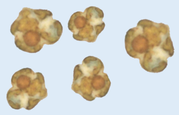
KARENIA BREVIS UTILIZATION OF DISSOLVED ORGANIC NITROGEN IN WASTEWATER AND STORMWATER POND EFFLUENT

Amanda Muni-Morgan[1,2], Mary G. Lusk[1] , Cynthia A. Heil[1] , Amy McKenna[3] , Patricia Scanlon Holland[1], Audrey Goeckner[4]

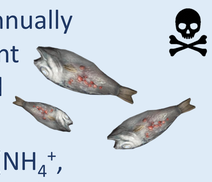
1. Soil and Water Quality Laboratory, Gulf Coast Research and Education Center, University of Florida, Institute of Food and Agricultural Sciences, 14625 CR 672, Wimauma, FL, USA 2. Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, USA 3. National High Magnetic Field Laboratory, Florida State University, 1800 E. Paul Dirac Dr., Tallahassee, FL, USA 4. Soil and Water Sciences Department, University of Florida, 1692 McCarty Dr, Gainesville, FL 32603, USA

Presenter:
Amanda Muni-Morgan
a.munimorgan@ufl.edu
@amuni16

BACKGROUND



- *Karenia brevis* is the toxic dinoflagellate species responsible for Florida red tide.
- Blooms of *K. brevis* occur almost annually in SW Florida[4] and impose significant human health[1], environmental, and economic impacts[7].



- *K brevis* can utilize both inorganic (NH_4^+, NO_3^-) and organic forms of nitrogen (N) in dissolved[5], and particulate[6] forms.
- There are over 76,000 urban stormwater ponds in Florida, which can be net exporters of nutrients, including bioavailable DON (BDON)[9].

- Florida leads the nation in reclaimed water use, with 0.63 mgd released into surface waters (2020)[3].

- The bioavailability of dissolved organic nitrogen (DON) in stormwater pond and municipal wastewater effluent to *K. brevis* has not been determined.

HYPOTHESIS

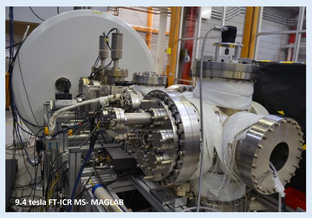
Nearshore Florida waters impacted by municipal wastewater discharges and outflows of urban stormwater ponds will contain a pool of readily available N to K. brevis and specific compound classes utilized in the DON pool can be identified through FT-ICR MS analysis.

METHODS

- Water samples were collected from outflows of 3 stormwater ponds aged 14, 18, and 34 yrs. (SWP 14, SWP 18, SWP 34) in Manatee County, FL, and one wastewater treatment plant (Southeast Water Reclamation Facility, Bradenton, FL).
- *K. brevis* cultures were inoculated with effluent in triplicate. Growth response was monitored for 21 days (508 hours) via microscopy.

- DON was extracted (SPE) at T_0 and T_{508} and analyzed at the National High Magnetic Field Lab using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS).

FT-ICR MS is an ultra high-resolution analysis that can identify thousands of organic compounds within a sample, providing specific measurements of molecular mass and elemental composition.



RESULTS

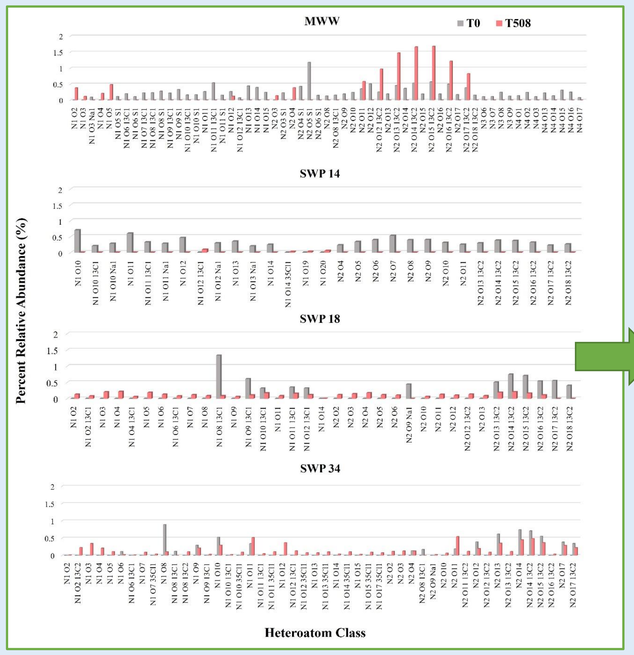


Fig. 1. Heteroatom class distributions of all N-containing compounds, comparing unique to T_0 ('removed') and unique to T_{508} ('produced') in municipal wastewater and stormwater pond effluents.

