

Defining the realized niche of the two major clades of *Trichodesmium*: a study on the West Florida Shelf

Kristina Confesor¹, Corday Selden^{1,2}, Kimberly Powell¹, Laura Donahue³, Travis Mellett^{4,5}, Salvatore Caprara⁴, Angela N. Knapp⁶, Kristen N. Buck⁴, P. Dreux Chappell¹

¹ Old Dominion University, Department of Ocean and Earth Sciences, Norfolk, Virginia, USA

² Rutgers University, Department of Marine and Coastal Sciences, New Brunswick, New Jersey, USA

³ Haverford College, Haverford, Pennsylvania, USA.

⁴ University of South Florida, College of Marine Science, St. Petersburg, Florida, USA

⁵ University of Washington, School of Oceanography, Seattle, Washington, USA

⁶ Florida State University, Department of Earth, Ocean, and Atmospheric Sciences, Tallahassee, Florida, USA

* Correspondence:

P. Dreux Chappell

pdchappe@odu.edu

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Abstract

The cyanobacterium *Trichodesmium* plays an essential role supporting ocean productivity by relieving nitrogen limitation via dinitrogen (N₂) fixation. The two common *Trichodesmium* clades, *T. erythraeum* and *T. thiebautii*, are both observed in waters along the West Florida Shelf (WFS). We hypothesized that these taxa occupy distinct realized niches, where *T. thiebautii* is the more oceanic clade. Samples for DNA and water chemistry analyses were collected on three separate WFS expeditions (2015, 2018, and 2019) spanning multiple seasons; abundances of the single copy housekeeping gene *rnpB* from both clades were enumerated via quantitative PCR. We conducted a suite of statistical analyses to assess *Trichodesmium* clade abundances in the context of the physicochemical data. We observed a consistent coastal vs. open ocean separation of the two clades: *T. erythraeum* was found in shallow waters where the concentrations of dissolved iron (dFe) and the groundwater tracer Ba were significantly higher, while *T. thiebautii* abundance was positively correlated with water column depth. The Loop Current intrusion in 2015 carrying associated entrainment of Mississippi River water brought higher dFe and elevated abundance of both clades offshore of the 50 m isobath, suggesting that both clades are subject to Fe limitation on the outer shelf. Whereas, previous work has observed that *T. thiebautii* is more abundant than *T. erythraeum* in open ocean surface waters, this is the first study to examine *Trichodesmium* niche differentiation in a coastal environment. Understanding the environmental niches of these two key taxa bears important implications for their contributions to global nitrogen and carbon cycling and their response to global climate change.

1 Introduction

Trichodesmium is an important genus of marine cyanobacteria that converts dinitrogen gas (N₂) into bioaccessible ammonia via N₂ fixation (Capone et al., 2005). Once fixed, new dissolved nitrogen (N) can be released, fueling local food webs (Capone, 2001) and, ultimately, playing an essential role in total ocean productivity by relieving N limitation (Carpenter and Capone, 2008). Indeed, *Trichodesmium*'s new N inputs are estimated at 60-80 Tg N yr (Bergman et al., 2013)—a large fraction of the ocean's total estimated N₂ fixation rate of 100-200 Tg N yr⁻¹ (Karl et al., 2002). Thus, *Trichodesmium*'s N contributions to the biosphere impacts both the global N and carbon (C) cycles, and is thought to influence global carbon dioxide (CO₂) sequestration on hundred-to-thousand-year timescales (Gruber and Sarmiento, 1997; Haselkorn and Buikema, 1997; Falkowski, 1998).

The *Trichodesmium* genus is composed of six different species, grouped into two major clades (Hynes et al., 2011; Rouco et al., 2014). *Trichodesmium thiebautii* and *Trichodesmium erythraeum* are considered the representative species of the two different clades (Rouco et al., 2014), with *T. thiebautii* being the more abundant clade in open ocean regions (Hynes et al., 2011; Chappell et al., 2012; Rouco et al., 2014). The groups differ in their ecophysiology, including in their optimal growth temperatures and iron (Fe) stress responses (Breitbarth et al., 2007; Chappell and Webb, 2010). Nevertheless, most culture-based studies rely on IMS101, a lab strain of *T. erythraeum* first isolated off the North Carolina coast roughly 30 years ago (Prufert-Bebout et al., 1993). Work with IMS101 has suggested that N₂ fixation rates increase commensurately with atmospheric CO₂ (Hutchins et al., 2007; Levitan et al., 2007); however, the growth and N₂ fixation responses of *T. thiebautii* and *T. erythraeum* differ under rising CO₂ concentrations (Hutchins et al., 2013). Understanding how the environmental sensitivities and realized niches of the two clades differ is essential to leveraging culture-based work and predicting their response to climate change.

Trichodesmium is found at tropical latitudes of the ocean (Capone et al., 2005), including along the West Florida Shelf (WFS) (Lenes et al., 2001). Surface waters beyond the 50 m isobath offshore of Tampa Bay bear low dFe concentrations (Lenes et al., 2001; Mellett and Buck, 2020) that are highly influenced by seasonal dust deposition (Mellett and Buck, 2020). Siderophore production in open ocean *Trichodesmium* colonies may enable the uptake of dust Fe via siderophore-mediated dissolution, making the dust Fe readily available to species with this capability (Basu et al., 2019). However, the *T. erythraeum* strain IMS101 is not known to produce siderophores (Basu et al., 2019), implying that differences in the capability to produce siderophores in *Trichodesmium* communities may lead to niche specialization related to dust deposition.

