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FLORIDA ATLANTIC UNIVERSITY
Department of Chemistry and Biochemistry
Charles E. Schmidt College of Science

Refinement and Application of Pigment-Based Chemotaxonomy to the Assessment of Periphyton Communities in the Everglades.

J. William Louda[1], Scot E. Hagerthey[2] , and Panne Mongkhonsri[1]

[1]Organic Geochemistry Group, Department of Chemistry and Biochemistry and the Environmental Sciences Program, Florida Atlantic University, Boca Raton, FL, USA

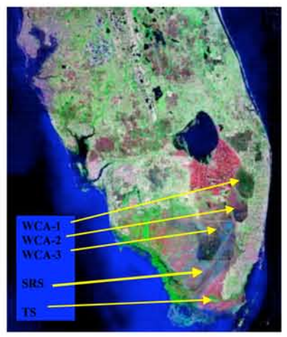
[2]South Florida Water Management District, Everglades Division, West Palm Beach, FL, USA

INTRODUCTION

Changes in periphyton community structure (taxonomic makeup) can occur rapidly in concert with physicochemical cues (nutrients, hydrology, temperature etc.) over the scales of weeks or even days. "At least three environmental gradients – hydroperiod/water depth, phosphorous concentration, and aspects of water chemistry involving the major ions, especially calcium - affect the taxonomic composition, growth characteristics, structure, and extent of calcite encrustation of Everglades periphyton." (Browder et al., 1994). During the **"replumbing"** of the modern Everglades that will occur during the implementation phases of the Comprehensive Everglades Restoration Plan (CERP, aka the Plan) all three of these **gradients may be influenced**. Therefore, methods that will detect any changes and allow rapid notification of mangers to such changes, under the concept of "adaptive management", are being explored.

In the present case, the utilization of **taxon-specific biomarker pigments** is being pursued in order to provide **rapid feedback over broad spatial and temporal scales**. These methods, termed pigment-based chemotaxonomy, will only allow taxonomic description to the Division / Class levels and will therefore not replace microscopy or the newer molecular approaches such as cladistics in detail (Genus species).

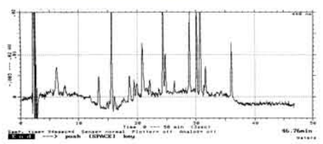
SITES, SAMPLES, MATERIALS & METHODS

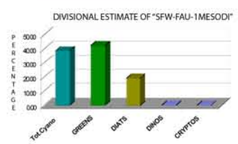


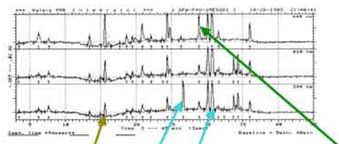
Samples are collected and frozen as soon as possible.
Maintained at -80o C.
Freeze dried
Extracted using methanol: acetone:dimethylformamide:water (MADW, 30:30:30:10) acc. Hagerthey et al., 2006.
Ground, sonicated, filtered.
High Performance Liquid Chromatography-Photodiode Array (HPLC-PDA) used for separation, identification and quantification (Beer's Law) of individual pigments.

Samples derive from SFWMD periphytometer stations and from anyone associated with CERP-RECOVER as cleared by SFWMD for submittal. Many samples have come from Dr. E. Gaiser's periphyton group at Florida International University as well as grab samples from Dr. Joel Trexler.

PIGMENT-BASED CHEMOTAXONOY-101



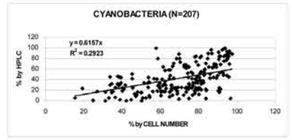


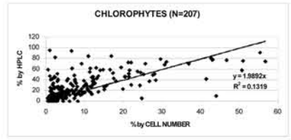


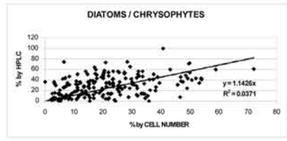
Separate and quantify individual pigments, apply simultaneous linear equations of matrix methods to determine the amount of taxon-specific chlorophyll-a and divide individual contributions by the total to obtain taxon percentages.

