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Eastern Oysters as Biomonitors of Emerging Contaminants in the Georgia Estuarine Environment

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Introduction

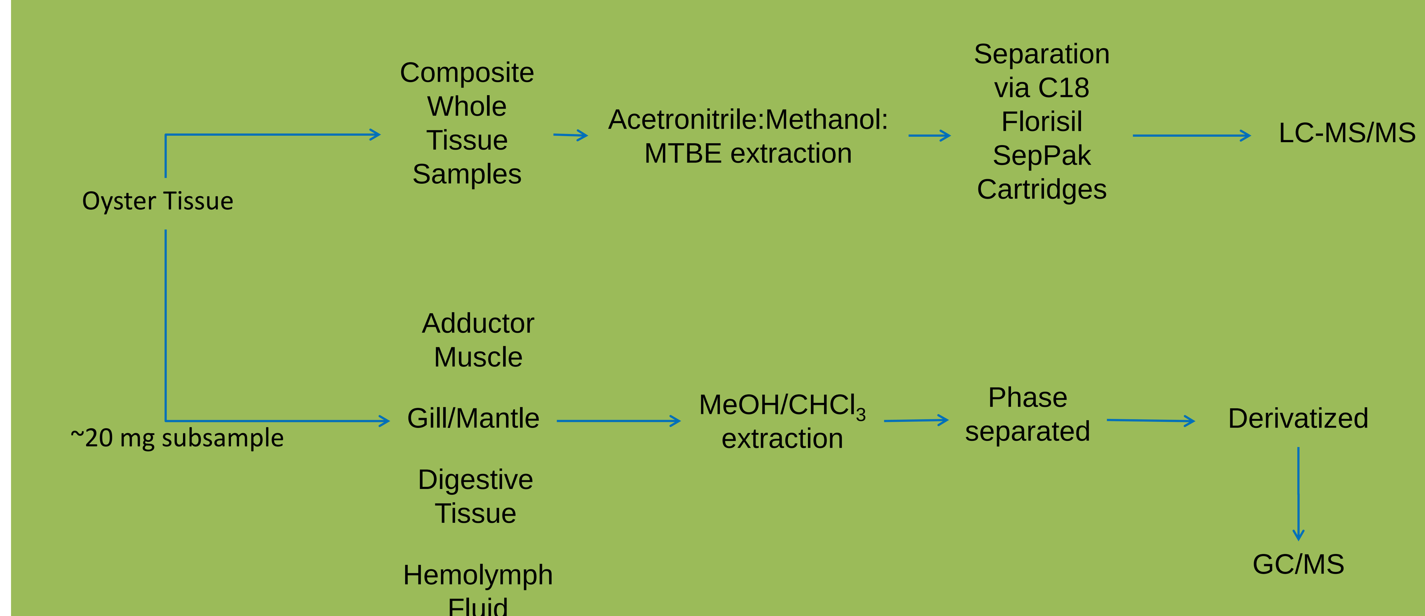
As populations in coastal zones continue to increase, anthropogenic sources of contaminants in estuarine and marine environments will be an increasingly complex problem. Increasing urbanization will place additional stresses not only on infrastructure systems, but also on the receiving environments around these highly populated areas. Alongside rising coastal populations, the quantity and variety of contaminants present will also increase, including emerging contaminants. Emerging contaminants are the thousands of chemicals without regulatory status whose health impacts are poorly understood. They are broadly grouped by chemical class as pharmaceuticals, personal care products, illicit drugs, pesticides and industrial chemicals. Because of their incomplete degradation in septic and wastewater treatment plants, emerging contaminants and their metabolites will enter coastal environments through sewage effluent releases and leaking septic tanks. These chemicals are continually released into estuaries and occur as complex mixtures at very low concentrations. Many emerging contaminants can exert significant effects on physiological systems at very low (ng/L to µg/L) concentrations, causing aquatic organisms to be at risk because of prolonged exposures. Sensitive segments of a population are at most risk to these exposures and could potentially suffer lifelong effects (Rosi-Marshall and Royer, 2012.

