Seasonal variability in diazotroph abundance and gene expression at a coastal N_2 fixation hotspot (Outer Banks, NC)

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Introduction

- N is present in nucleic acids, amino acids, and proteins, and is essential to all life. Marine microbial dinitrogen (N_2) fixation, the conversion of gaseous N_2 to bioavailable forms, is the primary source of new oceanic nitrogen (N). N availability often limits biological productivity in the ocean (Moore et al. 2013).
- Long considered to be a primarily warm, open-ocean process (Carpenter & Capone 2008), significant N_2 fixation rates have recently been observed in coastal environments, including along the Cape Hatteras front (Mulholland et al. 2012, Selden et al. 2021).
- To see if elevated N_2 fixation was a persistent feature in this region, N_2 fixation rates and N_2 fixer (diazotroph) abundance and gene expression were investigated through roughly monthly sampling at a field station (Jennette's Pier) in the Outer Banks, NC, from June 2019 to August 2020, as well as a day-long cruise around the pier in August of 2019.
- Diazotroph were targeted by their unique *nifH* gene sequences. Organisms investigated include:
  - Colonial cyanobacteria *Trichodesmium spp*. ("Tricho")
  - *Rhizosolenia* sp. heterocyst symbionts ("Het 1, 2, 3")
  - Unicellular cyanobacterial *Braarudosphaera bigelowii* endosymbionts UCYN-A1 and UCYN-A2
- The *nifH* gene is responsible for encoding a portion of the nitrogenase complex, which converts N_2 to more bioavailable forms of N.

Methods

Pier

- Surface ocean samples collected at Jennette's Pier, OBX, at mid-day once per month.
- Temperature and salinity were measured using a Castaway Conductivity, Temperature, Depth (CTD) sensor package, pictured to the right, lowered over the pier's edge.
- Chlorophyll-a, molecular, and nutrient samples were collected

Cruise

- Cruise sampling of the surface ocean occurred between ~10:00am and 3:00pm on 8/16/19, at 7 stations. Station #2 is Jenette's Pier.

General

- Gene abundance was measured by extracting DNA and subsequent analysis using quantitative PCR techniques (as in Selden et al. 2021).
- Gene expression was measured by reverse-transcription of RNA into cDNA, and subsequent analysis using quantitative PCR techniques (as in Selden et al. 2021).
- Relative expression was calculated by dividing cDNA abundance by DNA abundance. In cases where DNA was undetectable and cDNA was quantified, DNA was assumed to be equal to the effective limit of detection.
- Nitrogen fixation rates were quantified using the ^{15}N tracer method, described in White et al. 2020.

Photo 1. *Pier CTD sampling.*

Results

Pier

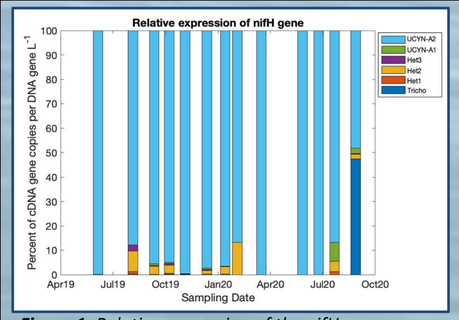

Figure 1. *Relative expression of the nifH gene among six diazotrophs. Values are indicative of relative activity of different diazotroph taxa.*

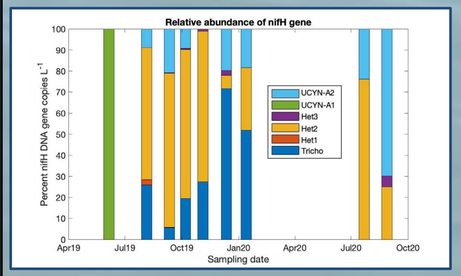

Figure 2. *Relative abundance of the nifH gene among six diazotrophs. Values are indicative of relative presence of diazotroph taxa.*

