Defining the environmental niche of the two main clades of *Trichodesmium*: a study on the West Florida Shelf

Kristina Confesor[1], Corday Selden[2], Kimberly Powell[1], Angela N. Knapp[3],
Kristen N. Buck[4], Laura Donahue[5], and Dreux Chappell[1]
[1]Old Dominion University, [2]Rutgers University, [3]Florida State University, [4]University of South Florida, [5]Haverford College

Introduction

- Dinitrogen (N_2) fixation is the process of taking widely abundant but mostly biologically inaccessible N_2 gas and converting it into more biologically accessible forms of the essential macronutrient nitrogen. Only a small fraction of organisms known as diazotrophs can perform biological N_2 fixation.
- *Trichodesmium* is one important diazotrophic genus of N_2-fixing marine cyanobacteria found in nutrient-poor tropical regions of the ocean with the ability to fix both atmospheric nitrogen and carbon (Capone, 2001; Gruber and Galloway 2008).
- *T. thiebautii (T.thi)* is the more abundant active clade in open ocean surface waters in comparison to *T. erythraeum (T.ery)* (Chappell et al. 2012). Prior studies have indicated the potential for niche separation of the two clades possibly associated with distance from the coast (Bergman et al. 2013; Rouco et al. 2014). None of these prior studies examined near shore samples, and this study sets out to explore potential differences in clade abundance near shore as well as correlations with hydrographic data.

Research Questions:

- Are there any trends in niche distribution on the West Florida Shelf (WFS)?
- What correlations exist between the two clades and hydrographic data?

Hypothesis:

- *T. thi* abundance will be higher further from the coast (stations with greater bottom depths or higher salinity) and the *T. ery* abundance will be higher closer to the coast (stations with lower bottom depths).

Methods

- 1-4 L of surface water collected via tow-fish or CTD Rosette was filtered onto 0.2 µm PES filters from transects on the West FL Shelf in June 2015, February 2018, and April 2019. Filters were extracted using Qiagen® Allprep RNA/DNA Mini Kit
- *Trichodesmium* clade abundance in extracted DNA was determined using the q-PCR procedure of Rouco et al. 2014 on a Step One Plus Real Time PCR thermal cyclerStandard curves for each major clade were prepared using *T. ery* and *T. thi rnpB* plasmids.
- Statistical analyses (Spearman's Correlation, Wilcoxon signed-rank, and Kruskal-Wallis) comparing clade abundances and distribution with environmental data were performed using Matlab.
- Bottom depths were obtained from ETOPO1(bedrock) data grid extraction from NOAA.gov using Python and Jupyter.

References

- Bergman, B., Sandh, G., Lin, S., Larsson, J., & Carpenter, E. J. (2013). *Trichodesmium*--a widespread marine cyanobacterium with unusual nitrogen fixation properties. FEMS microbiology reviews, 37(3), 286–302.doi:10.1111/j.1574-6976.2012.00352.x
- Capone, D. Marine nitrogen fixation: what's the fuss? (2001). Current Opinion in Microbiology 4: 341-348
- Chappell, P. D., Moffett, J. W., Hynes, A. M., & Webb, E. A. (2012). Molecular evidence of iron limitation and availability in the global diazotroph *Trichodesmium*. The ISME journal, 6(9), 1728–1739. doi:10.1038/ismej.2012.13
- Gruber, N., & Galloway, J. (2008). Gruber N, Galloway JN.. An Earth-system perspective of the global nitrogen cycle. Nature 451: 293-296. Nature. 451. 293-6. 10.1038/nature06592.
- Rouco, M., Joy-Warren, H., McGillicuddy, D., Waterbury, J., and Dyhrman, S. (2014). *Trichodesmium* sp. Clade distributions in the western North Atlantic Ocean. Limnology and Oceanography 59(6): 1899-1909.

