Phytosterols as tracers of terrestrial and wetland carbon to Ten Thousand Islands, Florida, USA:

Implications for trophic resource use in the eastern oyster, *Crassostrea virginica*

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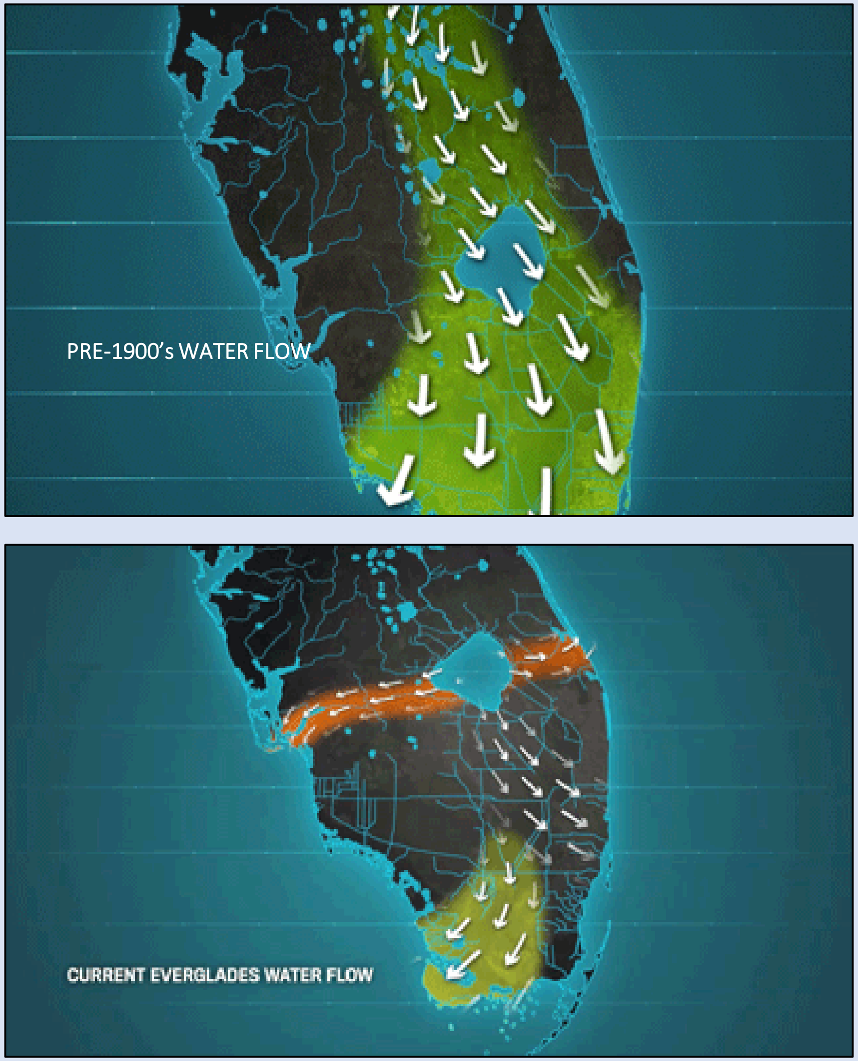
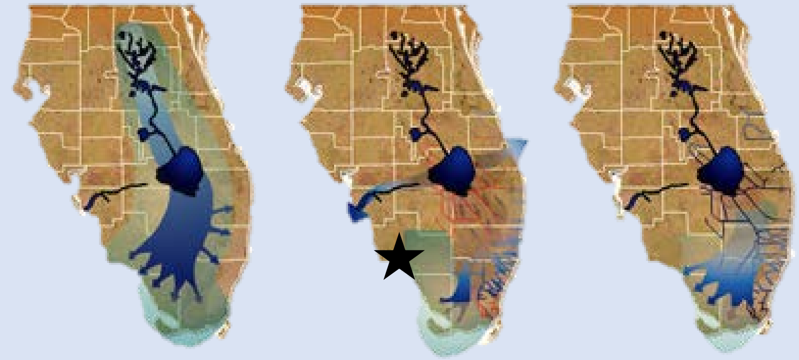
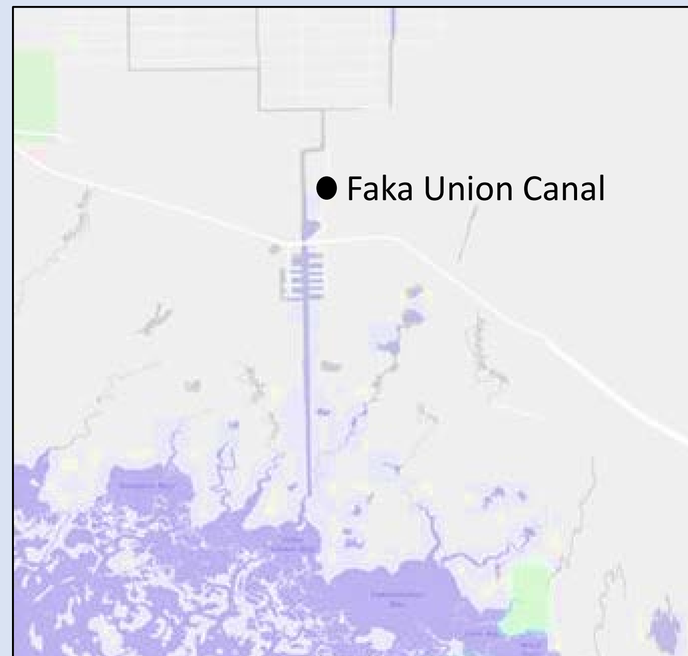