Low Fe has been shown to limit both N₂ fixation and growth of *Trichodesmium* (Berman-Frank et al., 2001; Kustka et al., 2003). *Trichodesmium* in the open ocean can be co-limited by phosphorus (P) and Fe (Mills et al., 2004; Basu et al., 2019), and has been shown to be more strongly P-limited in North Atlantic waters in comparison to the North Pacific (Sañudo-Wilhelmy et al., 2001; Sohm et al., 2008). Modeling work has shown that an increase in Fe availability can lead to N₂-fixation by *Trichodesmium* becoming P-limited rather than Fe-limited (Ye et al., 2012). Recently it has been found that some *Trichodesmium* species appear to not fix N at all, presumably as a result of evolutionary adaptations to resource availability (Delmont, 2021). Little is known about the physiology of the non-N₂-fixing *Trichodesmium* species, which have been identified solely through metagenomic analysis. Macronutrient concentrations at the WFS are negligible, and well below the half-saturation constant for phosphate uptake by *Trichodesmium* (Lenes et al., 2008), suggesting these trace macronutrient concentrations are likely to have little impact on clade distribution or inhibition of N₂ fixation by nitrate (Knapp et al., 2012).

Previous work examining clade distributions in the open ocean showed evidence of niche separation due to potential resource competition, where *T. thiebautii* distributions extended from the surface down to ≥ 80 m, while *T. erythraeum* was only observed in the mixed layer of the ocean (Rouco et al., 2016). Multiple studies have found that the *T. thiebautii* clade is more abundant than the *T. erythraeum* clade in open ocean waters (Hynes et al., 2011; Chappell et al., 2012; Rouco et al., 2014; Rouco et al., 2016). Whereas, N_2 fixation rates have been shown to positively correlate with dissolved Fe (dFe) concentrations in *T. thiebautii*-dominated open ocean waters (Chappell et al., 2012), the niche preferences of the two clades and the environmental controls on their abundance remain poorly constrained in coastal areas. Here, we assess the realized niches of the primary *Trichodesmium* clades (*T. erythraeum* and *T. thiebautii*) along the WFS by comparing the abundance of a housekeeping gene (*rnpB*) diagnostic of clade identity to physical and chemical variables. By elucidating clade-level niche preferences, this work provides insight into the sensitivity of this biogeochemically important genus to climate forcings.

2 Methods & Materials

2.1 Hydrographic Data

Samples were collected on the R/V Weatherbird II from June 18-21 in 2015 and from April 9-12 in 2019, as well as on the R/V Hogarth from February 27- March 2 in 2018 along the WFS (Figure 1 and Supplementary Figure 1). Surface salinity and temperature measurements were collected on all cruises using the ships' flow-through hydrographic systems (SeaBird). Hydrographic variables such as sea surface salinity and sea surface height (SSH) (Figure 1) for the calendar day and region that each cruise sampled were obtained using Daily CMEMS GLORYS12V1 global reanalysis ($0.083^\circ \times 0.083^\circ$ resolution) (Lien et al., 2021). SSH was used to outline the edge of the Loop Current for each sampling year, while low salinity indicated waters from the Mississippi river were entrained near the edge of the Loop Current in each sampling year. Samples for chemical analyses were obtained from the surface mixed layer as described below.

2.2 Macronutrient & Trace Metal Analyses

Surface (~ 2 m) dFe, barium (Ba), silicic acid (Si) samples were collected using a trace metal clean "towfish" system (Mellett and Buck, 2020). The specific sampling system and analytical details for trace metal and macronutrient concentration measurements are described in Mellett and Buck (Mellett and Buck, 2020). Briefly, trace metal samples were collected with a towfish sampling system and in-line filtered through $0.2 \mu\text{m}$ Acropak capsule filters into acid-cleaned low-density polyethylene bottles (dFe and Ba) or polypropylene tubes (Si and PO_4); samples for dissolved trace metals were acidified to pH ~ 1.8 with ultrapure hydrochloric acid (Optima HCl, Fisher; final concentration 0.024 M). Macronutrient samples (Si and PO_4 , and N+N) were stored frozen (-20°C) until analysis on a Lachat 8500 QuickChem analyzer using colorimetric methods (Parsons et al., 1984). Soluble reactive phosphorus (PO_4 or 'phosphate') and nitrate and nitrite ("N+N") concentrations were mostly below the detection limit (Supplementary Table 1), which is consistent with long term nutrient concentration measurements from the region (Heil et al., 2014). We note that the methodology we used for analyzing macronutrients does not incorporate techniques optimized for low-level concentration analysis, so PO_4 and N+N were excluded in later statistical analyses. Dissolved Fe samples were UV-oxidized, preconcentrated onto a Nobias PA1 chelating resin using an automated seaFAST-pico system, and the resulting eluents analyzed using standard addition on a Thermo Scientific Element XR Inductively Coupled Plasma Mass Spectrometer in medium resolution and counting mode at the University of South Florida (Hollister et al., 2020).

127 Dissolved Ba was measured after a 1:50 dilution in 5% ultrapure nitric acid and quantified by
128 standard addition within 24 hours of the dilution preparation directly on the Element XR.