Data

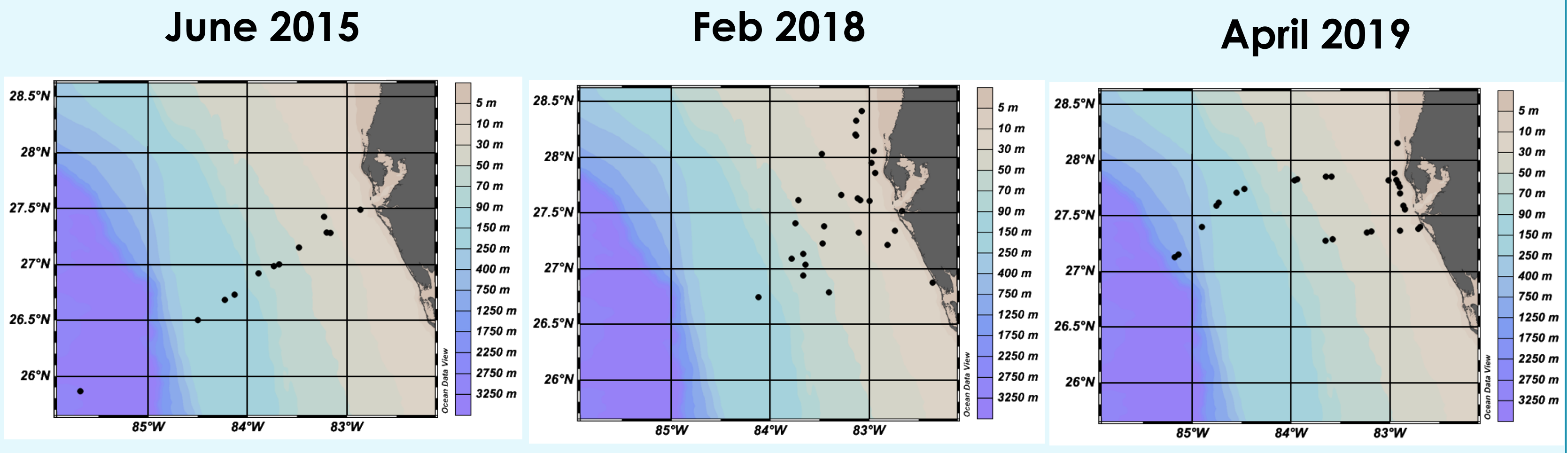

Figure 1. Maps of where samples were collected on the WFS in June 2015, February 2018, and April 2019.