Toxic effects of these chemical mixtures are difficult to determine, especially as the toxicities of chronic, low-level exposures are still not well understood. It is often impractical to detect all of the chemicals to which an organism is exposed at sub-lethal concentrations over its lifespan and the effects of these chemicals using traditional biomonitoring methods. These methods can only detect an organism's exposure at a point in time and without further studies, are unable to implicitly quantify possible toxic effects and potential population level impacts. Metabolomics is an emerging field that utilizes highly sensitive analytical techniques to measure changes in cellular or tissue level metabolites following exposure to contaminants, providing a snapshot of an organism's physiology at a specific time. Research has demonstrated that metabolomic data supports an Adverse Outcome Pathway (AOP) framework, which makes it possible to extrapolate from the molecular level effects of chemical exposure upwards to population level impacts (Ankley et al, 2010). Metabolomic analysis integrated with biomonitoring within an AOP approach would provide an innovative method to quantify the impacts of emerging contaminants on oyster populations that would not be possible by either method alone. Combining these techniques will provide a more holistic understanding of how emerging contaminants are potentially impacting the health of eastern oyster populations at Brunswick and Sapelo Island, GA.

Objectives

(1) Evaluate the eastern oyster as a sentinel species for emerging contaminants in tidal creeks
(2) Quantify the bioaccumulation of twenty-two pharmaceuticals, personal care products, pesticides and industrial contaminants in eastern oysters from tidal creeks at Brunswick and Sapelo Island, GA
(3) Determine relationships between oyster size and age for contaminant deposition in oyster tissues
(4) Develop indexes of oyster and environmental health for each sampling area
(5) Integrate metabolomic profiling of adductor muscle tissue with bioaccumulation data to infer potential population level impacts

Methods



Site Selection

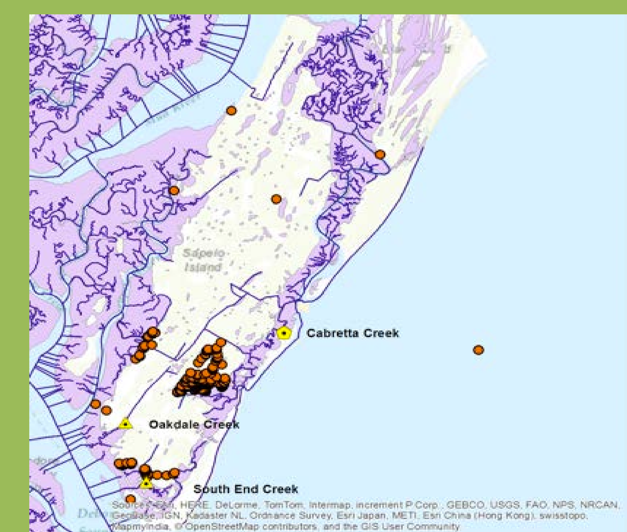
• Four study sites were chosen along the Brunswick River to coordinate with sites being sampled in an ongoing UGA MAREX study examining the effects of land use and septic tank densities on water quality. The sites on Sapelo Island were chosen to overlap with one site from the Fuller (2012) study, with two additional sites added for comparison.

Condition Index

• The oyster condition index (CI) is a non-specific measure of oyster health and was calculated for individual oysters (n = 5) per each sample trip and is based on the ratio of dry tissue mass to empty shell cavity space.

Data Analysis

• The statistical software RStudio (Version 0.98.501) and Excel 2013 were used to calculate the condition index and the analyte concentration data and create the graphs, respectively. Metabolomic data had residual solvent regions removed, the spectra were aligned and normalized to unit total intensity using Excel 2013. The maps of the Brunswick and Sapelo Island, GA sample sites were generated with ArcGIS 10.2.1 for Desktop. Effect size measures (mean +/- 95% CI) were utilized to quantify the differences in contaminant concentration within and between Brunswick and Sapelo sample sites, replacing null hypothesis significance testing.