129 2.3 DNA Sample Collection

130 Surface DNA samples were collected either via the “towfish” system (Mellett and Buck, 2020) or
131 from the ship’s Niskin bottle rosette and filtered onto 0.2 µm polyethersulfone (PES) filters using a
132 MasterFlex® peristaltic pumping system (Avantor™, Pennsylvania, USA). Up to 4 L of water was
133 filtered, with lower volumes of water (1-2 L) collected in 2015. In 2018 and 2019, lower volumes (~1
134 L) were sometimes collected in the very nearshore waters because of filter clogging. In 2015, flat
135 PES filters were stored with Qiagen® RLT Plus buffer (Qiagen, Germany), flash frozen in liquid N₂
136 at sea, and then stored at -80 °C until analysis. On the 2018 and 2019 cruises, Sterivex® cartridge
137 filters (MilliporeSigma, Burlington, MA) were preserved in RNAlater™ (Life Technologies,
138 Carlsbad, CA) at 4 °C for approximately 12-18 hours before being secured in a dry shipper for
139 transport and then storage at -80 °C until analysis.

140 2.4 DNA Extraction

141 Filters were extracted using the Allprep RNA/DNA Mini Kit (Qiagen, Germany) following the
142 manufacturer’s protocol with the addition of bead-beating and homogenization using the
143 QIAshredder® column (Qiagen, Germany). All extractions were performed in a HEPA filtered UV
144 sterilized AC600 PCR workstation (AirClean® Systems, Creedmore, NC). For samples collected on
145 Sterivex® filters, ethanol cleaned PVC pipe cutters were used to open the Sterivex® tube. The filter
146 was then cut out with autoclave-sterilized scalpel blades into two parts, then placed into RLT+ Buffer
147 tubes. DNA samples were eluted in 80 µL buffer EB and stored at -80 °C until qPCR analysis.

148 2.5 Quantitative Polymerase Chain Reaction (qPCR)

149 An established qPCR procedure was used for distinguishing between the two primary *Trichodesmium*
150 clades (Rouco et al., 2014). Clade-specific primers targeted the single-copy housekeeping gene *rnpB*,
151 which encodes for ribonuclease P and is frequently used in qPCR studies of cyanobacteria (Chappell
152 and Webb, 2010; Rouco et al., 2014). Quantitative PCR amplification was performed using a Step
153 One Plus Real Time PCR thermal cycler (Life Technologies, Carlsbad, CA). The only deviation from
154 the original protocol (Chappell and Webb, 2010; Rouco et al., 2014) was that clade-specific gene
155 abundances were absolutely quantified using standard curves prepared from *T. erythraeum* and *T.*
156 *thiebautii* *rnpB* plasmids generated and quantified following the protocol of Chappell and Webb (
157 2010). All standards, no-template controls, and samples were measured in triplicate with
158 PowerSYBR® Green Mastermix fluorescent dye (Life Technologies, Carlsbad, CA) using 96-well
159 plates. No-template control wells, used to monitor contamination, contained the qPCR master-mix,
160 clade-specific primers, and Rnase-free water. Samples were run as 1:10 dilutions of the original
161 extracted DNA with 2 µL of sample in a 20 µL reaction. Melt curves were incorporated to ensure that
162 single products were successfully amplified (Rouco et al., 2014) and all qPCR efficiencies were
163 above 93%.

164 Standard curves were created in triplicate with known gene abundances of serially diluted plasmid
165 standards of each clade. The critical threshold, the point at which fluorescence intensity crosses the
166 detectable level and corresponds to the initial abundance of DNA in samples (Kralik and Ricci,
167 2017), was determined by the Step One Plus software and values from the triplicate qPCR reactions
168 for each unknown were averaged and compared with the standard curve. Limits of detection and
169 limits of quantification were determined for each sample as described in Selden et al. (2021). In

subsequent statistical analyses, we chose a conservative approach whereby samples that were below detection limit (BDL, < 3 gene copies/PCR) were given the value of zero and samples that were detectable but unquantifiable (BQL, <10 gene copies/PCR) were given the value that was the limit of detection. Effective limit of quantification (ELOQ) are defined as 2000-3125 copies/L in 2015, 948-5000 copies/L in 2018, and 625 – 2000 copies/L in 2019.

2.6 Statistical Analyses

Kolmogorov-Smirnov tests were performed to test residuals for normality; however, residuals did not follow a normal distribution. Consequently, non-parametric tests (Spearman's Correlation and Kruskal-Wallis) using MATLAB, R2020a and ordination statistics (canonical correspondence analysis (CCA)) using the VEGAN package in RStudio (Dixon, 2003), were employed to compare clade abundances and correlations with environmental data. CCA was done with only one constrained axis. Environmental data evaluated for correlation with *Trichodesmium* spp. clades included dFe, Si, and Ba concentrations, as well as salinity and water column depth. Water column depths were obtained from ETOPO1 (bedrock) bathymetry data grid extraction (Amante and Eakins, 2009).

3 Results & Discussion

3.1 Physiochemical Data

On the WFS, surface dFe concentrations were highest near the coast (mean = 3.84 ± 3.14 nM, Table 1) and declined towards the shelf-break where waters were more oceanic (mean = 0.70 ± 0.62 nM, Table 1, Figure 2D-2F, & Supplementary Table 1). Areas near the shelf-break and west of the 50 m isobath are defined as offshore, while areas on the mid-shelf with bottom depths <50 m are defined as inshore (Figure 2). Concentrations of dFe were negatively correlated with water column depth (Spearman: 5.07×10^{-9} , Rho = -0.736). The dFe concentrations were likely elevated in surface waters on the shelf due to proximity to continental sources, including riverine and submarine groundwater discharge (SGD), and shallow water column mixing with shelf sediments.

