Application of an antibody-based biosensor for rapid assessment of PAH fate and toxicity at contaminated sediment sites

SRP Progress in Research
Biogeochemical Interactions Affecting Bioavailability for in situ Remediation
May 13, 1-3 pm EDT

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Application of an antibody-based biosensor for rapid assessment of PAH fate and toxicity at contaminated sediment sites

- PAH and their importance as environmental contaminants
    - Sources & concerns

- PAH biosensor, what is it and how do we make it?

- Biosensor applications to PAH fate and transport
    - Elizabeth River, VA: Evaluating PAH transport
    - Oil spill detection: ExxonMobil and Ohmsett

- Biosensor applications to PAH bioavailability/toxicity
    - Factors affecting bioavailability in sediments
    - Baltimore Harbor, MD: Toxicity of contaminated sediments

- Current and future work
    - Kristen Prossner's SRP Research-Bioaccumulation in oysters
    - Krisa Camargo SRP TAMU Research- Soil screening
    - Continued Technology Development-Sapidyne and VIMS
    - Fate and Toxicity Assessment

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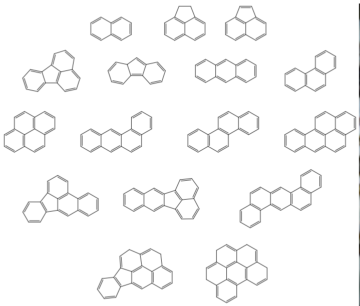
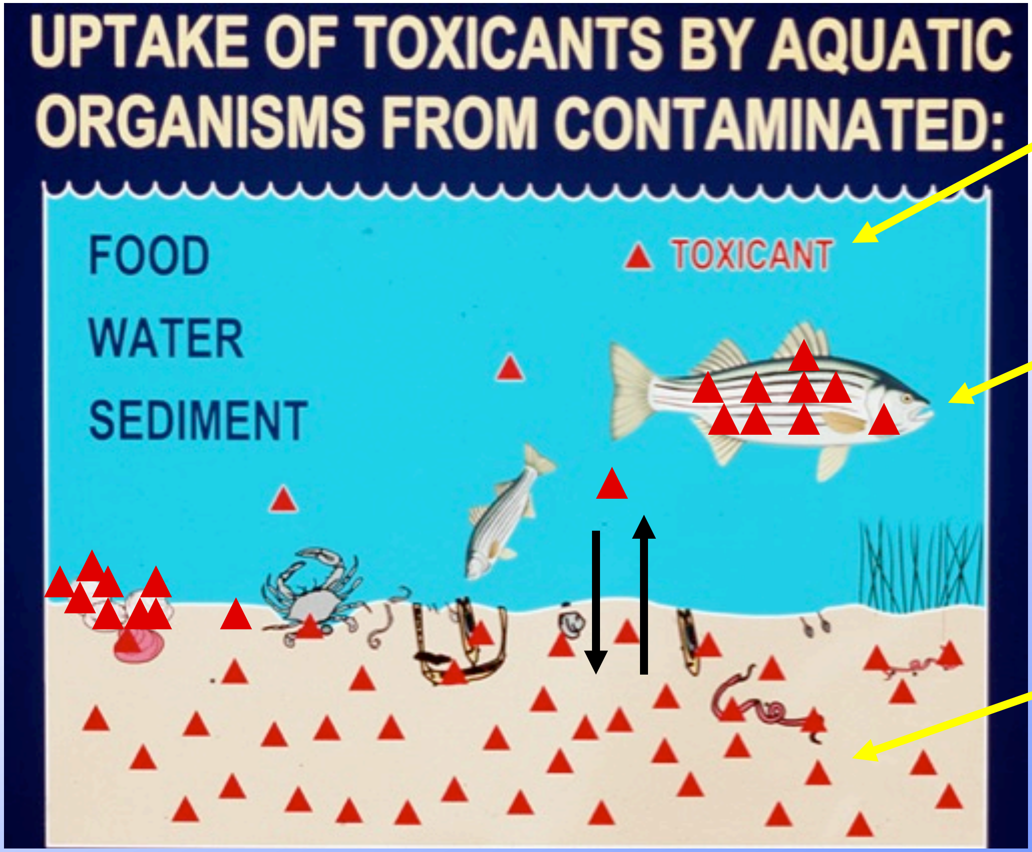
PAH: Bioavailability is governed by partitioning



Polycyclic Aromatic Hydrocarbons (PAH)
Potentially toxic and carcinogenic
Common target of Superfund cleanup (historical/legacy contaminants)
Oysters are potential vector for human exposure
Sources include: combustion products, creosote, oil

Superfund driven by reducing Human Risk



Limited water solubility
"hydrophobic" very low
concentrations in water

Under "equilibrium" conditions
High affinity for lipid
material "Lipophilic"
organic carbon rich
sediments and biota (bivalves)
are a "sink" or reservoir

NIEHS-SRP Research Focus

Can we predict how PAH fate will affect bioaccumulation from contaminated sediments?

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FTS Dura Dry Bulk Freeze Dryer 48 hours or until dry, aliquots removed for % solids, grain size, and organic carbon

2 days

Spike with surrogate standards

PCB 30, PCB 65, PCB 204, 1,1'binaphthyl, BDE-77, perinaphthenone, d-10 acenaphthene, d-12 chrysene, d-8 naphthalene, d-12 perylene, d-10 phenanthrene, and 1,4-dichlorobenzene

1-2 days

Dionex ASE 300 extracted

100% methylene chloride at 100°C and 1500psi

1 days

Copper Column to remove sulfur

1 day

HPLC-SEC

Waters HPLC with a Phenomenex Envirosep ABC GPC column in methylene chloride

1 day

Silica gel to remove polar compounds

1 day

Spike with Internal Standards

pentachlorobenzene, p-terphenyl, decachlorodiphenyl ether(DCDE), & BDE-166

1-2 days

Varian Saturn

GCMS-SIMS

Evaluate QA/QC
1-2 days

Available Analytical Methods for Organics can be **Slow and Expensive**

How slow?