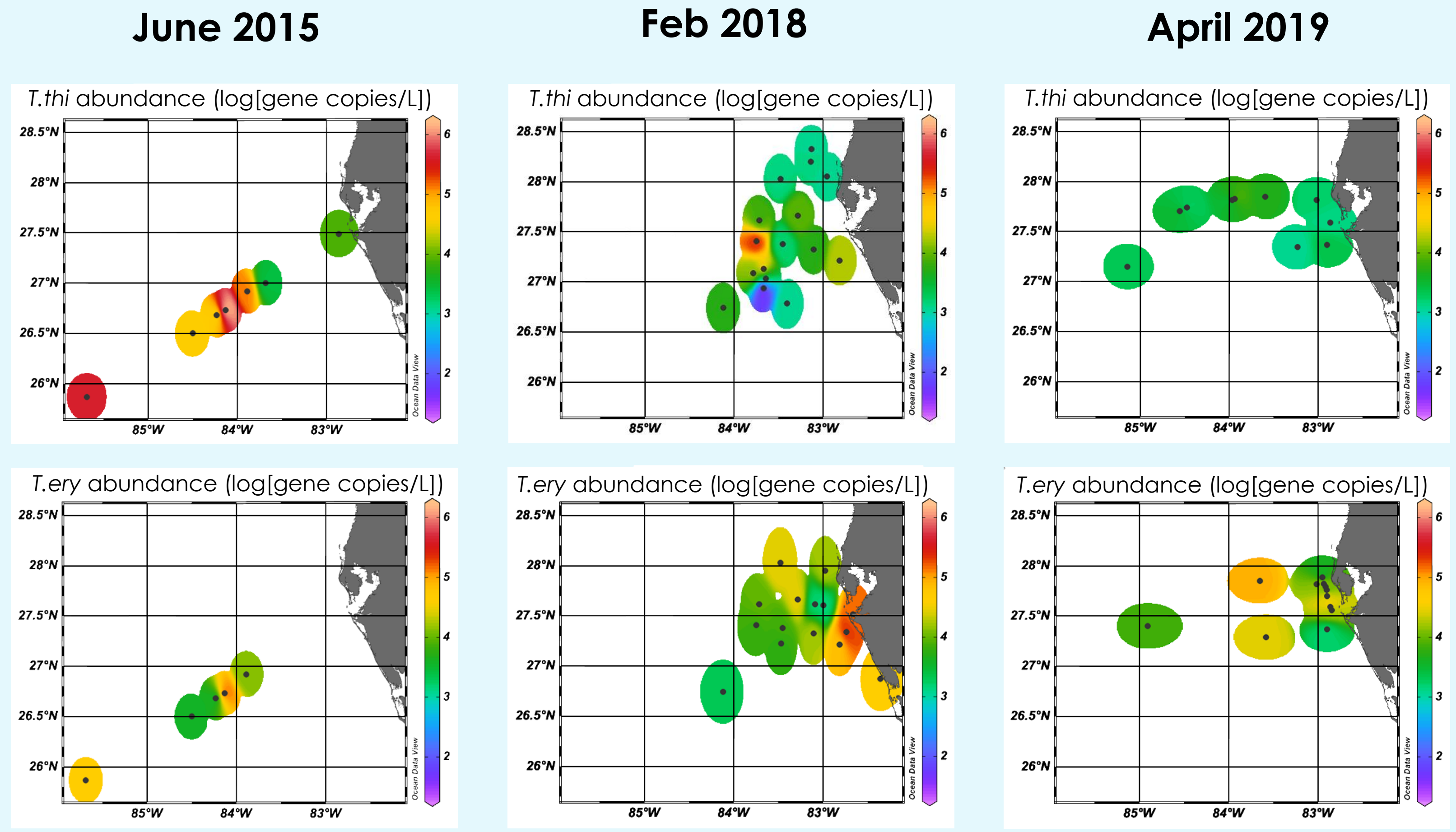

Figure 2. Niche distribution (log[gene copies/L]) of *T. thi* and *T. ery* clades *on* 2015, 2018, and 2019 cruises. Surface sample collection sites with no data were below quantification limits. (Effective limit of quantification (ELOQ): 2000-3125 copies/L in 2015, 948-5000 copies/L in 2018, and 625 – 2000 copies/L in 2019).