We used Ba and Si to trace continental runoff sources, including inputs from rivers and SGD (Shaw et al., 1998; Oehler et al., 2019). Continental runoff can deliver essential nutrients to macro- and microbiota living in coastal areas (Charette et al., 2013; Wang et al., 2018), and SGD in particular has been previously observed to be important on the inner WFS (<50 m) (Hu et al., 2006). As expected, Ba and Si were more abundant at stations with shallower bottom depths (Ba- Spearman: 1.38×10^{-12} , Rho=-0.817, Si- Spearman: 1.48×10^{-8} , Rho=-0.711, Table 1) and were also positively correlated with one another (Spearman: 3.48×10^{-07} , Rho=0.659), supporting the hypothesis that continental runoff was an important source of these elements on the inner shelf at the time of this study.

Based on salinity and SSH, we collected samples at or near the Loop Current edge on all three cruises (Figure 1) (Mellett and Buck, 2020). The Loop Current originates from warm Caribbean waters, enters the Gulf of Mexico through the Yucatan Channel and exits via the Florida Strait (Morrison et al., 1983). This water mass has been shown to be low in trace metals and macronutrients, but serves as a physical vector for bringing elevated dFe concentrations to the outer WFS region, by entrainment of Mississippi River plume water along the edge of the Loop that is advected south to the WFS (Mellett and Buck, 2020). We observed evidence of this entrainment across all three of our cruises (Figure 1 & Figure 2), though the effect on offshore dFe was most pronounced in 2018 against the backdrop of much lower wintertime surface dFe (Figure 2E).

3.2 *Trichodesmium* Clade Gene Abundance in Coastal vs. Offshore Samples

Our results support a distinct niche distribution between the two *Trichodesmium* clades along the WFS, where *T. erythraeum* dominates the inner shelf (Kruskal-Wallis: $p=0.0242$, $\text{Chisq}=5.08$, $\text{df}=66$, $\text{std.dev}=200,427$) and *T. thiebautii* dominates the outer shelf (Kruskal-Wallis: $p=0.000439$, $\text{Chisq}=12.4$, $\text{df}=66$, $\text{std.dev}=214,000$) (Figure 3). Absolute gene abundances of *T. thiebautii* consistently averaged between 1.0×10^5 - 1.0×10^6 gene copies/L offshore of the 50 m isobath (Figure 3A-3C), comparable with the average cells per L seen in prior studies at further offshore surface waters of the Atlantic (Rouco et al., 2014). In many inshore stations, *T. thiebautii* was below detection limits (Supplementary Table 1). *T. erythraeum*, on the other hand, had average ranges of 1.0×10^5 - 1.0×10^6 gene copies/L inshore of the 50 m isobath (Figure 3G-3I). In many offshore stations, *T. erythraeum* was not detectable (Supplementary Table 1). Notably, *T. erythraeum* was not detected inshore during the 2015 cruise (Figure 3G), but we ascribe this anomaly to a higher detection limit for that specific sampling event that precluded quantification (Figure 3G & Supplementary Table 1).

Altogether, our results support prior studies suggesting that *T. thiebautii* is the more oceanic clade (Chappell et al., 2012). It is important to note that on each cruise, stations sampled close to the Loop Current had elevated abundances of both *Trichodesmium* clades, which may have contributed to a higher mean abundance at offshore stations in 2015 (Figure 3). This may have resulted of the edge of the Loop Current delivering dFe and other nutrients to the shelf from entrainment of Mississippi River plume water as well as simultaneous dust deposition offshore in this season. The known SGD inputs inshore of the 50 m isobath (Hu et al., 2006) may also contribute to the differences in gene abundances and perceived environmental niches.

Before evaluating physicochemical drivers of clade distributions, we focused on water column depth as a way to distinguish between inshore and offshore samples. Water column depth and gene abundances were compared using multiple non-parametric tests (Spearman correlation and Kruskal-Wallis tests) to ensure that results were consistent regardless of analytical tool. Water column depth and *T. thiebautii* abundance were positively correlated (Spearman: $p=0.0009$, $\text{Rho}=0.4063$) while bottom depth and *T. erythraeum* abundance were negatively correlated (Spearman: $p=0.0123$, $\text{Rho}=-0.3114$). *T. erythraeum* was most often observed in waters <50 m depth. Based on these initial analyses, we distinguished stations deeper than 50 m as ‘offshore’ and those shallower as ‘inshore’. This distinction was used in subsequent statistical analyses. Additionally, prior work has indicated that WFS waters <50 m are more influenced by SGD (Hu et al., 2006), which is consistent with the elevated dFe and groundwater tracers observed inshore of the 50 m isobath (Figure 2 & 3, Table 1).

3.3 Gene Abundance Correlations with Physiochemical Data

To identify potential drivers of niche differentiation between the clades that might explain differences between inshore and offshore distributions, we examined correlations between each clade’s gene abundance with a number of physicochemical variables. Spearman correlations were employed to evaluate clade abundances with physiochemical data, while CCA was used to evaluate associations between both clades and environmental parameters. Using CCA, significant correlations were found between the two clades and dFe and Ba concentrations, as well as water column depth (Figure 4). Significant positive correlations were found between salinity and *T. thiebautii* abundance (Spearman Correlation (right tail): $p=0.0003$, $\text{Rho}=0.4079$), consistent with this being the more oceanic clade as salinity increased offshore (Figure 2A-2C). In the CCA analysis, there was no significant correlation

between the abundance of both clades with salinity; rather, water column depth was significantly correlated with the abundance both the clades (Figure 4). This is consistent with *T. thiebautii* being the more oceanic clade as water column depth increased, while *T. erythraeum* is the more coastal clade associated with shallower water columns.