(Everglades Foundation)



Historic Flow *Current Flow* *CERP Flow*

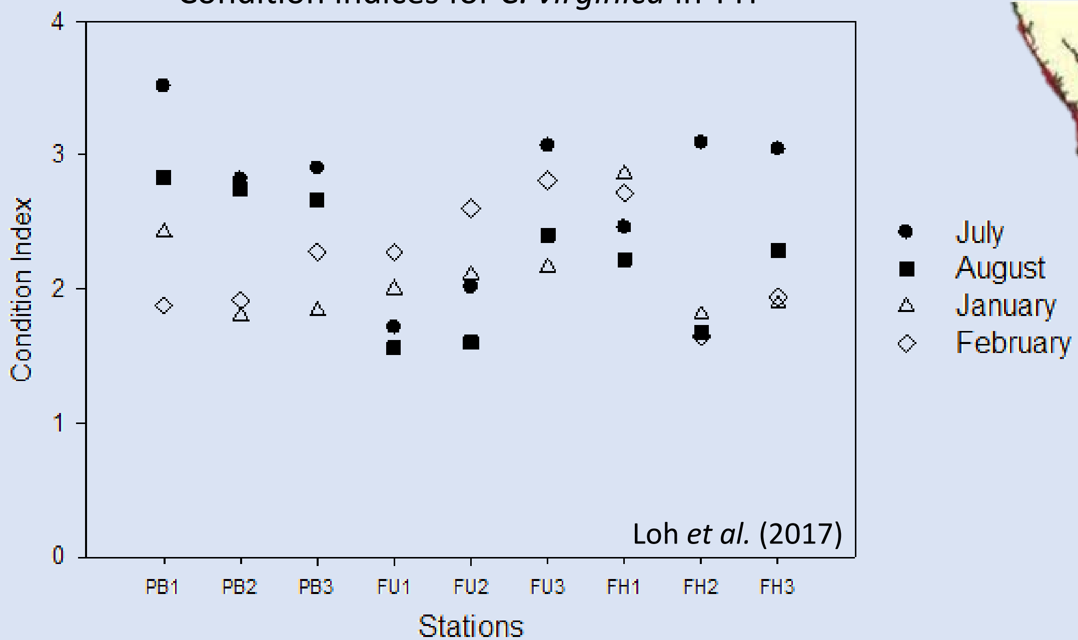
(U.S. Dept. Interior)

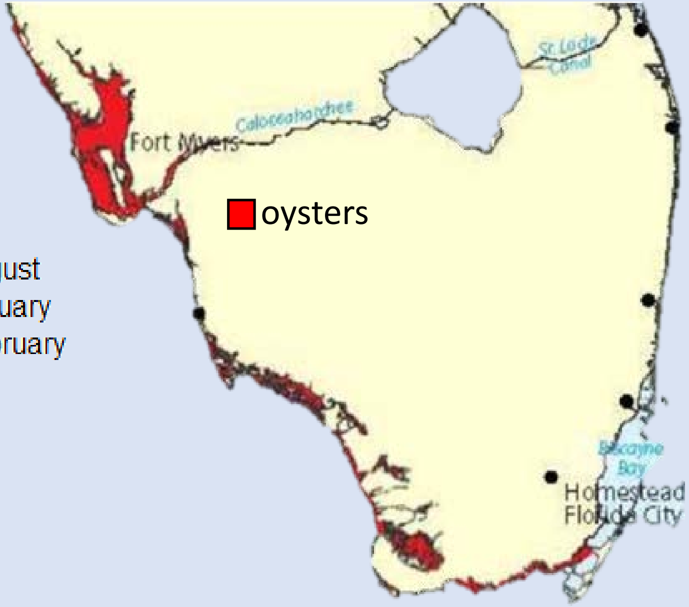


CERP = Comprehensive Everglades Restoration Plan

- **The eastern oyster (*Crassostrea virginica*)**
  - Distributed along Atlantic and Gulf coasts
  - Ecosystem service providers
    - Shoreline protection and stabilization
    - Water quality improvement
    - Substrate for mangrove propagation
  - “Canary in the coal mine”

Condition indices for *C. virginica* in TTI





Volety *et al.* (2014)