Environmental samples are extremely complex: 100,000's of compounds

Multiple steps to separate, isolate and concentrate the target molecules-
Instrument and time intensive

Days- Weeks up to $1000/sample (data point)

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Near real-time PAH analysis: VIMS Biosensor

Our Approach

Bio

Monoclonal Antibodies against Contaminants

Sensor

Electronic detection of mAb Binding

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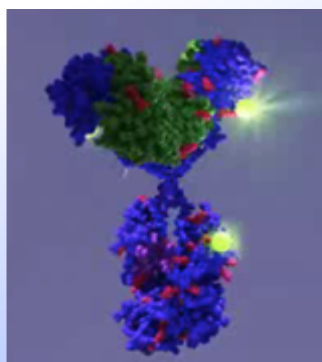

KinExA Technology

Sāpidyne Instruments
Boise, Idaho

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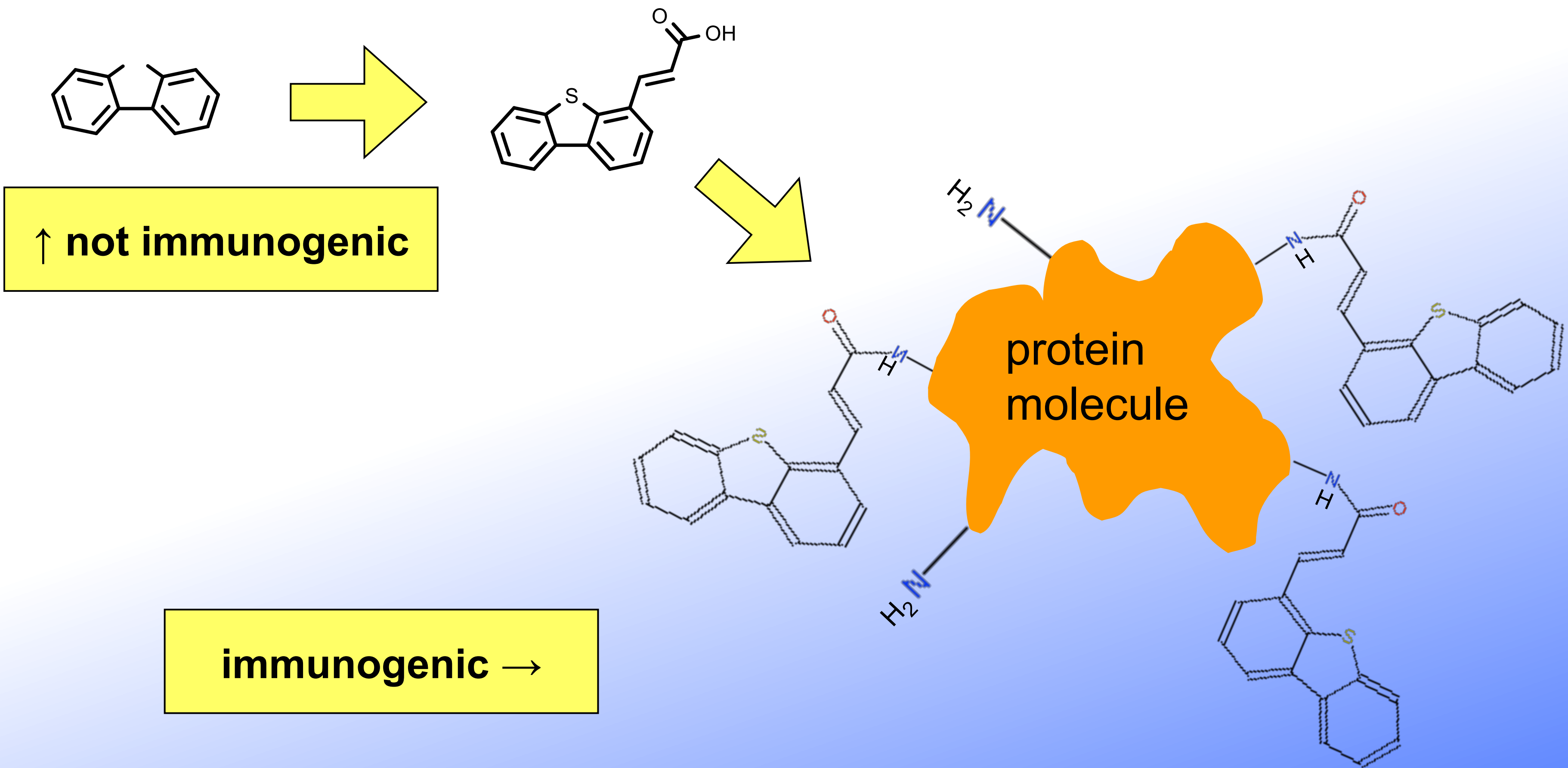
How to make new antibodies to PAH and other small targets?



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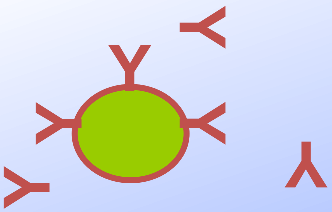
How to make antibodies to pollutants?

Pollutant

Pollutant

Immunize

Monitor sera for titer



Hybridoma-antibody producing cells

Provides an endless supply of antibodies in cell culture

Screening of Hybidomas an important step
Several month process from immunization to mAb
(Li et al 2016, Immunoassay and Immunochemistry)

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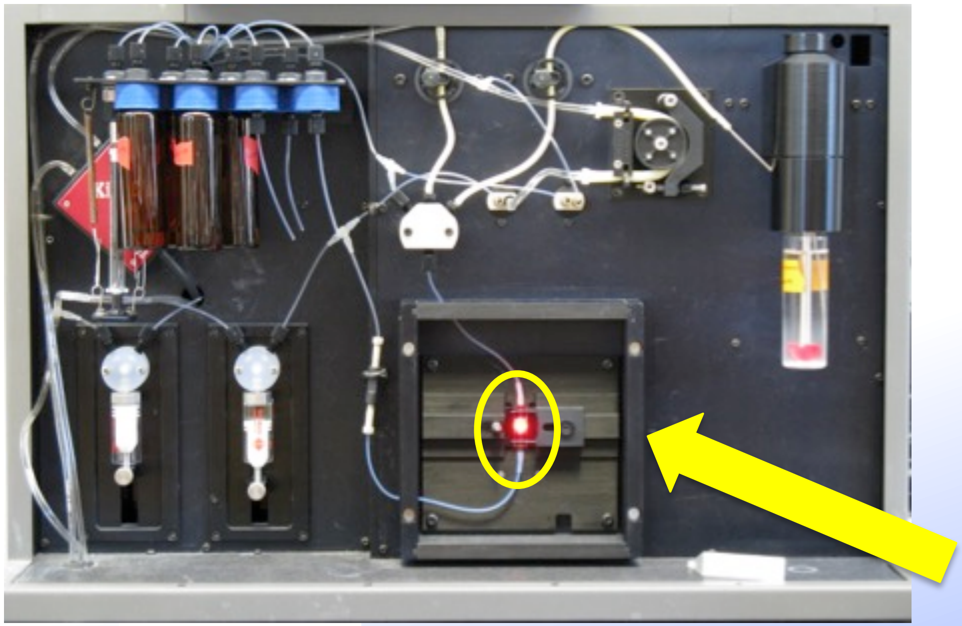
Goal: Quantification of mAb binding



