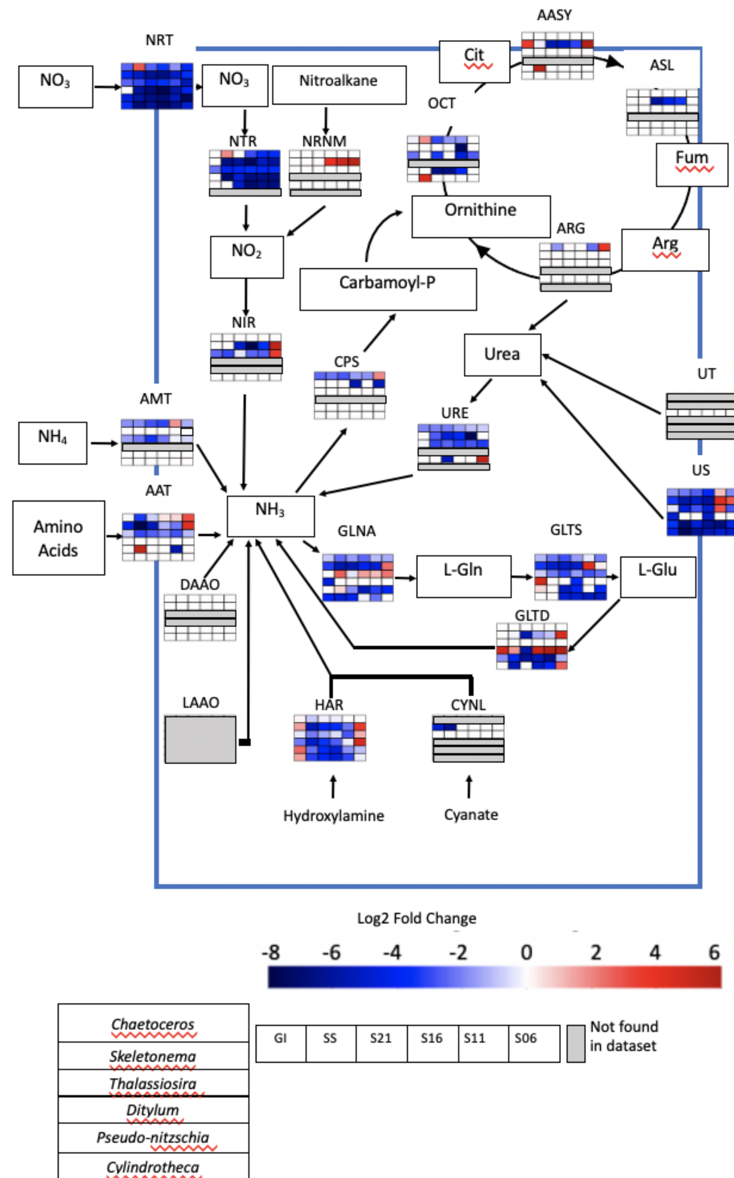



Figure 7: Log-fold change of nitrogen metabolism genes between the first response cruise and The second response cruise for *Chaetoceros, Skeletonema, Thalassiosira, Ditylum, Pseudo-nitzschia,* and *Cylindrotheca.* Blue values represent genes more highly expressed in the first response cruise and red values represent genes more highly expressed in the second response cruise. Bars shaded in light grey represent genes that were not found within the dataset. NRT: nitrate/nitrite transporter, NTR: nitrate reductase, NRNM: nitronate monooxygenase, NIR: nitrite reductase, AMT: ammonium transporter, AAT: amino acid transporter, DAAO: D-amino acid oxidase, LAAO: L-amino acid

oxidase, HAR: hydroxylamine reductase, CYNL: cyanate lyase, GLNA: glutamine synthetase, L-Gln: L-glutamine, GLTS: glutamate synthase, L-Glu: L-glutamate, GLTD: glutamate dehydrogenase, US: urea symporter, UT: urea transporter, URE: urease, CPS: carbamoyl phosphate synthase, Carbamoyl P: carbamoyl phosphate, OCT: ornithine carbamoyltransferase, Cit: citruline, AASY: arginosuccinate synthase, ASL: arginosuccinate lyase, Fum: fumerate ARG: arginase