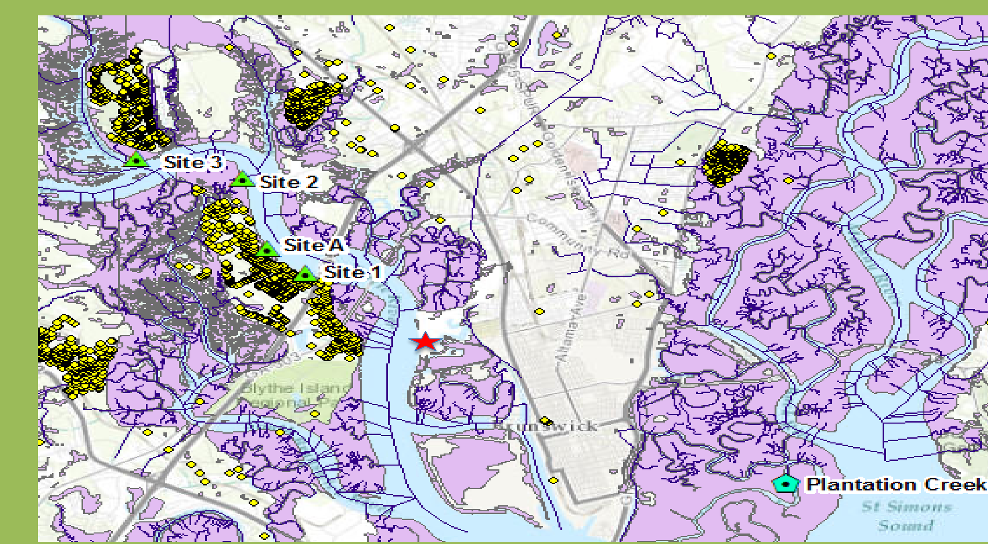


Figure 1: Maps of Sapelo Island (left) and Brunswick (right). Triangles are sample sites, pentagrams are presumed reference sites (Sapelo Island - Cabretta Creek; Brunswick - Plantation Creek). Colored circles are individual septic fields, the purple background identifies tidal marshes and the red star is a wastewater treatment plant.

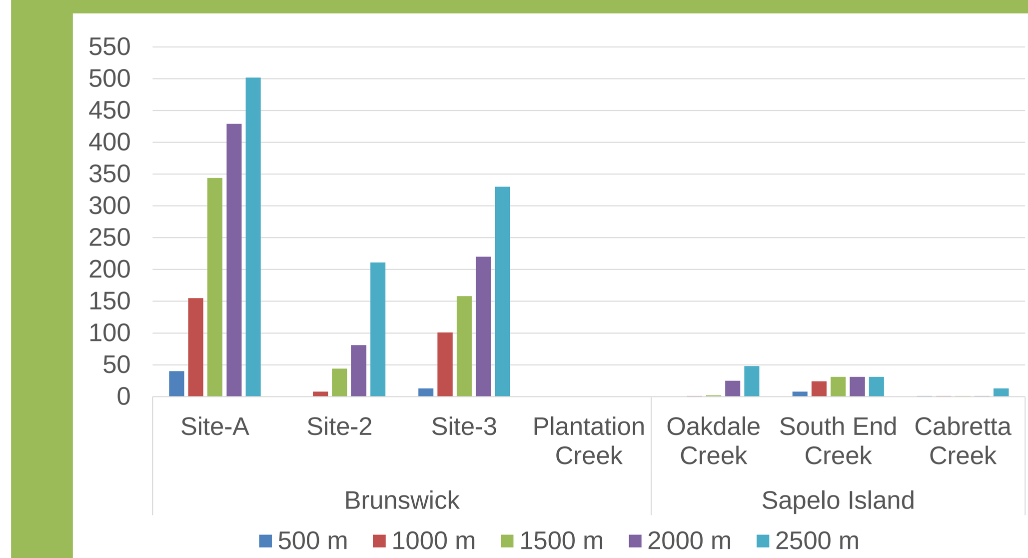


Figure 2: Quantity of septic sites within specific radii of each sample site.