Niche differentiation may also be related to ecological competition associated with dFe availability. In support of this hypothesis, *T. thiebautii* was negatively correlated with dFe concentration (Spearman Correlation, Table 2). CCA, but not Spearman, revealed a correlation between dFe and clade abundances (Figure 4), where *T. erythraeum* was positively correlated with dFe, but negative correlated with *T. thiebautii*. Prior studies have shown that in Fe-limited open ocean waters, *T. thiebautii* is the dominant *Trichodesmium* clade (Chappell and Webb, 2010); our results suggest that when dFe is higher inshore, the oceanic *Trichodesmium* clade representative, *T. thiebautii*, is outcompeted by *T. erythraeum*.

We proffer that this trend is driven by competition with *T. erythraeum* and other phytoplankton inshore, meaning that *T. thiebautii* is outcompeted by *T. erythraeum* when dFe from SGD and/or other sources is elevated inshore. This is supported by the observations that *T. thiebautii* was also negatively correlated with Ba and Si concentrations (Spearman Correlation, Table 2), while dFe and Ba were positively correlated with *T. erythraeum* (CCA, Figure 4), in particular during the Loop Current intrusion in 2015. The Loop Current intrusion resulted in enhanced offshore *T. erythraeum* abundance in 2015 (Fig 3G and Supplementary Table 1). However, we do not see *T. erythraeum* abundance elevated offshore in 2018 (Figure 3E and Supplementary Table 1), even though there was elevated dFe offshore as a result of the Loop Current intrusion (Figure 2E, Supplementary Table 1). This suggests that *T. erythraeum* is also Fe-limited offshore of the 50 m isobath on the WFS, potentially due to the lack of siderophore production mechanisms that open ocean *Trichodesmium* colonies have (Basu et al., 2019), except in situations where entrainment of dFe from continental runoff sources is carried by Loop Current to offshore locations.

4 Conclusion

Our findings show that the two main *Trichodesmium* clades, *T. thiebautii* and *T. erythraeum*, occupy distinct realized niches on the WFS. *T. thiebautii* is the more oceanic clade: its abundance was elevated in samples collected at deeper stations (>50 m), with elevated salinity and lower dFe. *T. erythraeum* is more coastal: it was most abundant at shallow stations with significant continental runoff inputs and higher dFe, and appears to outcompete *T. thiebautii* in coastal regions. These distinct niche occupations are likely due to resource competition between the two clades at stations with higher dFe. More work is needed to identify the metabolic pathways that distinguish the two clades and lead to the observed distinctions in distribution patterns. The intrusion of the Loop Current and associated entrainment of Mississippi River water brought higher dFe and elevates gene abundances of the two clades offshore of the 50 m isobath, suggesting that both clades are subject to Fe limitation on the outer shelf. Understanding the environmental niches of these two key taxa bears important implications for their contributions to global N and C cycling and their response to global climate change.

5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6 Author Contributions

ANK, KNB, and PDC designed the sampling program. CS, KC, and PDC designed the *Trichodesmium* analysis plan. All authors were involved in sample collection and/or analysis. KC, CS, and PDC wrote the initial manuscript draft. All other authors contributed to manuscript editing and approved the final manuscript.

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9 Data Availability Statement

The datasets analyzed for this study is available for direct download (Supplementary Table 1) and are archived in the Biological and Chemical Oceanography Data Management Office (<https://www.bco-dmo.org/project/814733>).

10 Figure Captions

Figure 1. WFS study region for June 2015 (1A), February-March 2018 (1B), and April 2019 (1C). Overlying sea surface salinity (note different color scales for each panel) from GLORYS12V1 (Lien et al., 2021). White dots indicate stations. Dashed, dotted, and solid lines indicate a SSH of 0 m, 0.2 m, and 0.4 m respectively, outlining the edge of the Loop Current.

Figure 2. Salinity (2A-2C) and dFe concentration (2D-2F) measurements from each sampling year plotted over bathymetry extracted from ETOPO1 bedrock (Amante and Eakins, 2009). Dashed line indicates the 50 m isobath which is used to distinguish between offshore and inshore samples and indicates where there are known SGD inputs inshore of this area (Hu et al., 2006).

Figure 3. Absolute gene abundance for *T. thiebautii* (3A-3C) and *T. erythraeum* (3G-3I), as well as relative gene abundance for *T. thiebautii* (3D-3F) and *T. erythraeum* (3J-3L) with contours reflecting bathymetry extracted from ETOPO1 bedrock (Amante and Eakins, 2009). In all panels, the dashed line indicates the 50 m isobath, which is used to distinguish between offshore and inshore samples and indicates where there are known SGD inputs inshore of this area (Hu et al., 2006). Triangles represent *Trichodesmium* spp. abundances below the detection limit (BDL), while squares represent data that is below the quantification limit (BQL). (Effective limit of quantification (ELOQ): 2000-3125 copies/L in 2015, 948-5000 copies/L in 2018, and 625 – 2000 copies/L in 2019).