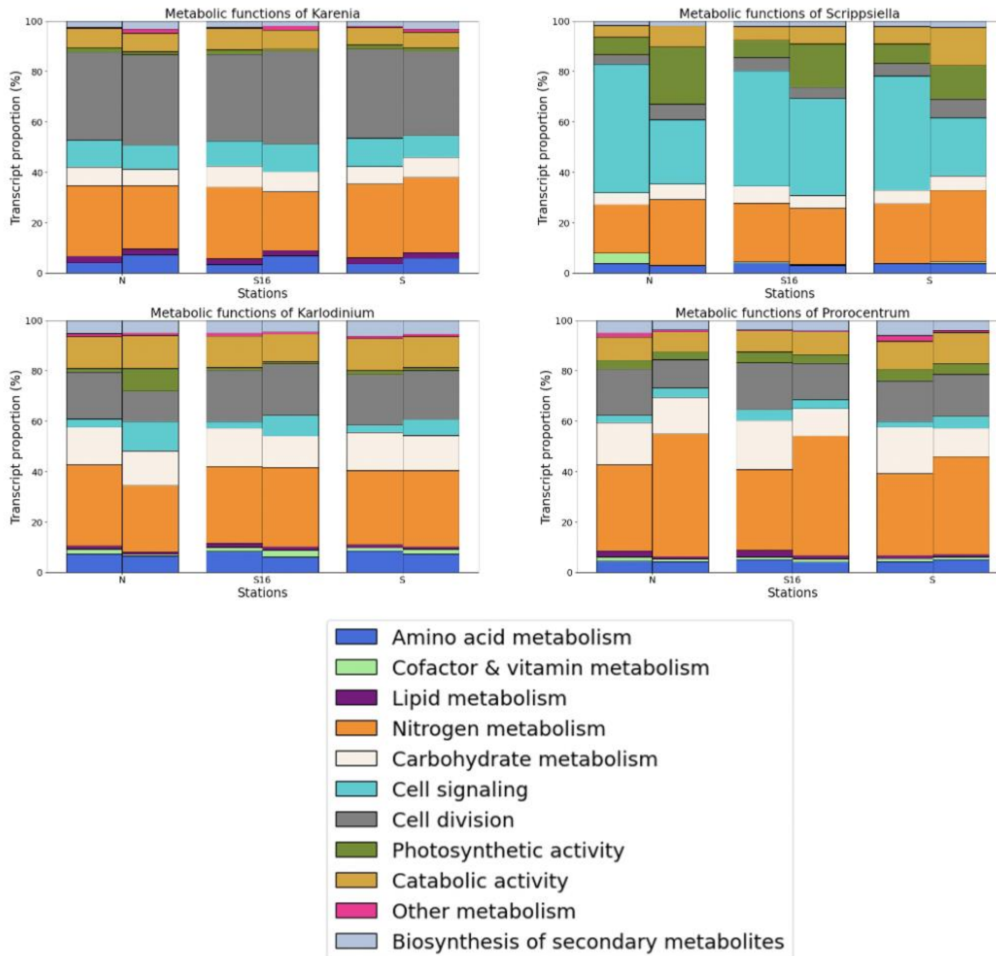



Figure 8: Overall metabolic functions of *Karenia, Scrippsiella, Karlodinium, Prorocentrum* post-Hurricane Harvey recovery metatranscriptome data from each station with the first response cruise (left bar) & the second response cruise (right bar). Stations were binned into north (N), S16 (station S16), and south (S) as results from ANOSIM indicated differences in nitrogen metabolism across those levels for the second response cruise stations. North stations were GI, SS, and S21 and south stations were S11 and S06. Functions were clustered based on the KEGG pathway database hierarchy. Functions with a cumulative percentage of greater than 10 % across all stations and timepoints were included.