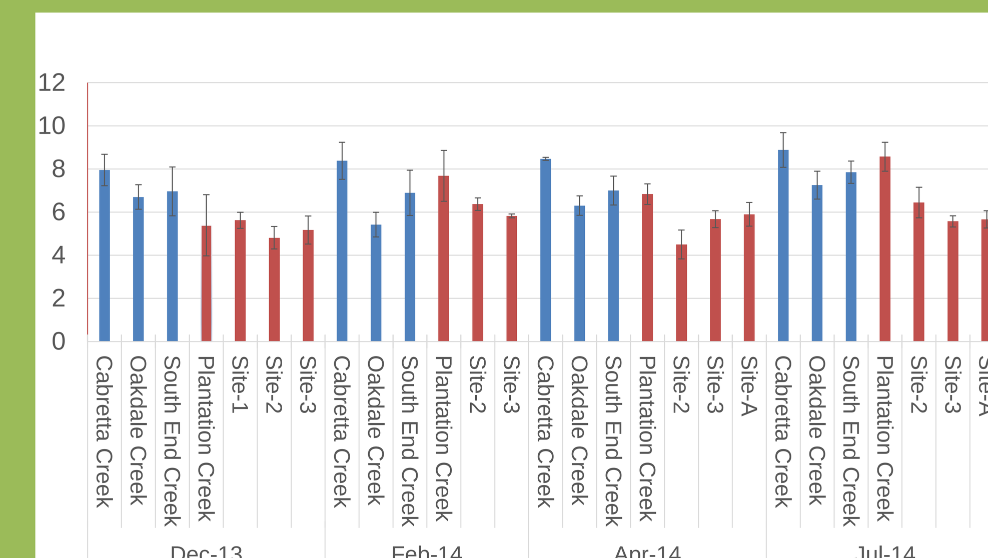
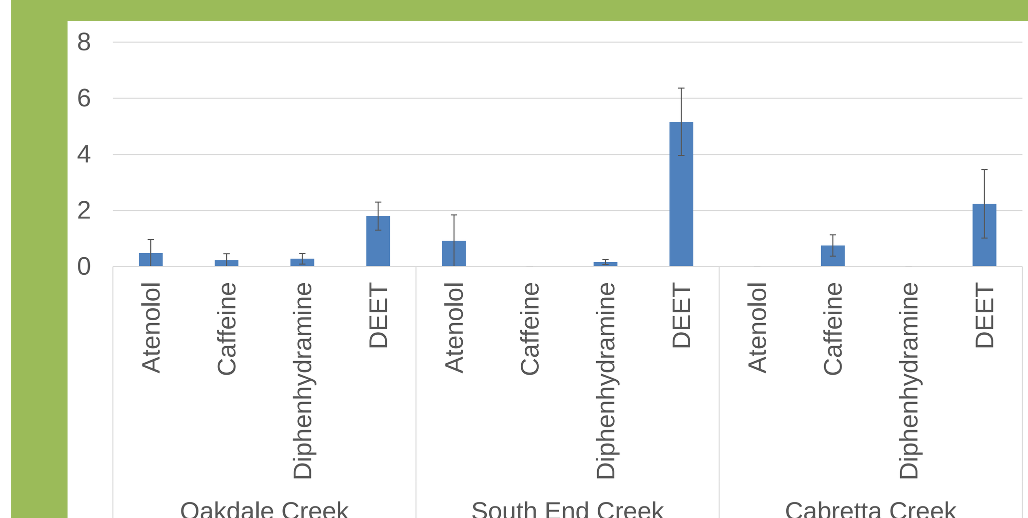


Figure 3: Condition Index (CI) values for oysters collected from Sapelo Island (blue) and Brunswick (orange), GA.