Figure 4. CCA model of gene abundances of both clades in relation to physiochemical data and water column depth (extracted from ETOPO1 bedrock (Amante and Eakins, 2009). Clades are

labeled on the data points, while blue arrows show the x-axis directionality of physiochemical data and environmental parameters. There was only one constrained axis as there were only two clades of interest. Significant environmental parameters and the two clades included dFe (nM) (p-value=0.001, Chi-square=0.116), Water column depth (m) (p-value=0.001, Chi-square=0.058), and Ba (nM) (p-value=0.001, Chi-square=0.490).

Supplementary Figure 1. WFS study region for June 2015 (1A), February-March 2018 (1B), and April 2019 (1C). Gray lines represent cruise track.

11 Tables

Table 1. Average values and standard deviations of physiochemical data from 2019 (first two columns) and from all three cruises combined (last two columns). Values from 2015 & 2018 are reported in Mellet and Buck (2020). Offshore is defined as west of the 50 m isobath, and inshore as all measurements east of the 50 m isobath.

Measurement	Offshore 2019	Inshore 2019	Offshore Total	Inshore Total
dFe (nM)	0.400 ± 0.15	2.81 ± 1.50	0.70 ± 0.62	3.84 ± 3.14
Ba (nM)	46.2 ± 5.7	59.1 ± 7.0	48.9 ± 3.8	61.1 ± 9.7
Si (uM)	1.07 ± 0.20	2.02 ± 1.08	0.95 ± 0.65	2.31 ± 1.76
Salinity	36.4 ± 0.1	35.0 ± 0.8	36.5 ± 0.2	35.4 ± 0.9

Table 2. Spearman individual correlations between *Trichodesmium* clades and chemical concentrations. Asterisks indicate a significant p-value.

Element concentration correlations	p-value	Rho
dFe & <i>T. thiebautii</i> (left-tail)*	0.0047	-0.3794
Ba & <i>T. thiebautii</i> (left-tail)*	0.0133	-0.3201
Si & <i>T. thiebautii</i> (left-tail)*	0.0082	-0.3446
Salinity & <i>T. thiebautii</i> (right-tail)*	0.000149	0.4284
Water Column Depth & <i>T. thiebautii</i> (right-tail)*	0.0000302	0.4698
dFe & <i>T. erythraeum</i> (right-tail)	0.368	0.0511
Ba & <i>T. erythraeum</i> (right-tail)*	0.0172	0.3059
Si & <i>T. erythraeum</i> (right-tail)	0.113	0.1779
Salinity & <i>T. erythraeum</i> (left-tail)	0.1486	-0.1293
Water Column Depth & <i>T. erythraeum</i> (left-tail)*	0.0200	-0.2516

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Figure 1. WFS study region for June 2015 (1A), February-March 2018 (1B), and April 2019 (1C). Overlying sea surface salinity (note different color scales for each panel) from GLORYS12V1 (Lien et al., 2021). White dots indicate stations. Dashed, dotted, and solid lines indicate a SSH of 0 m, 0.2 m, and 0.4 m respectively, outlining the edge of the Loop Current.

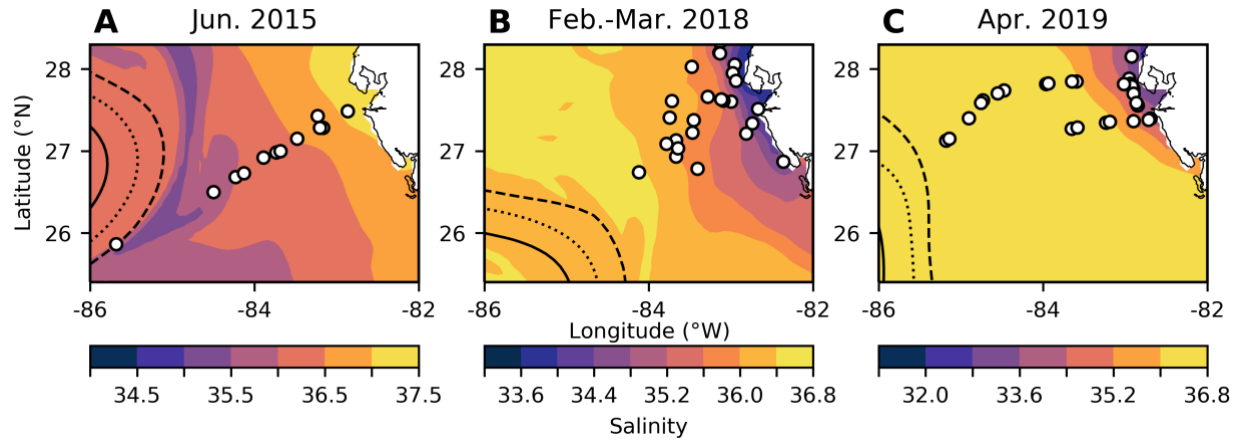


Figure 2. Salinity (2A-2C) and dFe concentration (2D-2F) measurements from each sampling year plotted over bathymetry extracted from ETOPO1 bedrock (Amante and Eakins, 2009). Dashed line indicates the 50 m isobath which is used to distinguish between offshore and inshore samples and indicates where there are known SGD inputs inshore of this area (Hu et al., 2006).