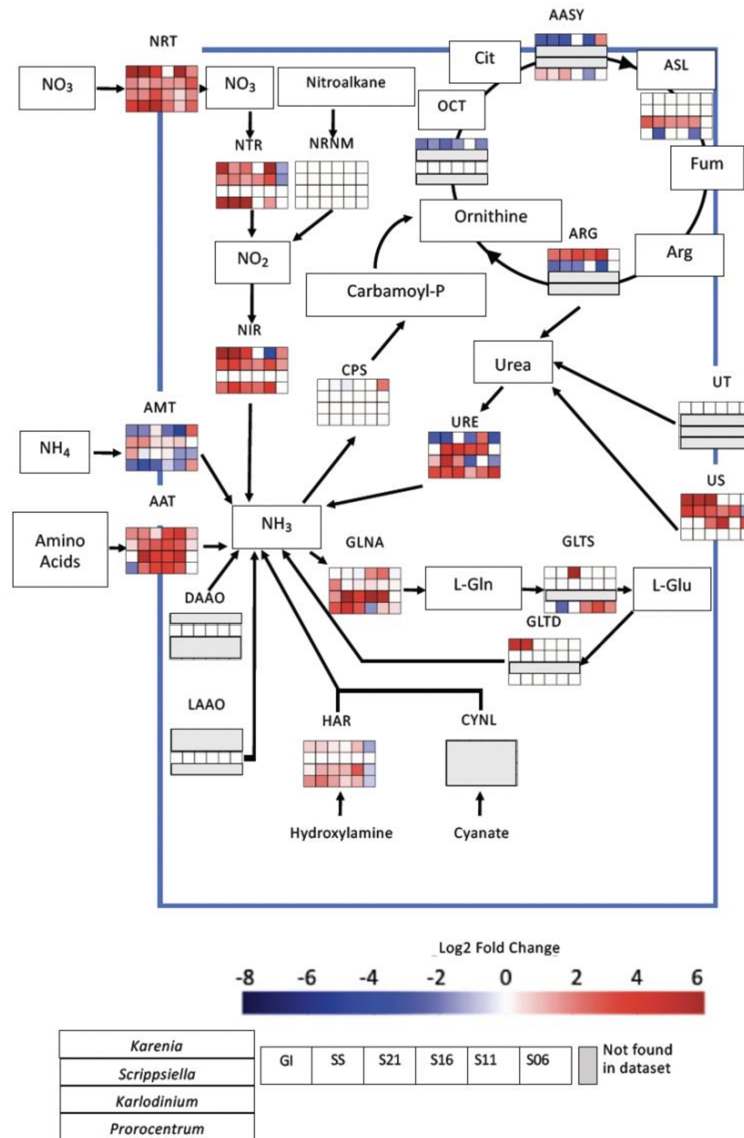



Figure 9: Log-fold change of nitrogen metabolism genes between the first response cruise and the second response cruise for *Karenia, Scrippsiella, Karlodinium,* and *Prorocentrum.* Blue values represent genes more highly expressed in the first response cruise and red values represent genes more highly expressed in the second response cruise. Bars shaded in light grey represent genes that were not found within the dataset. NRT: nitrate/nitrite transporter, NTR: nitrate reductase, NRNM: nitronate monooxygenase, NIR: nitrite reductase, AMT: ammonium transporter, AAT: amino acid transporter,

DAAO: D-amino acid oxidase, LAAO: L-amino acid oxidase, HAR: hydroxylamine reductase, CYNL: cyanate lyase, GLNA: glutamine synthetase, L-Gln: L-glutamine, GLTS: glutamate synthase, L-Glu: L-glutamate, GLTD: glutamate dehydrogenase, US: urea symporter, UT: urea transporter, URE: urease, CPS: carbamoyl phosphate synthase, Carbamoyl P: carbamoyl phosphate, OCT: ornithine carbamoyltransferase, Cit: citruline, AASY: arginosuccinate synthase, ASL: arginosuccinate lyase, Fum: fumerate ARG: arginase

A.

K. brevis
K. selliformis
K. mikimotoi
K. papilionacea

106
21,205
3,928
28
1,261
100
1,385
837
69
566
1,589
13
236
4
9

Length > 250 base pairs
K. brevis
K. mikimotoi
K. papilionacea
K. selliformis

B.

Nitrogen metabolism transcripts for Karenia species

K.brevis
K.papilionacea
K.selliformis

Number of transcripts

Stations

GI SS S21 S16 S11 S06

C.

Nitrogen genes expressed by Karenia species

K.brevis
K.papilionacea
K.selliformis

Read abundance

Stations

GI SS S21 S16 S11 S06

D.