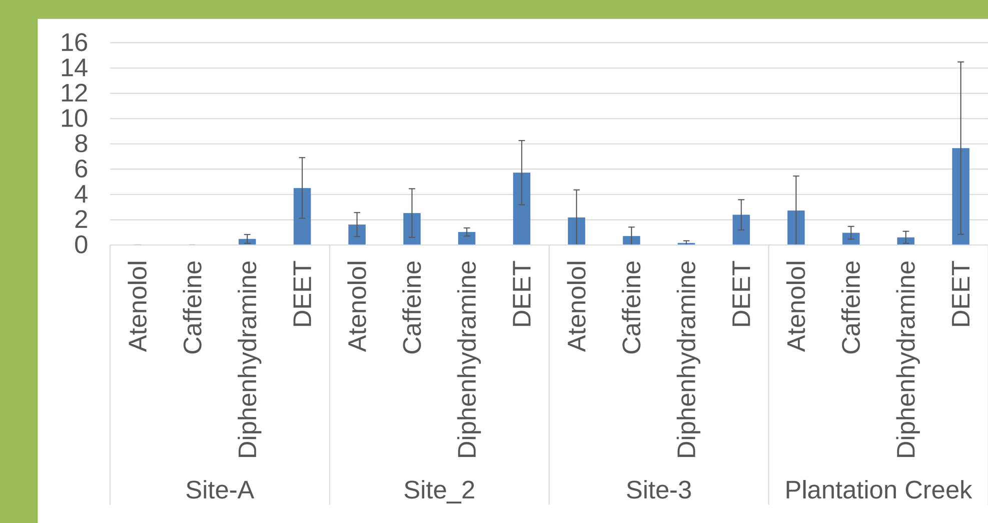


Figure 4: Target analyte concentrations for Sapelo Island (left) and Brunswick (right) grouped by sample site. All analytes were detected in the ng/g range.

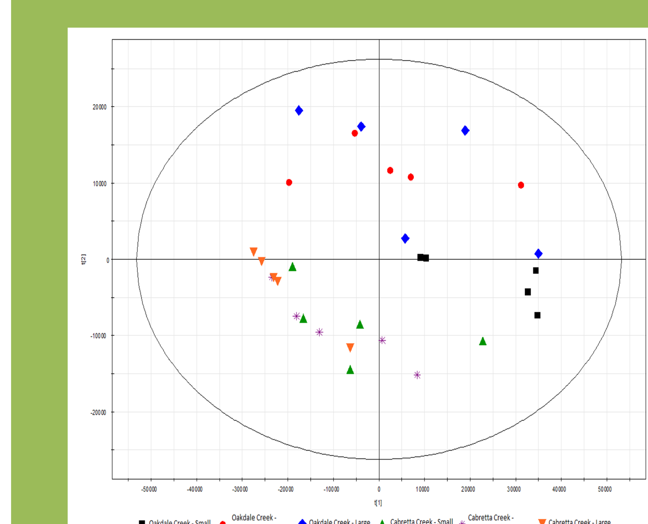


Figure 5: PLS-DA scores plots from the analysis of adductor muscle tissue polar extracts of Oakdale Creek (top) and Cabretta Creek (bottom) from Sapelo Island.

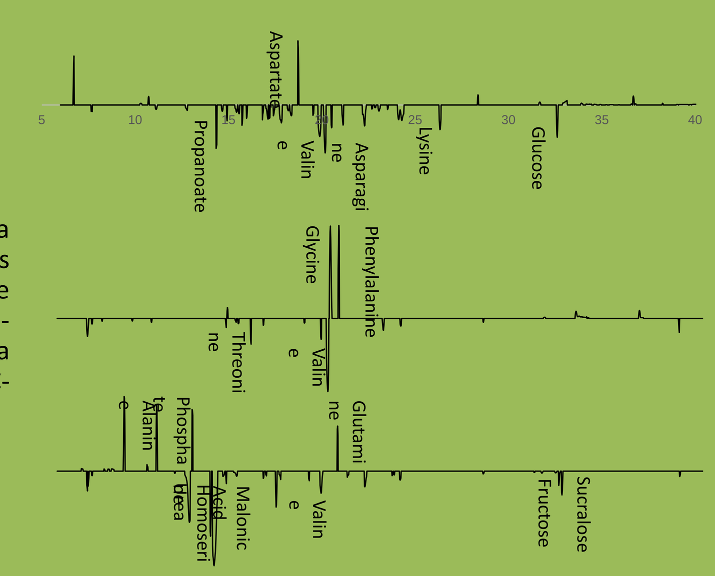


Figure 6: T-test filtered difference spectra (p ≤ 0.05) comparing polar metabolites responsible for class separation by size (large – top, medium – middle, small - bottom) for Oakdale Creek and Cabretta Creek from Sapelo Island identified by GC-MS/MS.