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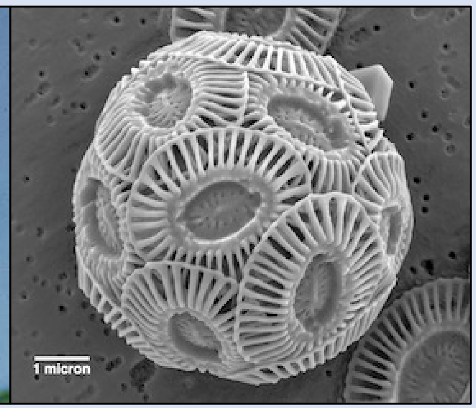
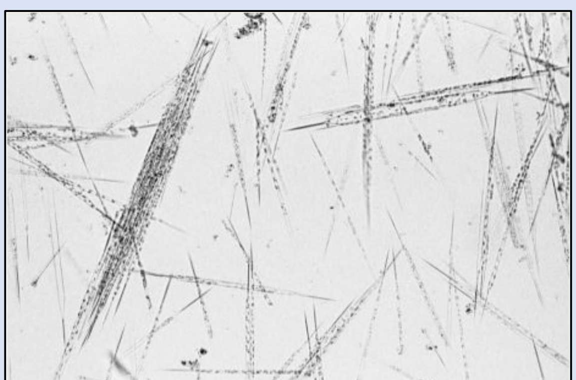
- **Assessing oyster diet using phytosterols**
  - Biochemicals in primary producers originating from C_{30} precursor
  - Well-preserved in estuarine environments
  - Determine organic matter sources – source specificity
  - Sterol proportions and configuration provides insight to community assemblages

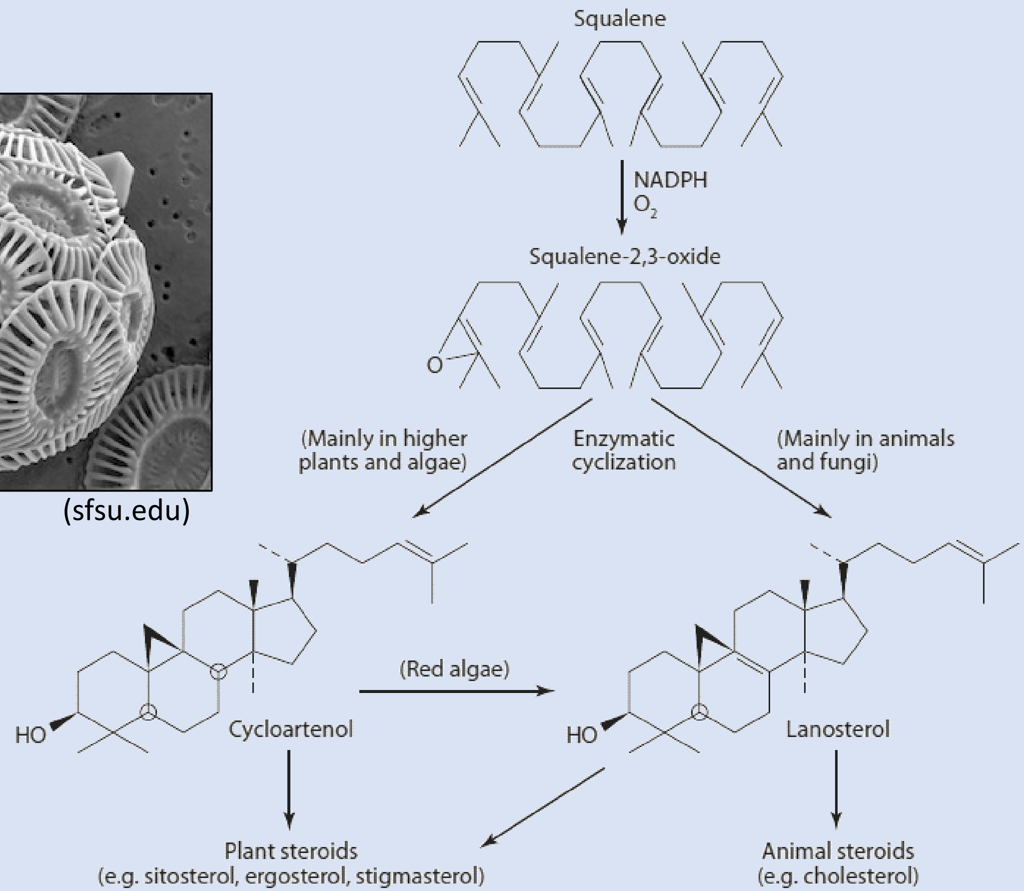
(uconn.edu)



(sfsu.edu)



(sms.si.edu)



Bianchi and Canuel (2011)

What is the pre-restoration impact on source contributions of terrestrial and aquatic organic matter as well as its quality and use as trophic resource?

1. Identify types and sources of sterols from POM, BMA, and oyster tissue.
2. Determine possible food source contributors to TTI.
3. Correlate biomarkers with food source quality.
4. Assess impact of altered freshwater discharge on *C. virginica* diet.

H_1: POM will comprise a mixture of phytoplankton and mangrove-derived organic matter.

H_2: There will be a greater abundance of phytoplankton-derived sterols in the wet season compared to the dry season

H_3: There will be a greater abundance of phytoplankton-derived sterols at sites with disproportionately high freshwater discharges (Faka Union Bay).