Inline Sensor (Biosensor) features:

1. Automated sample handling
2. Precise fluidics for analyzing small quantities accurately
3. Fluorescence emission/detection for heightened sensitivity

KinExA TECHNOLOGY

Sāpidyne Instruments

Boise, Idaho

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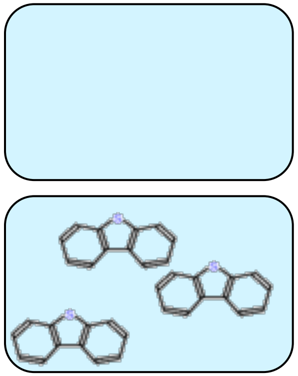
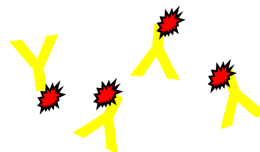
samples

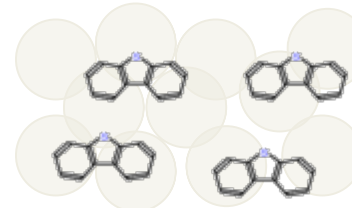
Sample with NO PAH

Sample with PAH



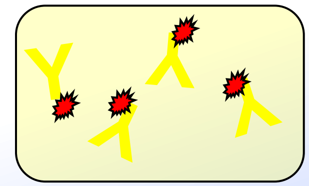


reagents



← Beads

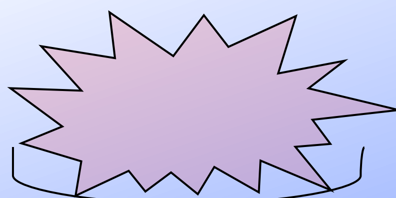
antigen



← AF647 labeled mAb

Fluorescent source →

high signal



Flow cell →

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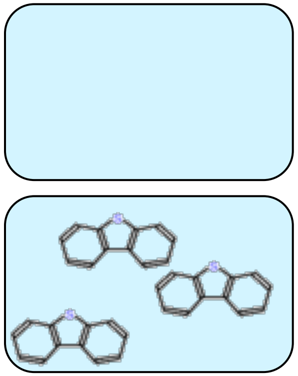
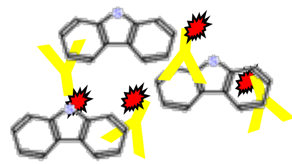
samples

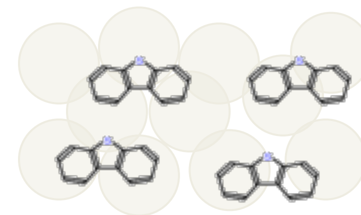
Sample with NO PAH

Sample with PAH



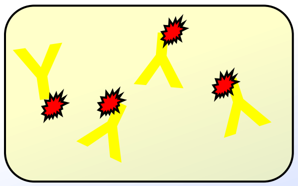


reagents



← Beads

antigen



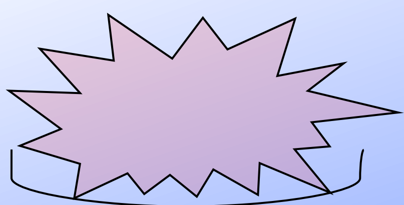
← AF647 labeled mAb

sample with NO PAH
= **high signal**

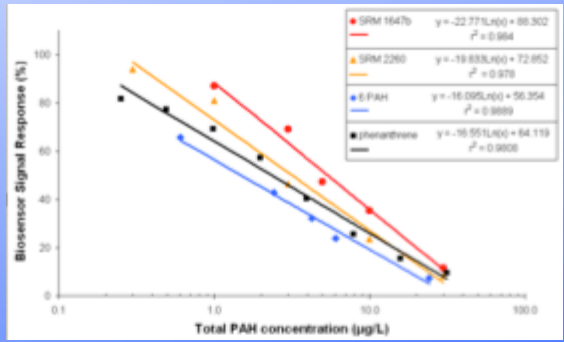
sample with high PAH
= **low signal**

Fluorescent source →

low signal



Flow cell →



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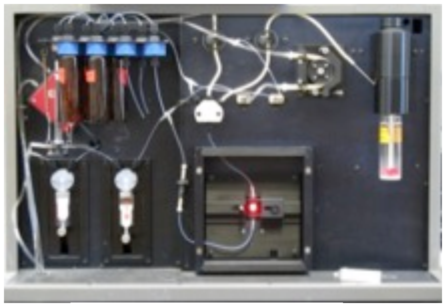
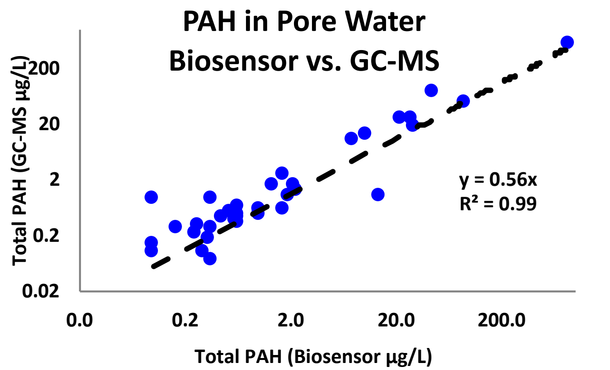
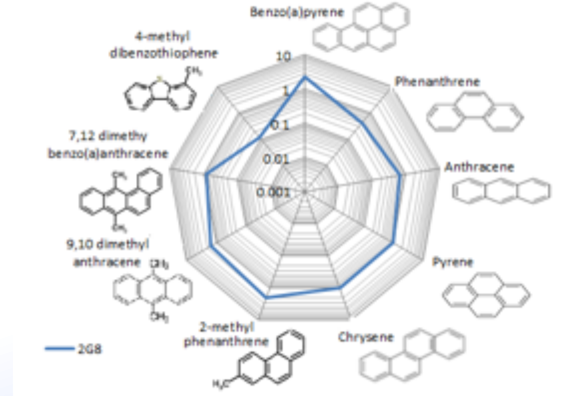
VIMS Antibody Biosensor: new technology for contaminant analysis allows quantification at low concentrations at new spatial and temporal scales



Sāpidyne Instruments Inc.