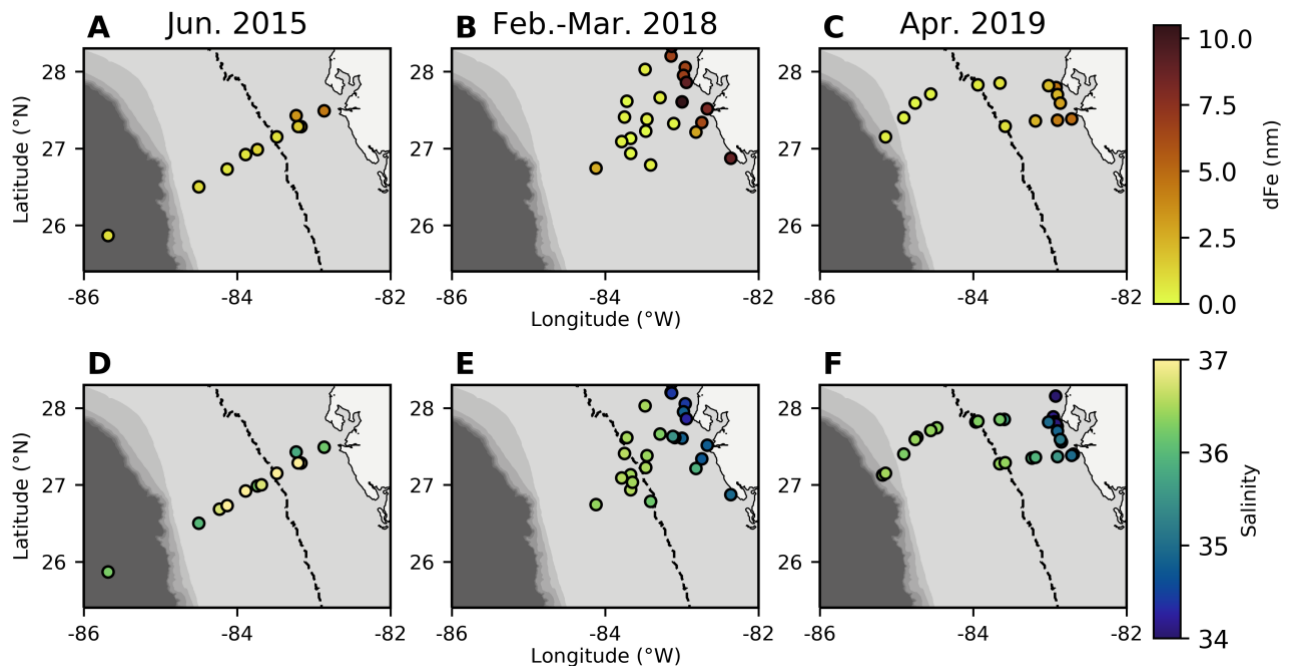
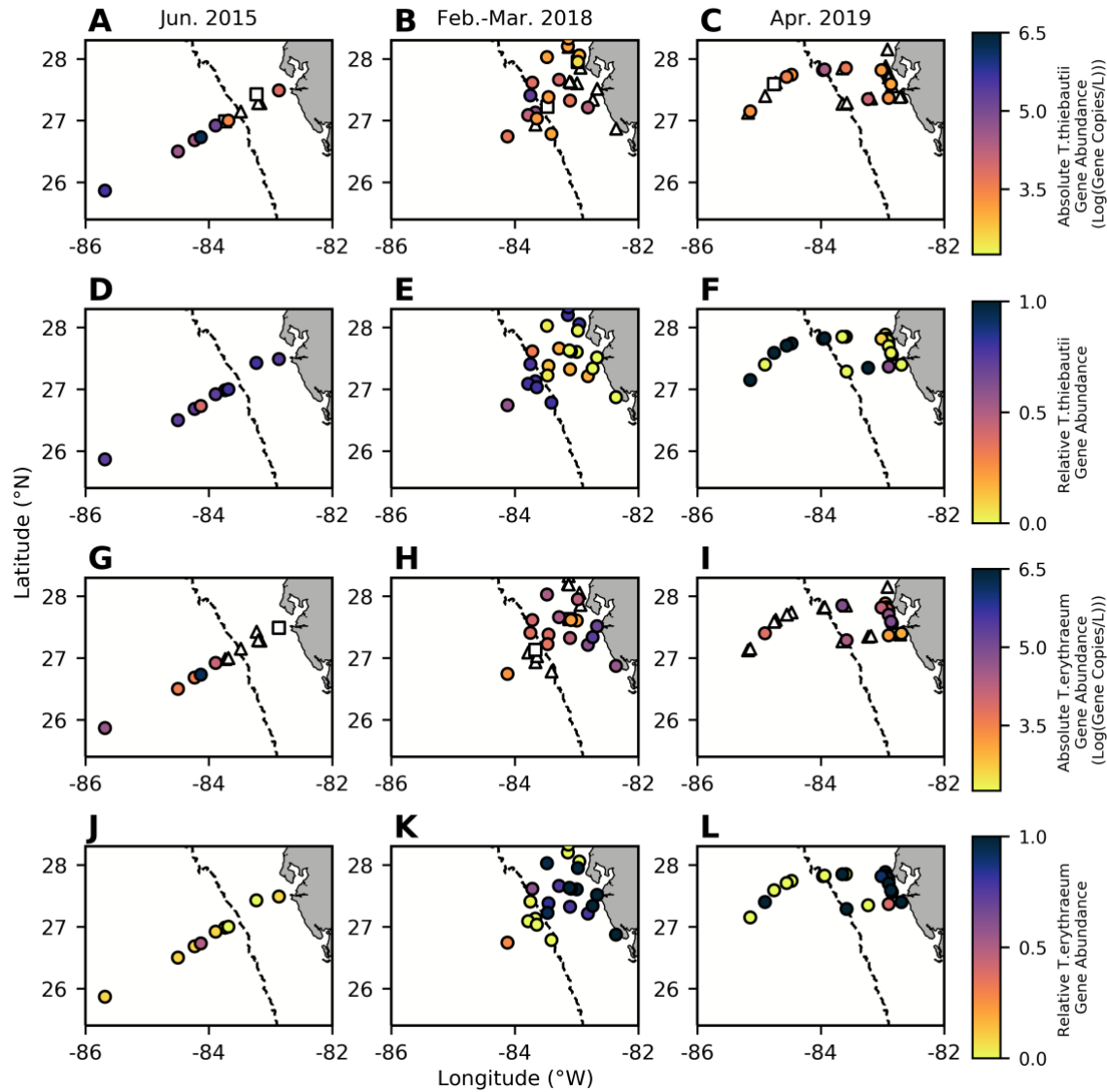
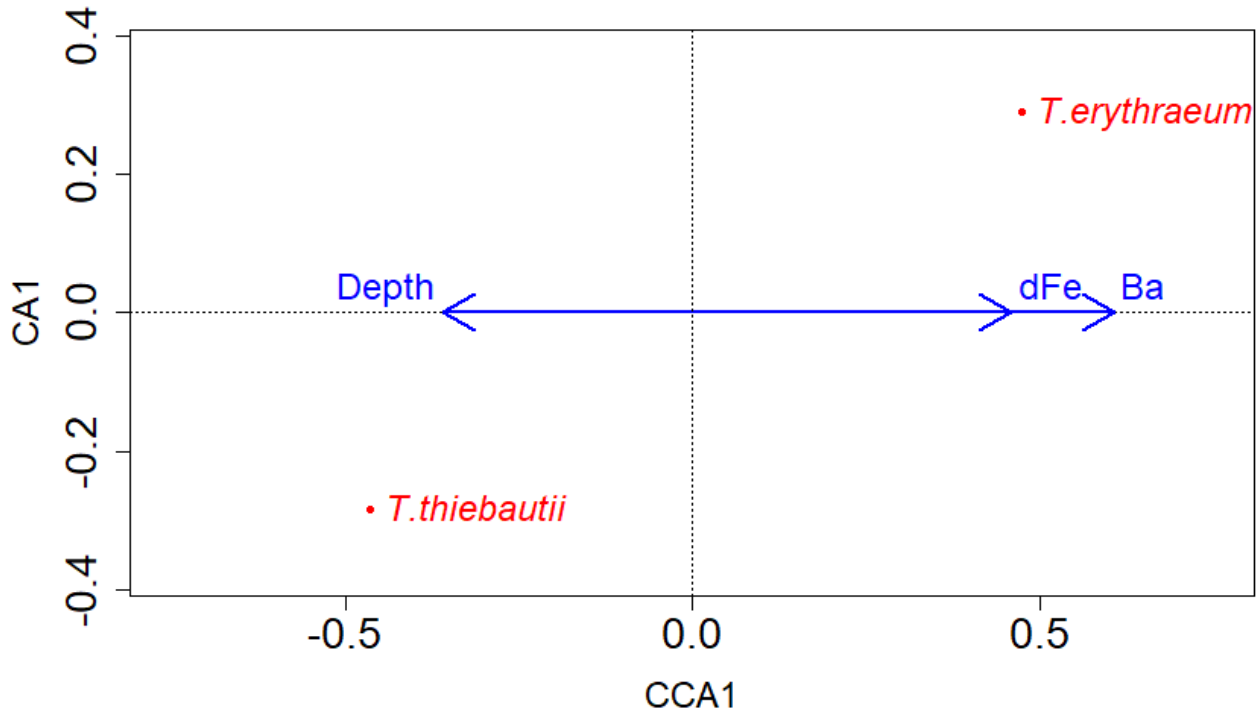