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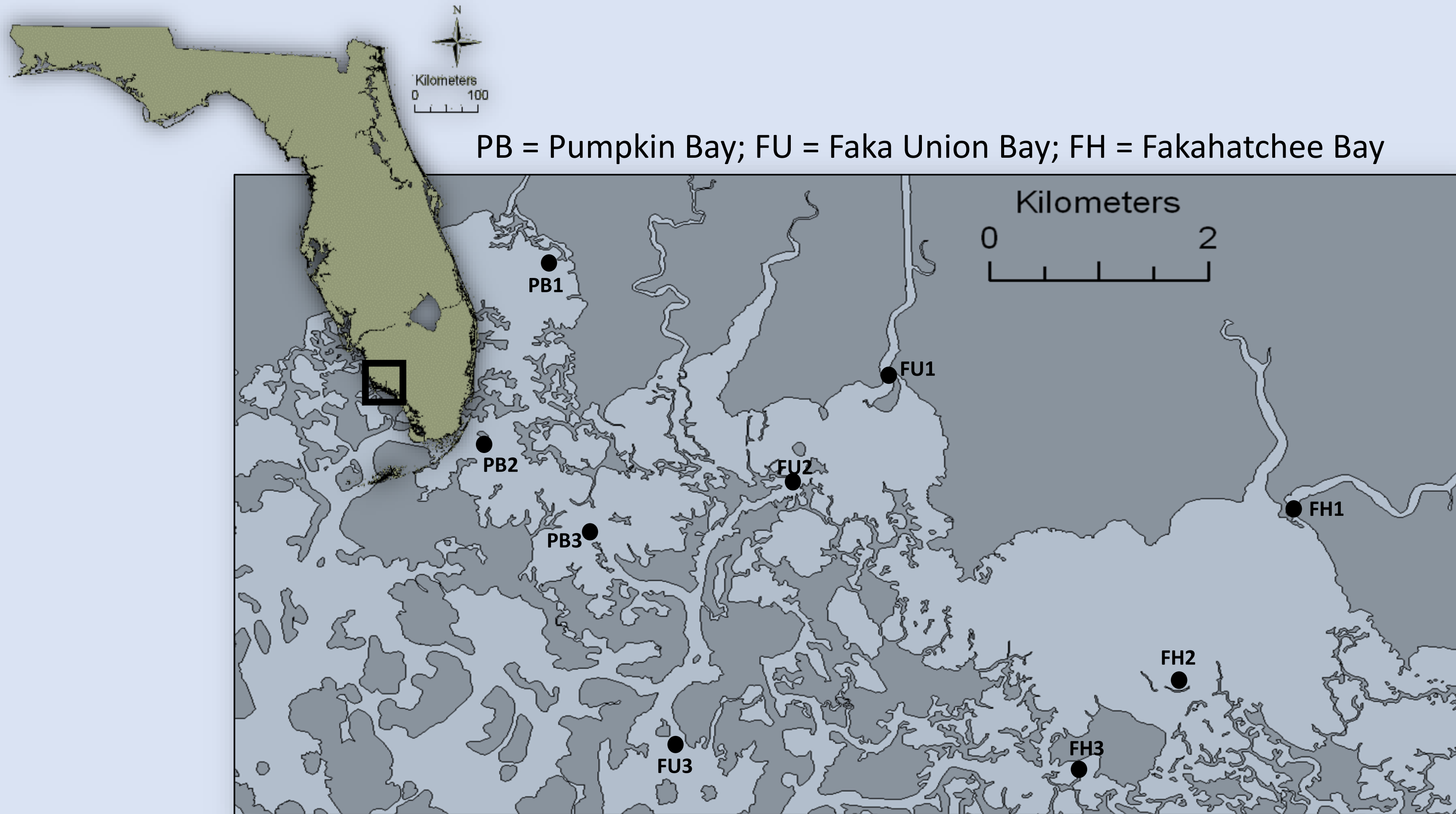
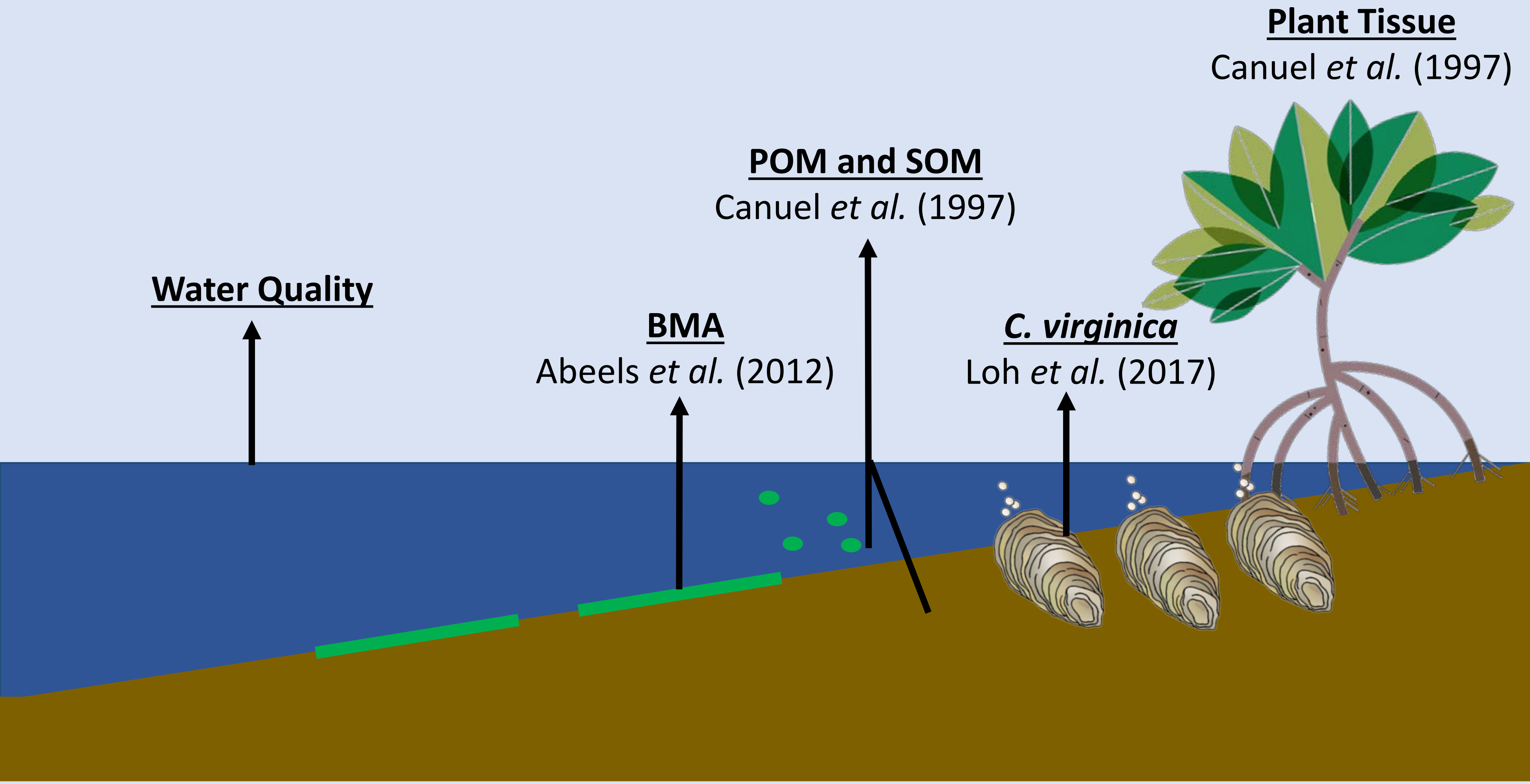