Good correlation to GC-MS



2G8 Affinity for a wide range of PAH (3-5 ring)

SMALL **volume samples (1-5 ml)**
FAST **analysis (8 m) near real-time**
LOW **concentrations (0.1 ppb total PAH)**

Environmental Fate Studies: spatial and temporal resolution to identify sources and transport mechanisms

Toxicity Evaluation: spatial and temporal resolution to understand what is driving bioavailability and toxicity

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PAH selective antibodies (Spier et al., 2009, Anal. Biochem., Spier et al., 2011, Environ. Chem. Tox.; Xin et al., 2016, J. Immunoassay and Immunochemistry, Xin et al. 2016, Sensing and Bio-sensing Research

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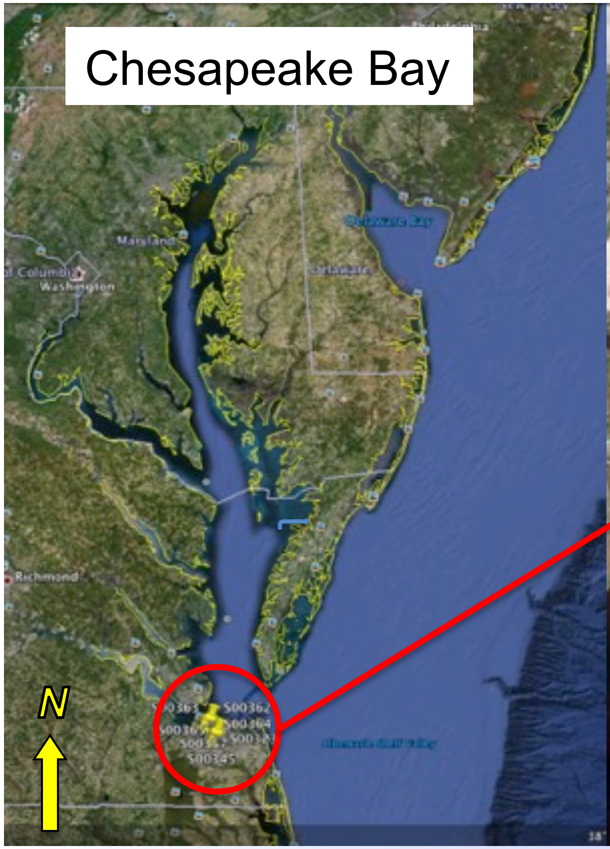
Study Site Money Point: Contaminated with PAH and DNAPL from Historical Creosote Facilities in the Southern Branch of the Elizabeth River, VA



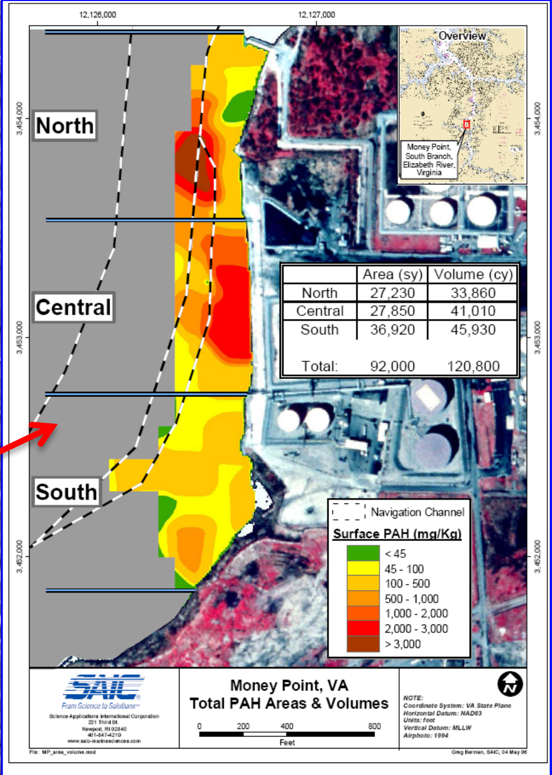


Figure 3.2-8. Money Point nearshore area partitioned into North, Central, and South sub-areas for area and volume estimates.

Methods are needed to better understand and predict PAH transport at sediment remediation sites to assure long-term success

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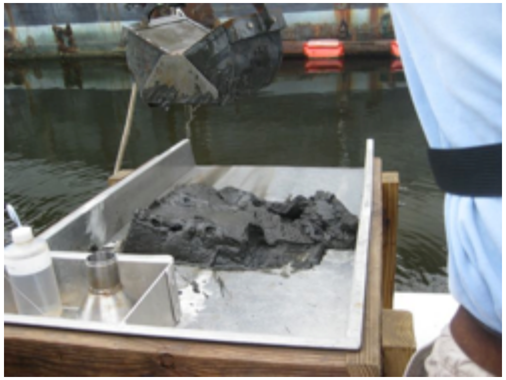
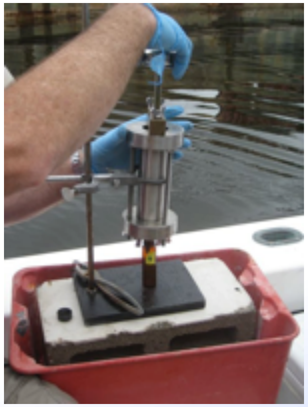
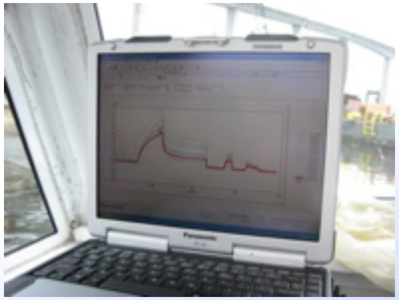
Methods: Porewater sampling surface sediments