Species = K. brevis

Species = K. papilionacea

Species = K.selliformis

Gene

Amino acid transporter
Ammonium transporter
Nitrate/nitrite transporter
Nitrate reductase
Nitrite reductase
Nitronate monooxygenase
Carbamoyl phosphate synthetase
Ornithine carbamoyltransferase
Arginosuccinate synthetase
Arginosuccinate lyase
Arginase
Urease
Urea transporter
Urea symporter
Glutamine synthetase
Glutamate synthase
Glutamate dehydrogenase
Cyanate lyase
Hydroxylamine reductase
L-amino acid oxidase
D-amino acid oxidase

Species
K. brevis
K. papilionacea
K.selliformis
Percentage
0
8
16
24
32
40

E.

$NO_3$
NRT
$NO_3$
Nitroalkane
Cit
AASY
ASL
OCT
NTR
NRNM
Fum
Ornithine
ARG
Arg
$NO_2$
Carbamoyl-P
NIR
Urea
CPS
UT
AMT
URE
$NH_4$
AAT
US
Amino Acids
$NH_3$
GLNA
L-Gln
GLTS
L-Glu
DAAO
GLTD
LAAO
HAR
CYNL
Hydroxylamine
Cyanate

Log2 Fold Change
-8 -6 -4 -2 0 2 4 6

K. brevis
K. mikimotoi
K. papilionacea
K. selliformis
GI SS S21 S16 S11 S06
Not found in dataset

Figure 10: Summary of nitrogen metabolism genes for the four different *Karenia* species. All the *Karenia* genus transcripts were BLAST'd against *K. brevis, K. papilionacea, K. mikimotoi,* and *K. selliformis* reference transcriptomes assembled by the Velvet Oases approach. For each transcript from Hurricane Harvey multiple species were matched to. The four-way Venn diagram contains the number of transcripts for each combination of matches for which the match was greater than 250 base pairs (A). The total number of nitrogen metabolism transcripts with a greater than 250 base pair matches for three of the species, *K. brevis* (orange), *K. papilionacea* (brown), and *K. selliformis* (yellow). The first response cruise abundances are on the left of the station label and the second response cruise response cruise abundances are on the right. There were no nitrogen metabolism transcripts that matched to *K. mikimotoi* that were greater than 250 base pairs. (B). The total number of nitrogen metabolism reads for transcript matches greater than 250 base pairs for three of the species, *K. brevis* (orange), *K. papilionacea* (brown), and *K. selliformis* (yellow). There are two y-axes to represent the order of magnitude higher number of reads *K. selliformis* at the northern stations during the second response cruise. The first response cruise abundances are on the left of the station label and the second response cruise response cruise abundances are on the right (C). Percentage of nitrogen metabolism genes expressed by the three different species of *Karenia, K. brevis* (orange), *K. papilionacea* (brown), *K. selliformis* (yellow) at each station. The size of the bubble corresponds to the total number of reads for a gene at a particular station divided by the total number of reads for that gene multiplied by one hundred (D). Log fold change of nitrogen metabolism genes between response cruises for *K. brevis, K. mikimotoi, K. papilionacea,* and *K. selliformis* at each station. Blue values represent genes more highly expressed in the first response cruise and red values represent genes more highly expressed in the second response cruise. Bars shaded in light grey represent genes that were not found within the dataset. NRT: nitrate/nitrite transporter, NTR: nitrate reductase, NRNM: nitronate monooxygenase, NIR: nitrite reductase, AMT: ammonium transporter, AAT: amino acid transporter, DAAO: D-amino acid oxidase, LAAO: L-amino acid oxidase, HAR: hydroxylamine reductase, CYNL: cyanate lyase,

GLNA: glutamine synthetase, L-Gln: L-glutamine, GLTS: glutamate synthase, L-Glu: L-glutamate, GLTD: glutamate dehydrogenase, US: urea symporter, UT: urea transporter, URE: urease, CPS: carbamoyl phosphate synthase, Carbamoyl P: carbamoyl phosphate, OCT: ornithine carbamoyltransferase, Cit: citruline, AASY: arginosuccinate synthase, ASL: arginosuccinate lyase, Fum: fumerate ARG: arginase (E).

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