N
Kilometers
0 100
PB = Pumpkin Bay; FU = Faka Union Bay; FH = Fakahatchee Bay
Kilometers
0 2
PB1
PB2
PB3
FU1
FU2
FU3
FH1
FH2
FH3

(Airphoto – Jim Wark)

- **Field Sampling**
  - Wet (August 2012) and dry (February 2013) season



Total Lipid Extracts
(CEM MARS 5 Digestion System)

Bligh and Dyer (1959)

↓

Base Saponification (KOH)

↓

Neutral Fraction | Acid Fraction (archived)

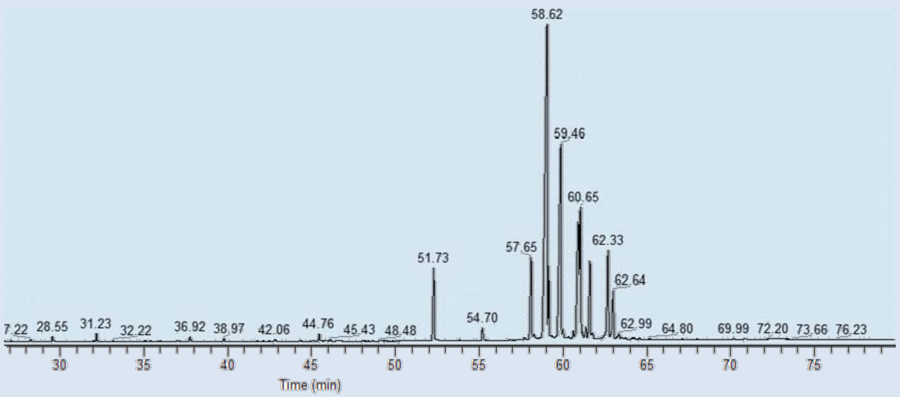
Neutral Fraction

↓

Column Separation
(Alcohols & Sterols)

↓

Derivatization to
Trimethylsilyl Ethers (TMS)



↓

Gas Chromatography w/ Flame Ionization Detection (GC-FID)
Gas Chromatography w/ Mass Spectrometry (GC-MS)

↓

Resulting GC peaks identified (MS) by retention times of known standards and quantified (FID) relative to a 5α(H)-cholestane internal standard

Canuel and Martens (1993); Loh *et al.* (2008); Waterson and Canuel (2008)

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- **Sterols of interest (in order of elution)**