- Real-time analysis can be used to map [PAH] in water/sediment porewater in the field

- Dissolved phase (0.47 µm) porewater samples are collected and analyzed on board and up to 30 stations can be surveyed in 1 day

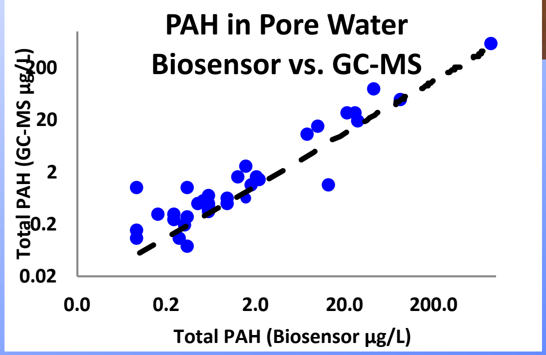






- Small volume samples analyzed on board by biosensor and larger volume samples can be brought back to the lab for GC-MS

- Good correlation between biosensor & GC-MS in complex environmental samples



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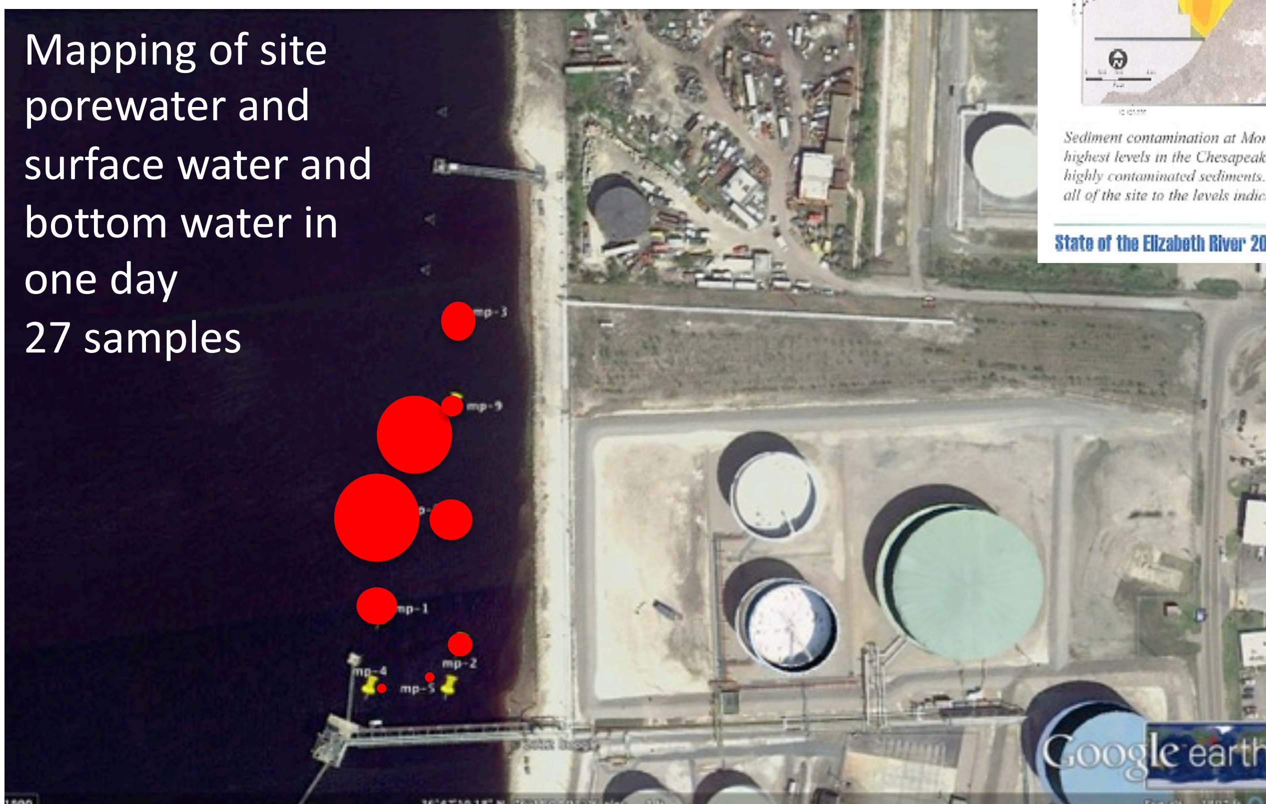
Results: Money Point, Phase 2
Mapping water/porewater in a day

Southern branch Money Point Phase 2 (MP) Site Survey 08-09-12

Id	Conc(ug/L)	Station
1	0.08	MP-5 Bot
2	0.12	MP-5 Surf
3	0.25	MP-4 Bot
4	0.2	MP-4 Surf
5	0.11	MP-1 Bot
6	0.19	MP-1 Surf
7	0.3	MP-7 Bot
8	0.13	MP-7 Surf
9	0.1	MP-2 Bot
10	0.15	MP-2 Surf
11	0.1	MP-8 Bot
12	0.07	MP-8 Surf
13	0.07	MP-6 Bot
14	0.09	MP-6 Surf
15	3	MP-9 Bot
16	0.1	MP-9 Surf
17	0.13	MP-3 Bot
18	0.08	MP-3 Surf
19	190	MP-3 PW
20	120	MP-9 PW
21	400	MP-6 PW
22	450	MP-7 PW
23	230	MP-8 PW
24	130	MP-2 PW
25	220	MP-1 PW
26	50	MP-5 PW
27	50	MP-4 PW

Surface water <1μg/L-3μg/L
Porewater 50μg/L – 450 μg/L
Phase 2 remediation area



Sediment contamination at Money Point includes some of the highest levels in the Chesapeake Bay. Red areas indicate the highly contaminated sediments. Funding is in hand to restore all of the site to the levels indicated in green. Source: SAIC