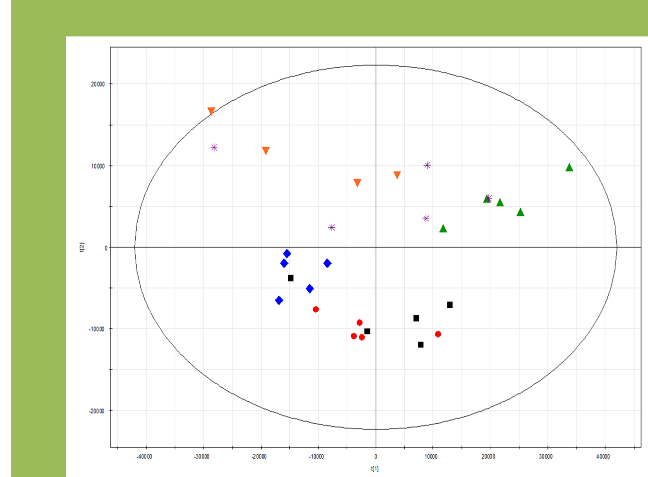


Figure 7: PLS-DA scores plots from the analysis of adductor muscle tissue polar extracts of Site-3 (bottom) and Plantation Creek (top) from Brunswick.

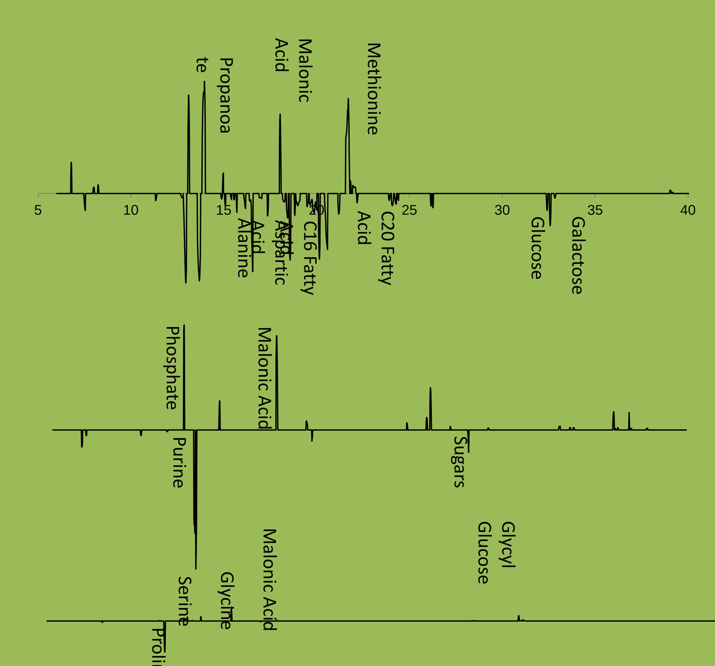


Figure 8: T-test filtered difference spectra (p ≤ 0.05) comparing polar metabolites responsible for class separation by size (large – top, medium – middle, small - bottom) for Site-3 and Plantation Creek from Brunswick identified by GC-MS/MS.

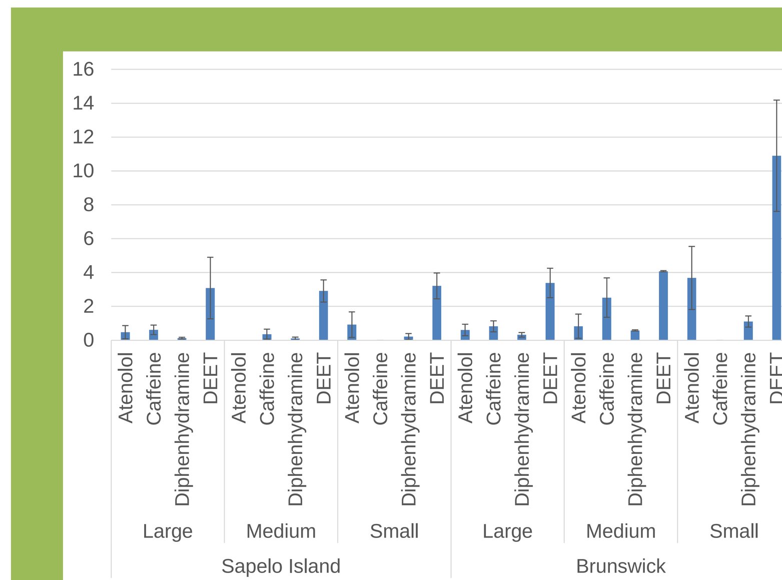


Figure 9: Analyte concentrations (ng/g dry tissue) in oysters collected from Sapelo Island (left) and Brunswick (right) grouped by oyster size.