Biomarker	Source
phytol	side-chain of chl. *a*
27-nor-24-methylcholesta-5,22-dien-3β-ol (occelasterol)	silicoflagellates and marine algae
cholesta-5,22-dien-3β-ol (22-dehydrocholesterol)	diatoms and rhodophytes
24-methylcholesta-5,22-dien-3β--ol (brassicasterol)	diatoms, haptophytes, prymnesiophytes
24-methylcholesta-5,24(28)-dien-3β-ol (Me cholesterol)	diatoms and chlorophytes
24α-methylcholest-5-en-3β-ol (campesterol)	chlorophytes and higher plants
24-ethylcholesta-5,22-dien-3β-ol (stigmasterol)	diatoms, haptophytes, chlorophytes
23,24-dimethyl-5α(H)-cholest-22-en-3β-ol	diatoms, haptophytes, chlorophytes
taraxer-14-en-3β-ol (taraxerol)	mangrove triterpenoid
24-ethylcholest-5-en-3β-ol (β sitosterol)	higher plants and cyanobacteria
24-ethyl-5α(H)-cholest-3β-ol (stigmastanol)	diatoms, haptophytes, chlorophytes
24-ethylcholesta-5,24(28)-dien-3β-ol (iso/fucosterol)	brown algae (mostly marine)
4α,23,24-trimethylcholest-22-en-3β-ol (dinosterol)	dinoflagellates
4α,23S,24R-trimethyl-5α (H)-cholestan-3b-ol (dinostanol)	dinoflagellates

terrestrial; freshwater; marine

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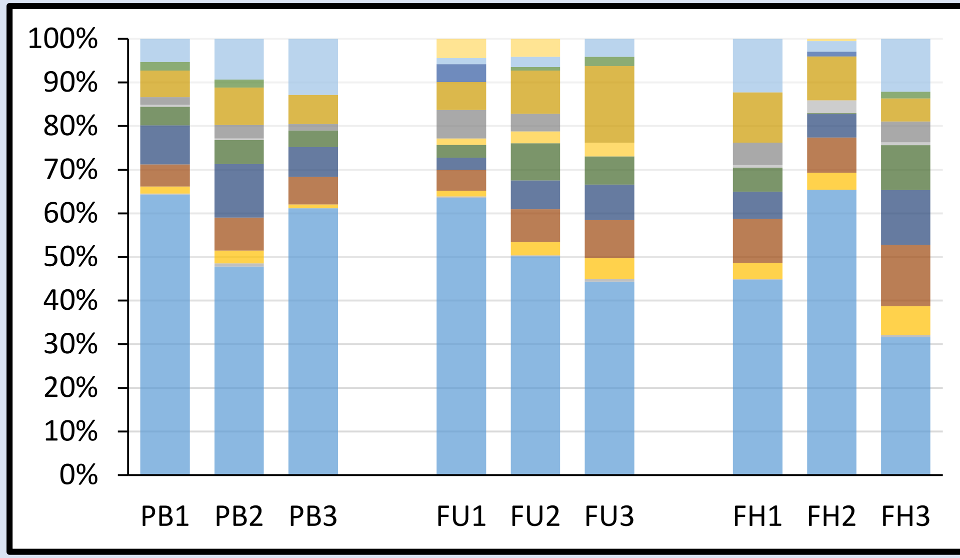
- **POM sterols**

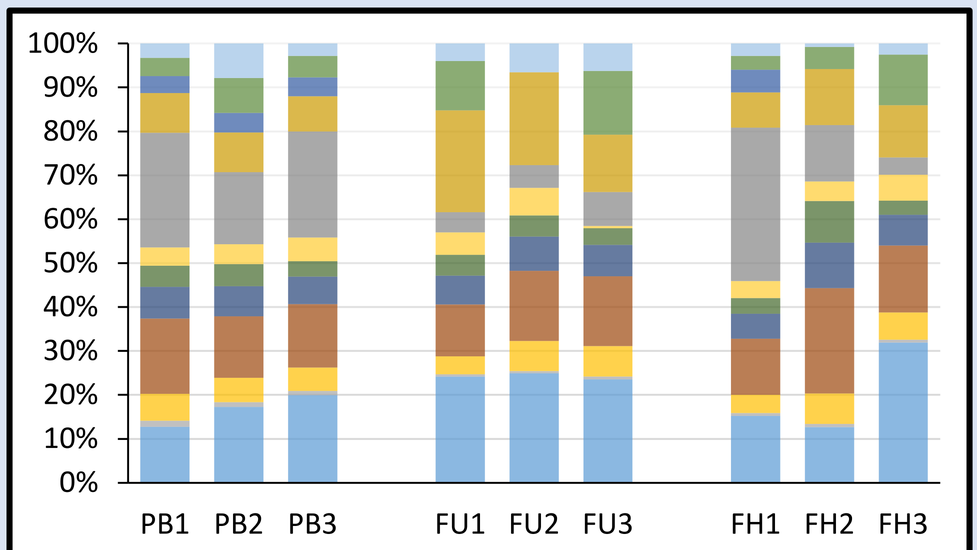
- 4a,23S,24R-trimethyl-5a(H)-cholestan-3b-ol
- 4α,23,24-trimethylcholest-22-en-3β-ol (dinosterol)
- 24-ethylcholesta-5,24(28)-dien-3β-ol
- 24-ethyl-5α(H)-cholest-3β-ol (stigmastanol)
- 24-ethylcholest-5-en-3β-ol (β sitosterol)
- taraxer-14-en-3β-ol (taraxerol)
- 23,24-dimethyl-5α(H)-cholest-22-en-3β-ol
- 24-ethylcholesta-5,22-dien-3β-ol (stigmasterol)
- 24-methylcholest-5-en-3β-ol (campesterol)
- 24-methylcholesta-5,24(28)-dien-3β-ol (Me cholesterol)
- 24-methylcholesta-5,22-dien-3β-ol (brassicasterol)
- cholesta-5,22-dien-3β-ol
- 27-nor-24-methylcholesta-5,22-dien-3β-ol (occelasterol)
- Phytol