ΣCHL*a* = **[1.1(ZEA-ECH)* + 11.0(ECH)] + [3.2(CHL*b*)] + [1.2(FUCO)] + [1.5(PERI)] + [3.8(ALLO)]**

DIVISION ESTIMATES; HPLC VS. CELL







Desired relationship is y = 1x with R^2 = 1.00

CHLa estimate relates better to BIOMASS

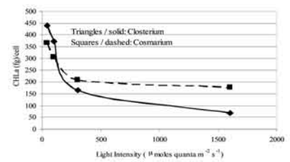
Resultant equations will be used to estimate adjustments in estimator coefficients. That is, with chlorophytes estimator is likely 2x too large (y = 1.99x) etc.

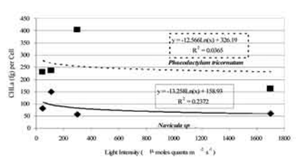
Future microscopic data must include biomass !

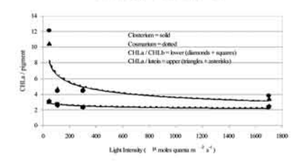
NON-FUNDED SPINOFFS WITH DIRECT APPLICATION / ADVANTAGES TO THE PROJECT.

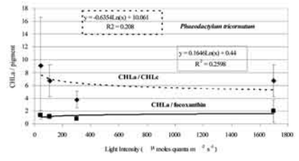
- **Ms. Cidya Grant** (Ph.D. student)-investigating effect of light on pigment ratios and the relationship of chlorophyll-a to biomass (as total organic C, protein, carbohydrate, cell #).
- **Mrs. Maria West** (Ph.D. student)- beginning a project on the ultrastructure of periphyton mats and their changes with alterations in nutrient regimes.
- **Ms. Jaime Browne** (M.S. student): Mathematical modeling of periphyton and the application of the CHEMTAX algorithm. Comparison of CHEMTAX to OGG's existing multiple simultaneous equation methodology.

ASSESSING THE INFLUENCE OF LIGHT ON CHEMOTAXONOMY



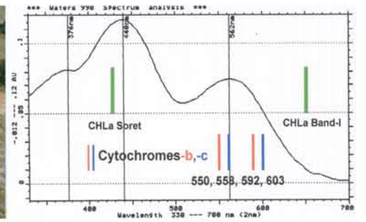






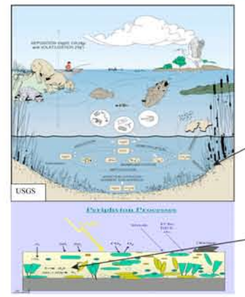
LOW LIGHT = HIGH CONCENTRATIONS
RELIABLE RATIOS (CHLa to CHLb,FUCO, PERI, ALLO, ECHIN)
Others (CHLa to ZEA, CHLs-c, lutein) are problematic

VISIBLE LIGHT SUNSCREEN PIGMENT – HMM ?



This pigment (λ_{max} = 440, 562 nm) is reddish-brown ("mahogany") colored and appears in periphyton of low hydroperiod high light conditions. Also found in cultures of *Scytonmea hoffmani* grown in high (300-1,600 µmol quanta m^{-2} s^{-1}) light conditions. Suggested here to serve to photoprotect the αβ bands of cytochromes and the Soret of Chlorophylls.

PIGMENT APPLICATIONS IN DETECTING POTENTIAL MERCURY METHYLATION SITES



Bacteriochlorophylls-a (purple-S bacteria) and –c/-d (Green-/Brown-S bacteria) exist in various periphyton samples. (pE indicators).

Cleckner et al., in 1999 first related anoxic microzones in periphyton to sites of active mercury methylation.

Our studies with CERP- RECOVER are also tracing these bacterial pigments.

CLOSING

Pigment based chemotaxonomy describes taxon specific chlorophyll-a and in that light relates to biomass. Presently our "ground truthing" efforts reveal low correlation to microscopic data since biomass is not available. Cell numbers are better than "unit" counts for correlation to hplc but still not reflecting reality (i.e. biomass).

Significant strides are being made in the relation of pigment data to light field and mathematical models are being undertaken (again, this will require biomass data).

Sunscreen pigments are being considered as probes to reflect / hindcast hydroperiod.

Bacterial pigments are being investigated as probes for anoxic microzones elated to mercury methylation.

REFERENCES

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Cleckner, L. B., Gilmour, C.C., , Hurley, J.P., and Krabbenhoft, D.P. (1999) Mercury methylation in periphyton of the Florida Everglades. *Limnol. Oceanogr.* **44**: 1815-1825.

ACKNOWLEDGEMENT

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