- MWW contained the largest pool of BDON (45%).
- SWP 14 contained 41% BDON, while SWP 18 and SWP 34 contained 10% and 13% BDON, respectively.
- Depletions of compounds in MWW and SWP 14 correspond to unsaturated hydrocarbon, lignin/CRAM, protein, and amino sugar-like compounds, with notable depletions among all other groups (Fig. 2).
- Increases in DON compounds were observed for SWP 18 and SWP 34, corresponding to lignin and carboxyl-rich alicyclic molecules (CRAM), which have structural similarity to terrestrial humic compounds[10].

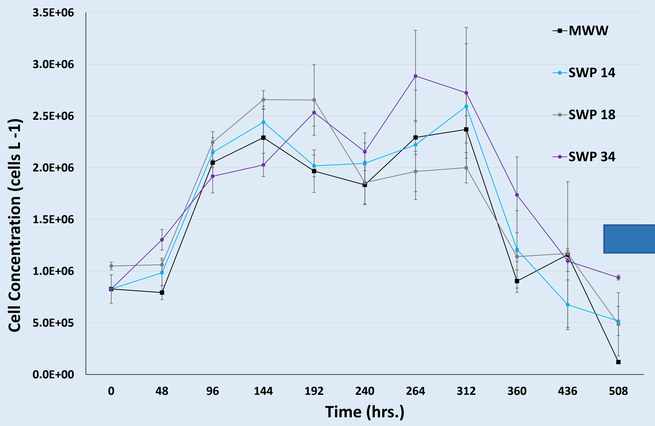


Fig. 3. Effects of municipal wastewater and stormwater effluent on *Karenia brevis* growth response. Each data point is the average of three replicates (±S.E.).

- Negative-ion ESI FT-ICR MS analysis identified 11,247-16,138 species at T_0, and 6,682-19,750 species at T_{508}.
- Notable depletions across all heteroatom classes were observed for MMW and SWP 14 (Fig.1).
- Transformations of N-chloro compounds were observed only in the SWP samples.
- SWP 18 and SWP 34 exhibited increases (167 % and 194 %, respectively) (Fig. 1) across heteroatom classes at T_{508}.
- Transformations of organosulfur compounds were only observed in MWW, observed at T_{508}.

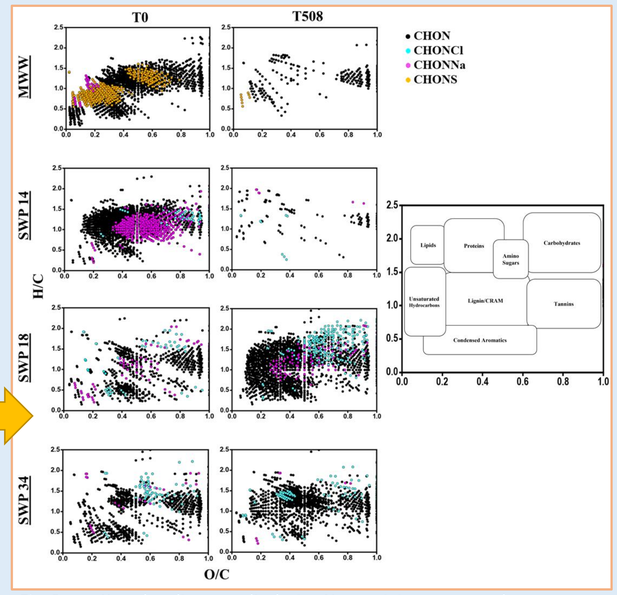


Fig. 2. van Krevelen diagrams displaying N-containing compounds comparing T_0 ('removed') vs. T_{508} ('produced') compounds. The H/C vs. O/C values can be correlated to a biomolecular class.

- *K. brevis* cells within all treatments responded to additions after 48 hrs. except for SWP 34, which exhibited no lag period in response.
- Specific growth rates (μ day^{-1}) for MWW, SWP 14, SWP 18, and SWP 34 were 0.48, 0.39, 0.37, and 0.21, respectively.
- Maximum cell concentrations were in excess of 2.0 x 10^6 cells L^{-1} and were not significantly different from each other (p = 0.791)
- Growth of cells was consistent between all samples until ~ T_{312}, where cell concentrations steadily declined until T_{508} when the experiment was terminated.