terrestrial; **freshwater**; **marine**

Wet



Dry



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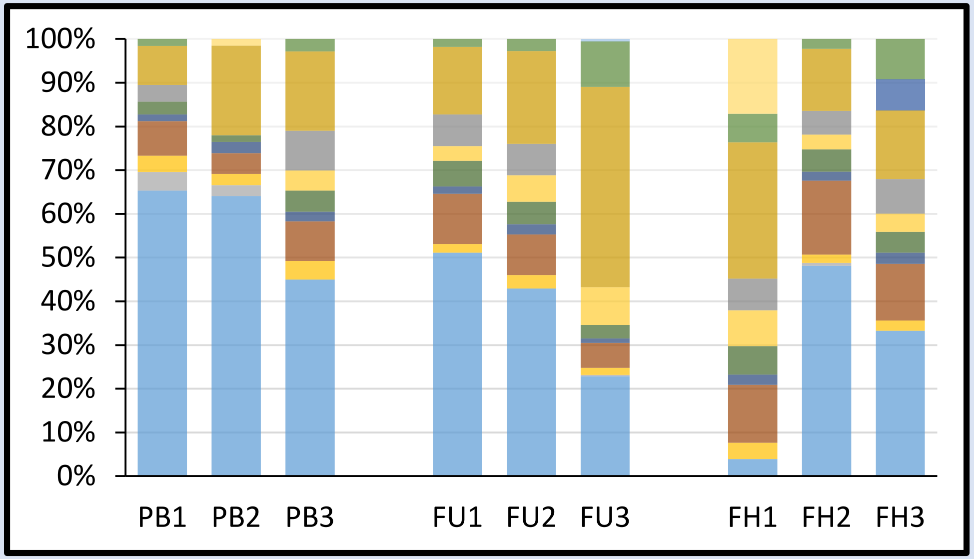
- **BMA sterols**

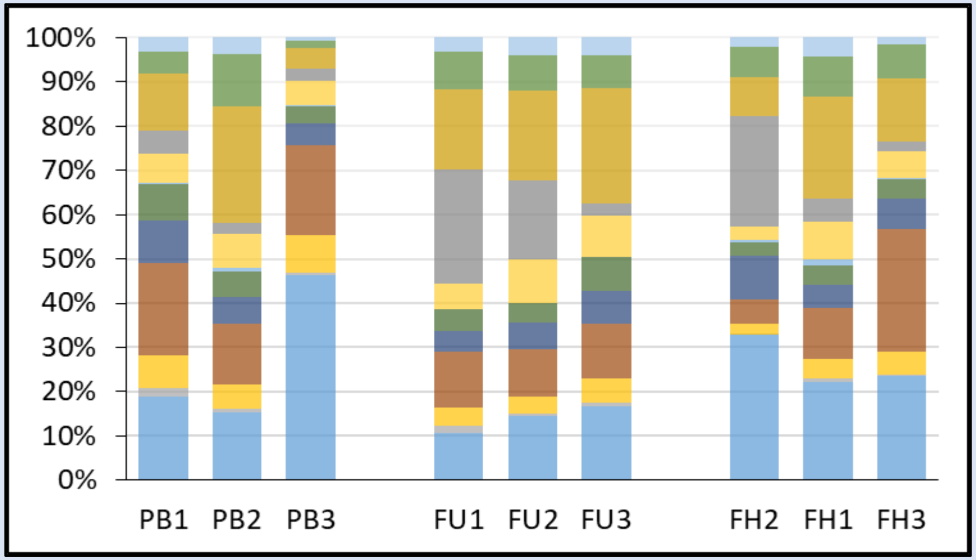
- 4a,23S,24R-trimethyl-5a(H)-cholestan-3b-ol
- 4α,23,24-trimethylcholest-22-en-3β-ol (dinosterol)
- 24-ethylcholesta-5,24(28)-dien-3β-ol
- 24-ethyl-5α(H)-cholest-3β-ol (stigmastanol)
- 24-ethylcholest-5-en-3β-ol (β sitosterol)
- taraxer-14-en-3β-ol (taraxerol)
- 23,24-dimethyl-5α(H)-cholest-22-en-3β-ol
- 24-ethylcholesta-5,22-dien-3β-ol (stigmasterol)
- 24-methylcholest-5-en-3β-ol (campesterol)
- 24-methylcholesta-5,24(28)-dien-3β-ol (Me cholesterol)
- 24-methylcholesta-5,22-dien-3β-ol (brassicasterol)
- cholesta-5,22-dien-3β-ol
- 27-nor-24-methylcholesta-5,22-dien-3β-ol (occelasterol)
- Phytol