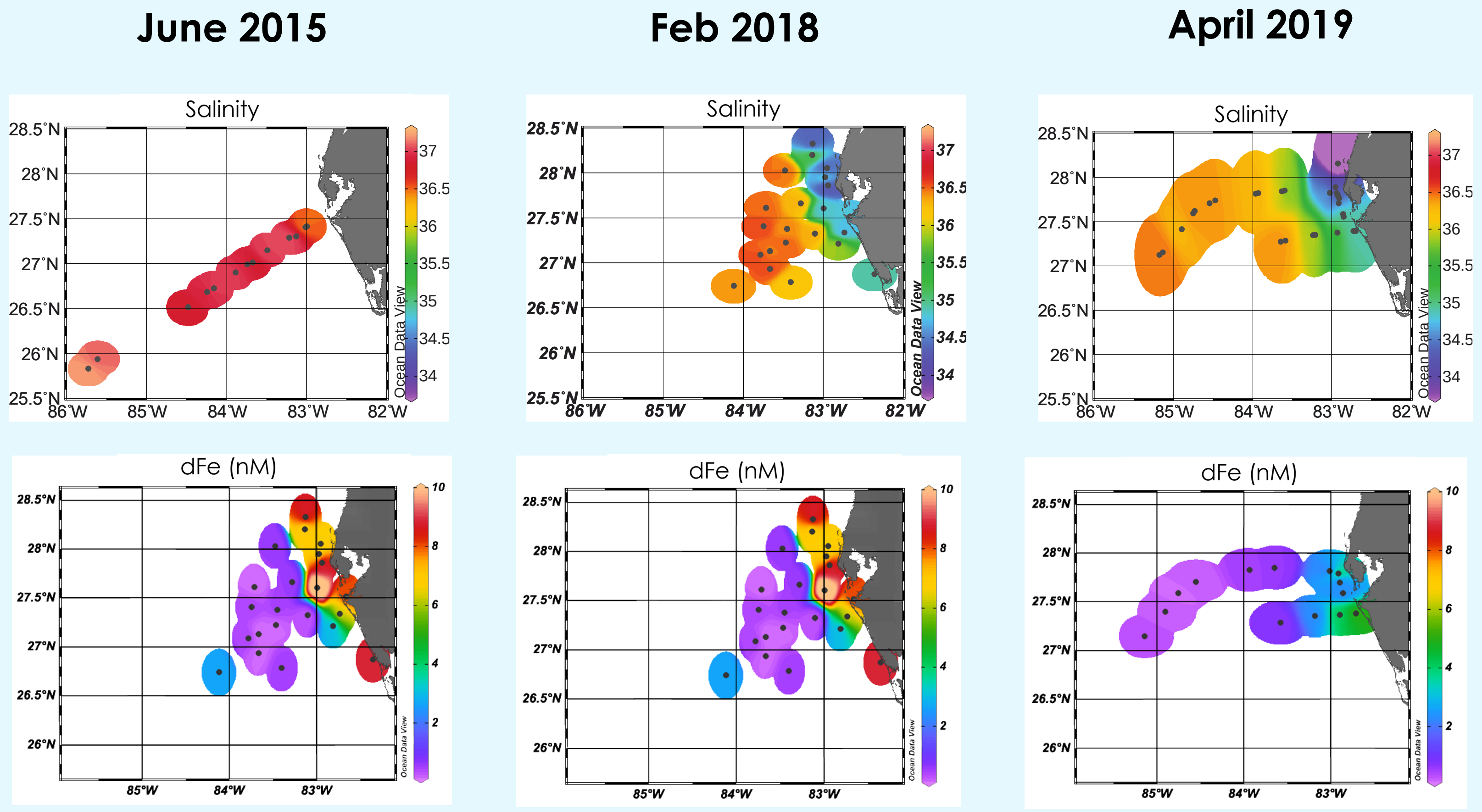

Figure 3. Surface salinity and dissolved iron (dFe, units in nM) concentration sample collections on WFS research cruises in 2015, 2018, and 2019.

Results & Discussion

Test	df	Std.dev	z-value	signedrank	Rho	Chi-sq	p-value
Wilcoxon (bottom depth > 50 vs clades)			3.5093	199			0.00044934
Wilcoxon (bottom depth < 50 vs clades)			-3.7527	78			0.00017495
Spearman (Salinity vs T.thi)					0.4079		0.00030636
Spearman (Bottom Depth vs. T.thi)					0.4063		0.00086400
Spearman (Bottom Depth vs. T.ery)					-0.3114		0.0123
Spearman (Bottom Depth vs. dFe)					-0.7513		4.2068×10^{-9}
Kruskal-Wallis (T.Thi < 50 m vs T.Thi > 50 m)	63	0.8351				7.66	0.0056
Kruskal-Wallis (T.Ery < 50 m vs T.Ery > 50 m)	63	0.9589				7.85	0.0051

Table 1: Statistical Analysis results. Bottom depth range is between 0-500 m, with 50 m marking the location of isobaths along the WFS.

- There exists a difference between the two clades at bottom depths > 50 (Wilcoxon: p-value=0.00044934, z-value=3.5093, sign rank=199), and bottom depths < 50 (Wilcoxon: p-value=0.00017495, z-value=-3.7527, sign rank=78).
- There is a correlation between bottom depth and the *T.thi* (Spearman: p=0.00086400, Rho=0.4063), *T.ery* (Spearman: p=0.0123, Rho=-0.3114), and dFe (Spearman: 4.2068×10^{-9}, Rho=-0.7513) variables, but not between dFe and each of the two clades.
- There is also a correlation between salinity and *T. thiebautii,* where as salinity increases, so does gene abundance (Spearman Correlation (right tail): p= 0.00030636, Rho=0.4079)
- Further data analysis suggests that *T.thi* and *T.ery* abundance is different above and below the 50 m isobath, with less T.thi below, (Kruskal-Wallis: p=0.0056, Chi-sq=7.66, df=63, std.dev=0.83510) as well as *T.ery* abundance being more abundant below the 50 m isobath (Kruskal-Wallis: p=0.0051, Chi-sq=7.85, df=63, std.dev=0.99589).
- Both 2015 and 2019 sampling transects sampled the edge of the loop current, which appears to be associated with an offshore increase in *T. erythraeum* abundance.

Future Studies:

- Future analysis will include an exploration of the 50 m isobath significance on clade gene abundance differences. Additionally, adding a multivariable analysis of clades in comparison to hydrographic data may also give a clearer picture of any differences between the two clades.

Acknowledgements

Support for K.C. came from the National Science Foundation (NSF) through a Research Experience for Undergraduates (REU) grant to the Department of Ocean, Earth and Atmospheric Sciences at Old Dominion University. K.C. received additional support from the ASLO Multicultural Program. We thank the Captain and crew of the R/Vs Weatherbird II and Hogarth. Partial funding for shiptime on the R/Vs Weatherbird II and Hogarth was generously provided by Florida Institute of Oceanography grants to K.N.B. and A.N.K. Remaining costs for field sampling came from the start-up funds of A.N.K., P.D.C., and K.N.B. at their respective institutions. Additional funding for A.N.K. was provided by an FSU Planning Grant (CRC# 036701) and NSF-OCE#1537314, for K.N.B. and S.C from NSF-OCE-1446327, and for PDC from the Jeffress Trust Awards Program in Interdisciplinary Science.