State of the Elizabeth River 2008

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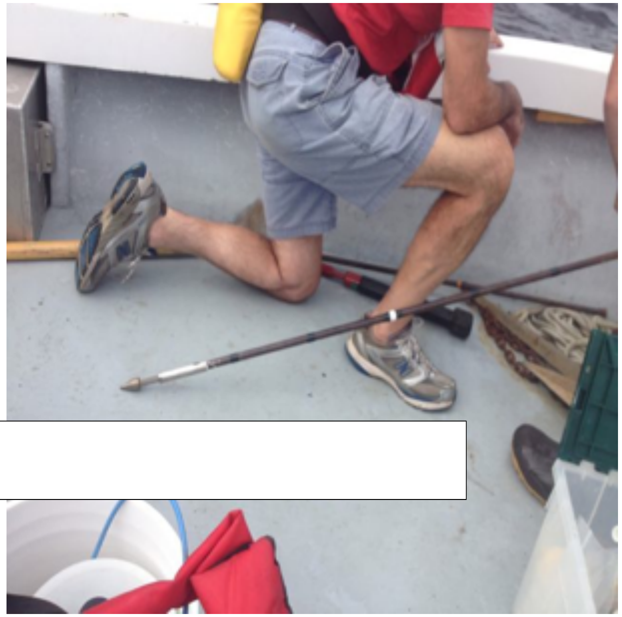
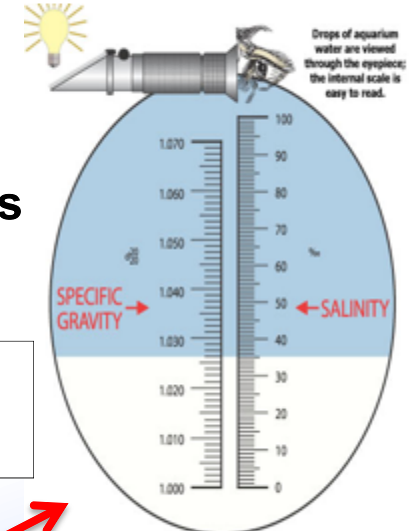
PAH Transport within sediment : Methods

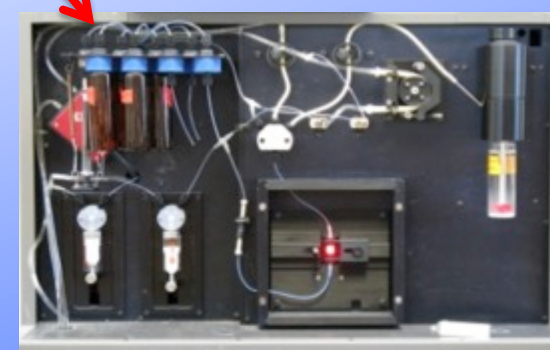


In-situ porewater measurements



Salinity by refractometer

Total PAH by biosensor



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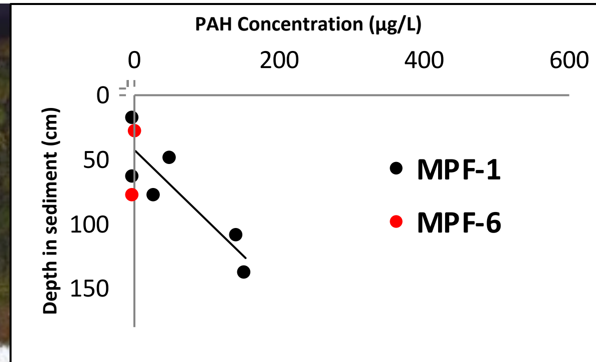
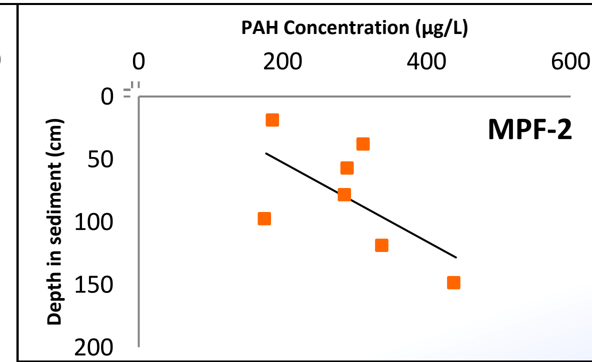
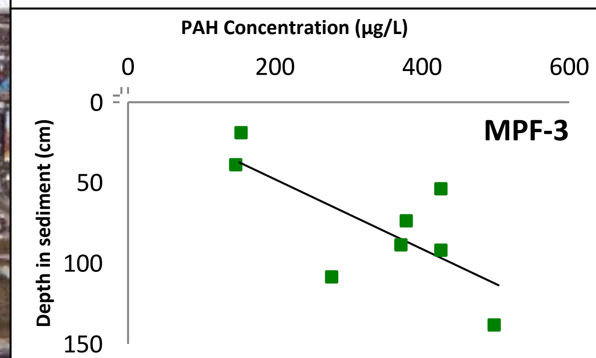
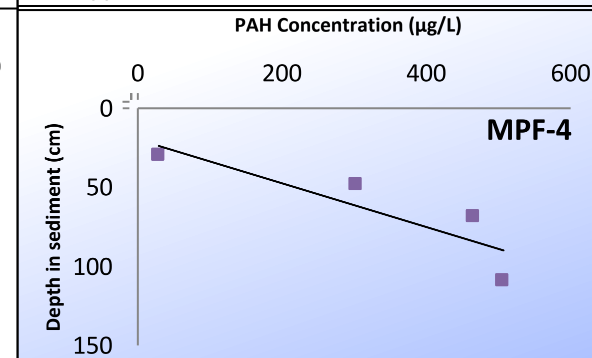
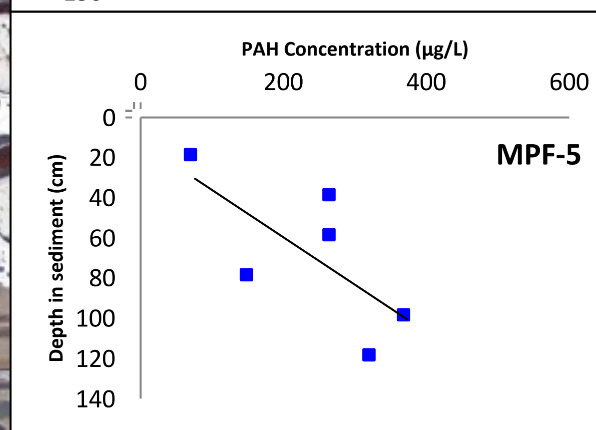
Porewater PAH Concentration Profiles within the Sediment at Money Point