terrestrial; freshwater; marine

Wet



Dry



- ***C. virginica* sterols**

- 4a,23S,24R-trimethyl-5a(H)-cholestan-3b-ol
- 4α,23,24-trimethylcholest-22-en-3β-ol (dinosterol)
- 24-ethylcholesta-5,24(28)-dien-3β-ol
- 24-ethyl-5α(H)-cholest-3β-ol (stigmastanol)
- 24-ethylcholest-5-en-3β-ol (β sitosterol)
- taraxer-14-en-3β-ol (taraxerol)
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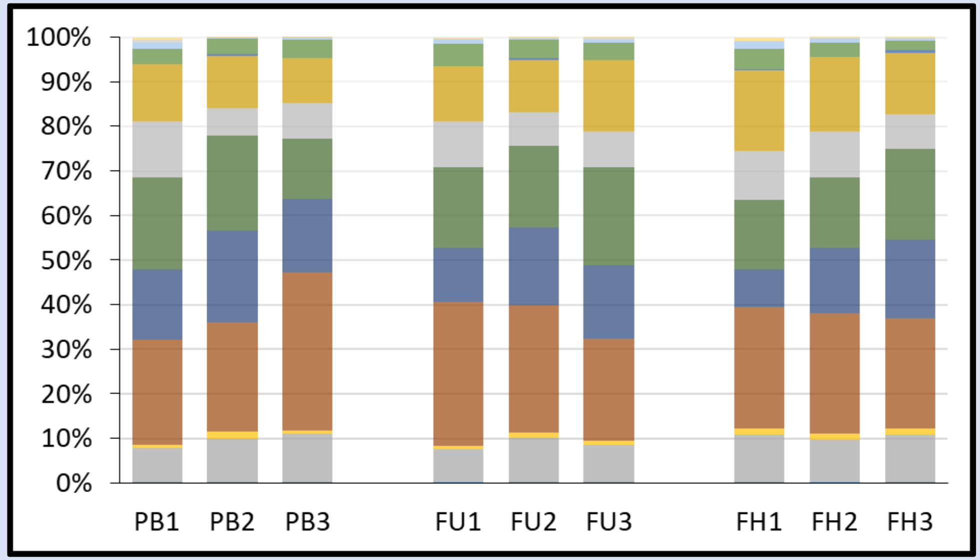
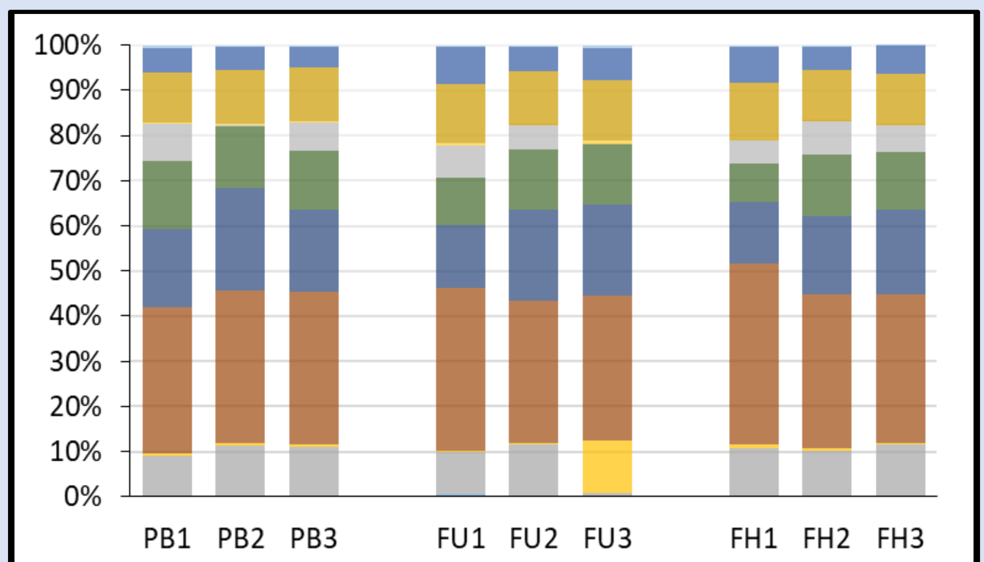
- 24-methylcholesta-5,22-dien-3β-ol (brassicasterol)
- cholesta-5,22-dien-3β-ol
- 27-nor-24-methylcholesta-5,22-dien-3β-ol (occelasterol)
- Phytol

terrestrial; freshwater; marine

Wet



Dry



- **Preliminary Conclusions**
  - TTI POM contains both autochthonous and allochthonous sources of OM.
  - *C. virginica* preferentially ingests diatoms, haptophytes, and chlorophytes rather than mangrove-derived organic matter and dinoflagellates.
  - High rates of freshwater discharge may increase POM availability, but not necessarily utilization by *C. virginica.*
  - In summary, *C. virginica* diet is sustained mostly by aquatic phytoplankton-derived POM underscoring the importance of high quality, readily available trophic resources.
- **Continuing Work**
  - Apply statistical tests (PCA, MANOVA) to determine significant differences in sterol composition across sample types and field sites.

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Thank you for your attention
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