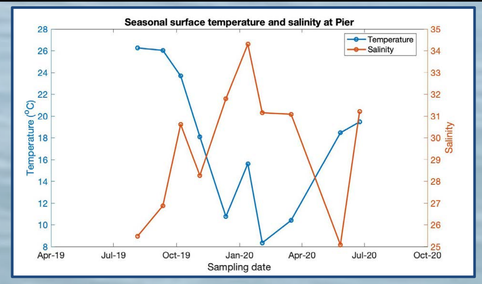

Figure 3. *Temperature and salinity measurements.*

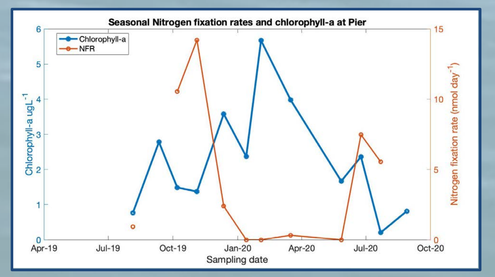

Figure 4. *Nitrogen fixation rates and chlorophyll-a measurements.*

Cruise

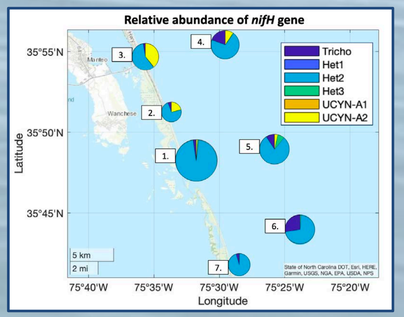

Figure 5. *Relative abundance of the nifH gene among six diazotrophs. Pie charts are sized to Nitrogen fixation rate values.*

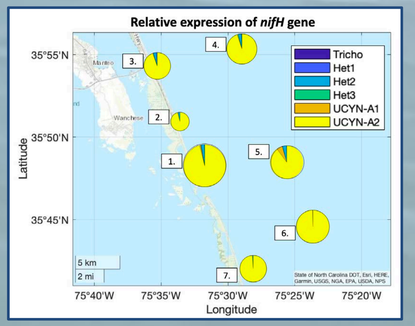

Figure 6. *Relative expression of the nifH gene among six diazotrophs. Pie charts are sized to Nitrogen fixation rate values.*

Station	1	2	3	4	5	6	7
Nitrogen fixation rate (nmol N/day)	27.6	Not measured	13.4	15.0	15.15	18.9	10.7

Table 1. *Nitrogen fixation rates observed on Cruise.*

Trends:

- Endosymbiont UCYN-A2 was consistently the most active diazotroph, as indicated by *nifH* expression, in both Pier and Cruise samples. It was also not the most abundant diazotroph investigated here, with the exception of the 8/28/20 pier sample.
- Higher Nitrogen fixation rates were observed where chlorophyll-a concentrations were lower.

Discussion

- This project investigated coastal N inputs in the context of the global ocean N cycle and budget, and explored chemical and physical factors that affect these processes.
- Variability in Nitrogen fixation rates does not seem to be directly related to diazotroph community composition, temperature, or salinity, but may follow a seasonal cycle (higher in summer, lower in winter), as temperature does.

Future Directions

- Additional sampling at different sites is necessary to continue investigating the prevalence of significant coastal ocean Nitrogen fixation rates.
- Monitored movement of the Cape Hatteras front through stationary sensors of salinity and temperature, inshore and offshore, would provide clarity as to the relationship of the front and Nitrogen fixation rate variability.

References:

Carpenter, E. J., and D. G. Capone. 2008. Nitrogen fixation in the marine environment, p. 141-198. In D. G. Capone, Deborah A. Bronk, Margaret R. Mulholland, and Edward J. Carpenter [ed.], Nitrogen in the Marine Environment. Academic Press.

Moore, C. M., Mills, M. M., Arrigo, K. R., Berman-Frank, I., Bopp, L., Boyd, P. W., Galbraith, E. D., Geider, R. J., Guieu, C., Jaccard, S. L., Jickells, T. D., La Roche, J., Lenton, T. M., Mahowald, N. M., Marañón, E., Marinov, I., Moore, J. K., Nakatsuka, T., Oschlies, A., … Ulloa, O. (2013). Processes and patterns of oceanic nutrient limitation. *Nature Geoscience*, *6*(9), 701–710. https://doi.org/10.1038/ngeo1765

Mulholland, M. R., Bernhardt, P. W., Blanco-Garcia, J. L., Mannino, A., Hyde, K., Mondragon, E., Turk, K., Moisander, P. H., & Zehr, J. P. (2012). Rates of dinitrogen fixation and the abundance of diazotrophs in North American coastal waters between Cape Hatteras and Georges Bank. *Limnology and Oceanography*, *57*(4), 1067–1083. https://doi.org/10.4319/lo.2012.57.4.1067

Selden, C., Chappell, P.D., Clayton, S., Macías-Tapia, A., Bernhardt, P., Mulholland, M. 2021. A coastal N2 fixation hotspot at the Cape Hatteras front: Elucidating spatial heterogeneity in diazotroph activity via supervised machine learning. *Limnology and Oceanography*, in press, DOI: 10.1002/lno.1172

White, A. E., Granger, J., Selden, C., Gradoville, M. R., Potts, L., Bourbonnais, A., Fulweiler, R. W., Knapp, A. N., Mohr, W., Moisander, P. H., Tobias, C. R., Caffin, M., Wilson, S. T., Benavides, M., Bonnet, S., Mulholland, M. R., & Chang, B. X. (2020). A critical review of the $^{15}N_2$ tracer method to measure diazotrophic production in pelagic ecosystems. *Limnology and Oceanography: Methods*, *18*(4), 129–147. https://doi.org/10.1002/lom3.10353

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