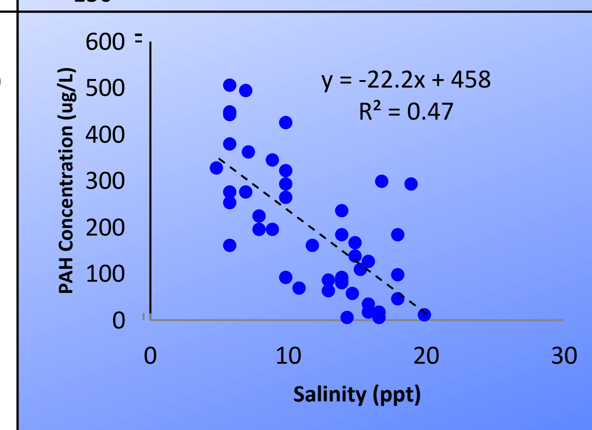












Saline surface water is mixing with more contaminated fresh pore water at depth in the sediment

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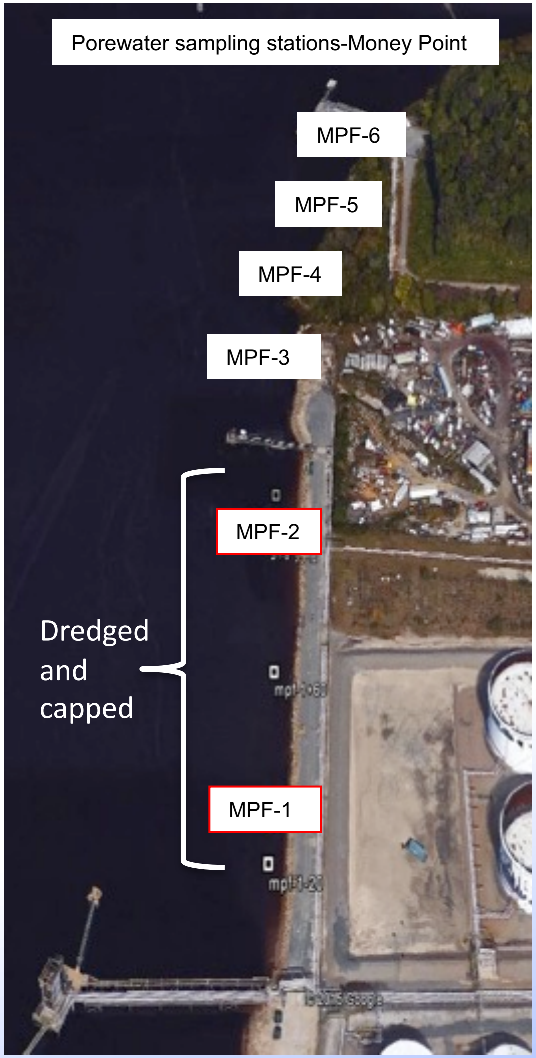
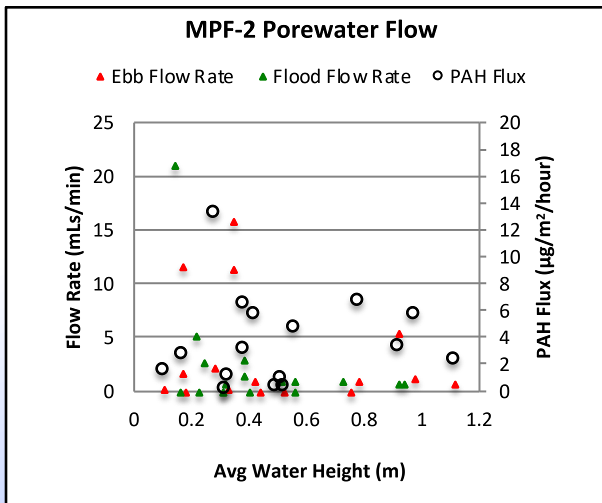
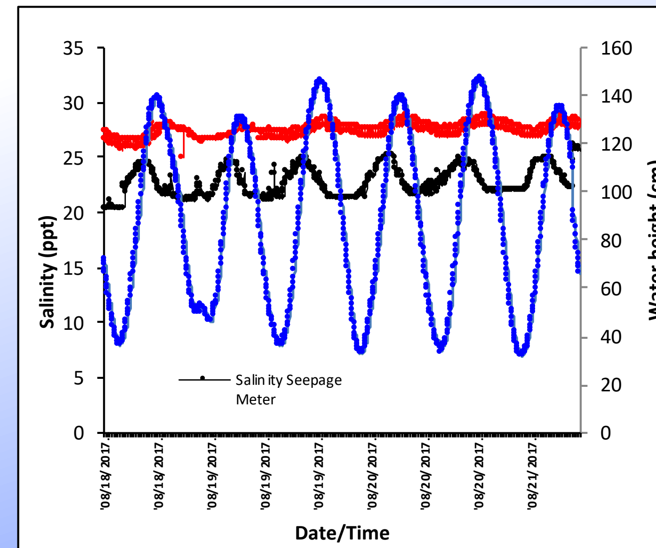
PAH Flux Transport to the water column: Seepage meter/Biosensor data



Seepage Meters
Direct hourly flow measurements
PAH concentrations by biosensor
Short-term concentration/flux measurement



Highest flux at remediated sites with coarse sediment cap and low total PAH



CTD data logger provides evidence of tidal driven advection

Data from the Biosensor is now helping to guide future remediation plans to limit flux to the water column.
Revisit problem sites and engineered caps in new areas