Figure 3. Absolute gene abundance for *T. thiebautii* (3A-3C) and *T. erythraeum* (3G-3I), as well as relative gene abundance for *T. thiebautii* (3D-3F) and *T. erythraeum* (3J-3L) with contours reflecting bathymetry extracted from ETOPO1 bedrock (Amante and Eakins, 2009). In all panels, the dashed line indicates the 50 m isobath, which is used to distinguish between offshore and inshore samples and indicates where there are known SGD inputs inshore of this area (Hu et al., 2006). Triangles represent *Trichodesmium* spp. abundances below the detection limit (BDL), while squares represent data that is below the quantification limit (BQL). (Effective limit of quantification (ELOQ): 2000-3125 copies/L in 2015, 948-5000 copies/L in 2018, and 625 – 2000 copies/L in 2019).



514 **Figure 4.** CCA model of gene abundances of both clades in relation to physiochemical data and
 515 water column depth (extracted from ETOPO1 bedrock (Amante and Eakins, 2009). Clades are
 516 labeled on the data points, while blue arrows show the x-axis directionality of physiochemical data
 517 and environmental parameters. There was only one constrained axis as there were only two clades of
 518 interest. Significant environmental parameters and the two clades included dFe (nM) (p-value=
 519 0.001, Chi-square=0.116), Water column depth (m) (p-value=0.001, Chi-square=0.058), and Ba (nM)
 520 (p-value=0.001, Chi-square=0.490).



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