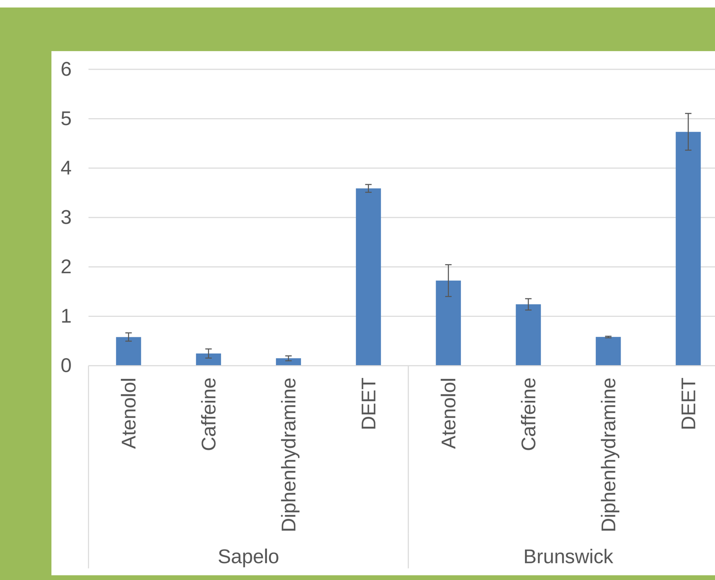


Figure 10: Analyte concentrations (ng/g dry tissue) in oysters collected from Sapelo Island (left) and Brunswick (right), combining all sites.

Summary & Conclusions

- Condition Index (CI) values for oysters collected Sapelo Island (SI) and Brunswick (BR) suggest that the oyster populations on Sapelo Island are more robust. (SI, mean = 7.33 CI [6.89, 7.77]; BR, mean = 5.98 CI [5.62, 6.34])
- Brunswick had higher levels of contamination than sites on Sapelo Island for all contaminants examined. Similar trends in the relative abundance of contaminants were observed for both locations (Figure 10).
- DEET had the highest average concentration at both locations (SI, mean = 3.59 ng/g CI [3.51, 3.67]; BR, mean = 4.74 ng/g CI [4.36, 5.11]), followed by atenolol (SI, mean = 0.58 ng/g CI [0.50, 0.66]; BR, mean = 1.72 ng/g CI [1.40, 2.04]), caffeine (SI, mean = 0.25 ng/g CI [0.15, 0.34]; BR, mean = 1.24 ng/g CI [1.13, 1.36]), diphenhydramine (SI, mean = 0.15 ng/g CI [0.10, 0.20]; BR, mean = 0.58 ng/g CI [0.57, 0.60]). Carbamazepine was not detected in any sample.
- In Brunswick, site A had the lowest levels of contamination, with the other sites having slightly higher, but relatively uniform levels of contaminants (Figure 4). Data suggest tidal influences along the Brunswick River transporting contaminants upstream.
- At Sapelo Island, Oakdale Creek had the highest level of contamination, followed by South End Creek and Cabretta Creek.
- Oysters at both locations (SI and BR) displayed similar trends in contaminant body burdens according to their size (Figure 9). Data may imply a threshold where caffeine begins to be accumulated with increasing size.
- PLS-DA analysis displays class clustering for both reference site to sample site comparisons and between oyster sizes within each sample site (Figures 5 and 7).
- Metabolomic analysis indicates that unique physiological processes are occurring in oysters collected from the reference sites compared to sample sites for both Sapelo Island and Brunswick. Mostly different metabolites were identified between locations, possibly correlating with varying contaminant exposures (Figures 6 and 8).

Upcoming Research

- Complete analyses of oyster samples collected bimonthly since October 2013, continuing though March 2015.
- Complete metabolomic analysis of tissues collected from all sites in June 2014 (adductor muscle, gill/mantle, digestive gland and hemolymph).
- Elucidate metabolic pathways from the identified metabolites to infer population level effects
- Determine age-related deposition of emerging contaminants in oyster tissues to evaluate the use of native oysters as contaminant biomonitors.
- Expand target analytes to include more pharmaceuticals, personal care products, pesticides and industrial contaminants.
- Collect sediment, suspended solids and composite water samples from each site and analyze for target analytes (June 2014-March 2015).

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