DISCUSSION

- The higher % BDON in the MWW and SWP 14 samples suggests that these effluents contained a higher % of DON compounds known to be bioavailable e.g., in biomolecular groups such as carbohydrates, amino acids, and proteins.
- Labile compounds provide a potential source of amino acids, in addition to vitamins and cofactors for *K. brevis* growth.
- DON compositional changes could be due to partial mineralization of semi-labile DON, resulting in an increase of degradation products with greater recalcitrance[8], or DIN assimilation, as phytoplankton release an average of 25-41 % DON after DIN uptake[2,].

CONCLUSIONS

- This work provides evidence of wastewater and urban stormwater pond DON utilization and transformation by a *K. brevis*-bacteria consortia confirmed through bioassay and FT-ICR MS analysis.
- Environmental management and monitoring efforts must expand to include analysis of DON in wastewater and advanced treatment technologies should be considered.
- DON removal efficiencies of stormwater ponds are important for downstream ecosystem health.
- Preventing wastewater discharges to coastal waters can potentially play a role in mitigating *K. brevis* proliferation once a bloom reaches nearshore waters.

REFERENCES

1. Baden, D. G.; Mende, T. J., Amino acid utilization by *Gymnodinium breve. Phytochemistry* **1979,** *18*, (2), 247-251.
2. Bronk, D. A.; Glibert, P. M.; Ward, B. B., Nitrogen uptake, dissolved organic nitrogen release, and new production. *Science* **1994,** *265*, (5180), 1843-1846.
3. FDEP 2020 Reuse Inventory. https://floridadep.gov/sites/default/files/2020Reuse_Inventory_Report_Approved_2.pdf (31 January 2022).
4. Geesey, M.; Tester, P., *Gymnodinium breve*: Ubiquitous in Gulf of Mexico waters?, Toxic Phytoplankton Blooms in the Sea TJ Smayda, Y. Shimizu, 251–255. In Elsevier Sci., New York: 1993.
5. Heil, C. A.; Dixon, L. K.; Hall, E.; Garrett, M.; Lenes, J. M.; O'Neil, J. M.; Walsh, B. M.; Bronk, D. A.; Killberg-Thoreson, L.; Hitchcock, G. L., Blooms of *Karenia brevis* (Davis) G. Hansen and Ø. Moestrup on the West Florida Shelf: Nutrient sources and potential management strategies based on a multi-year regional study. *Harmful Algae* **2014,** *38*, 127-140.
6. Jeong, H. J.; Park, J. Y.; Nho, J. H.; Park, M. O.; Ha, J. H.; Seong, K. A.; Jeng, C.; Seong, C. N.; Lee, K. Y.; Yih, W. H., Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. *Aquatic Microbial Ecology* **2005,** *41*, (2), 131-143.
7. Larkin, S. L.; Adams, C. M., Economic consequences of harmful algal blooms: Literature summary. *Economics of Harmful Algal Blooms: Literature Review. Final report for Gulf of Mexico Alliance Project* **2013,** *100304*.
8. Lechtenfeld, O. J.; Kattner, G.; Flerus, R.; McCallister, S. L.; Schmitt-Kopplin, P.; Koch, B. P., Molecular transformation and degradation of refractory dissolved organic matter in the Atlantic and Southern Ocean. *Geochimica et Cosmochimica Acta* **2014,** *126*, 321-337.
9. Lusk, M.; Toor, G., Biodegradability and Molecular Composition of Dissolved Organic Nitrogen in Urban Stormwater Runoff and Outflow Water from a Stormwater Retention Pond. *Environmental Science and Technology* **2016,** *50*, (7), 3391-3398.
10. Nebbioso, A.; Piccolo, A.; Lamshöft, M.; Spiteller, M., Molecular characterization of an end-residue of humeomics applied to a soil humic acid. *RSC Advances* **2014,** *4*, (45), 23658-23665.

ACKNOWLEDGEMENTS

We express gratitude to Kylie Chapman for assistance in collecting the stormwater samples, Stephen Suau who provided the reclaimed water sample, as well as funding provided by the University of Florida, Andrew and Judith Economos Charitable Foundation, The Charles and Margery Barancik Foundation, and NOAA ECOHAB Grant #NA19NOS4780183.