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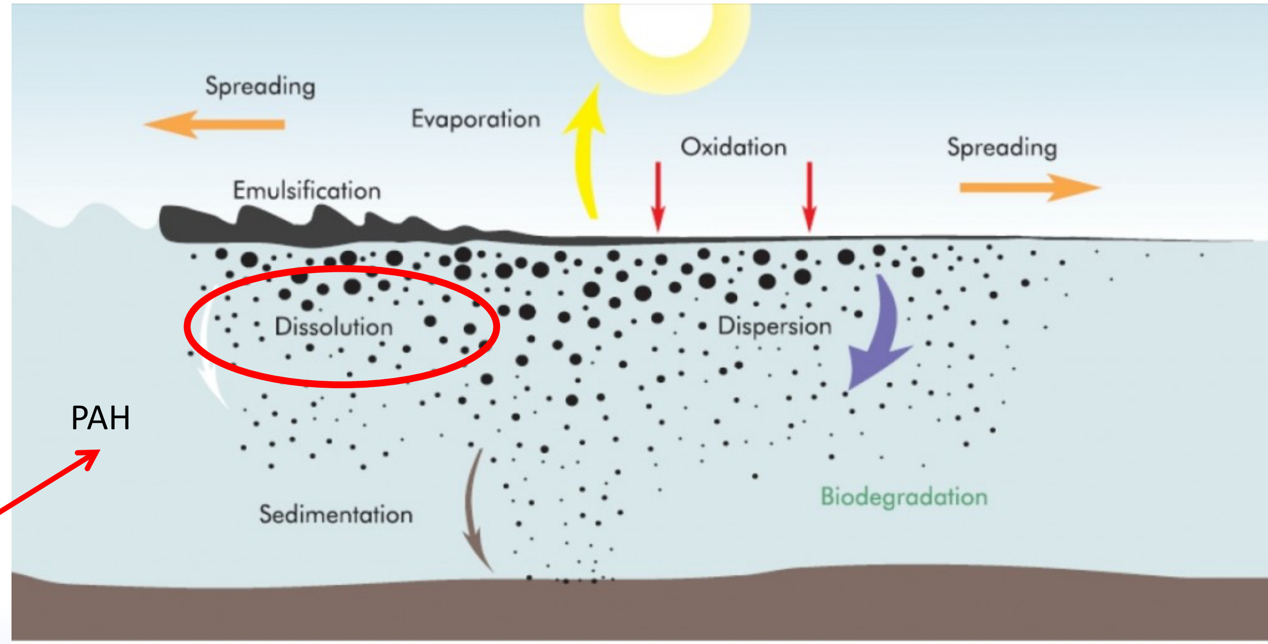
Can the Biosensor help to better understand the fate and effects of oil?



PAH

Dissolution is important for the exposure and bioavailability to aquatic organisms.

While PAH are a minor component in the total hydrocarbons in oil they represent a major fraction of the dissolved potentially toxic compounds

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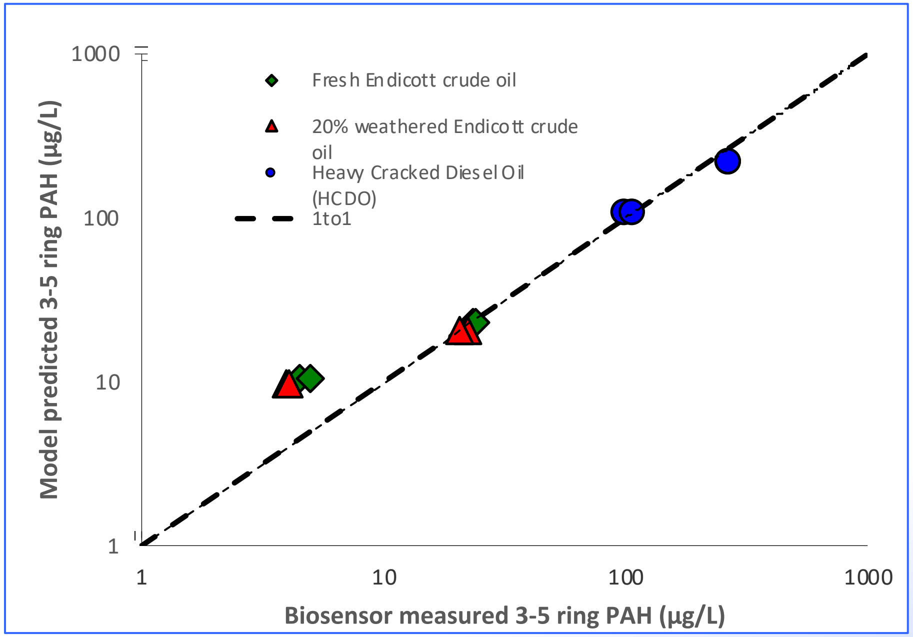
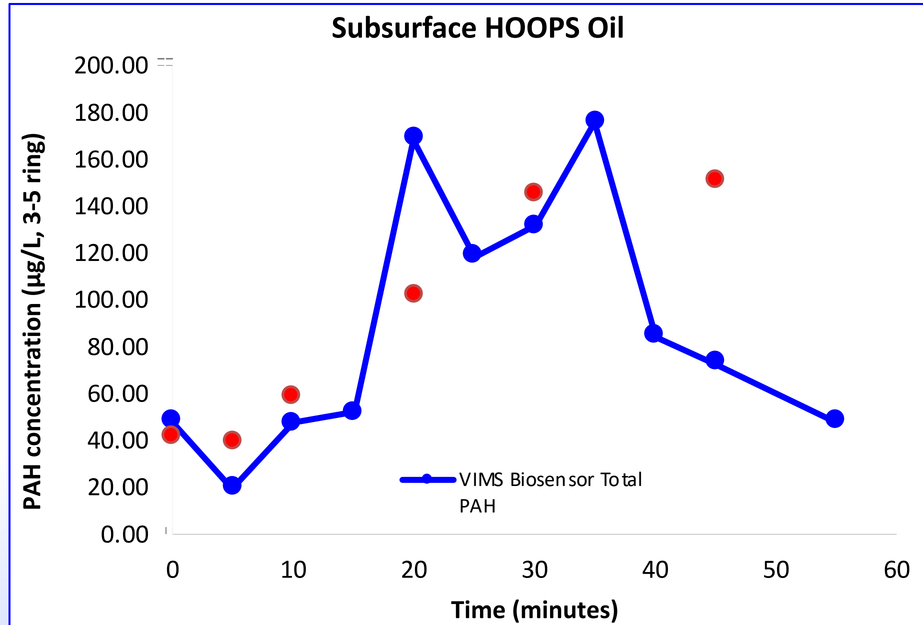
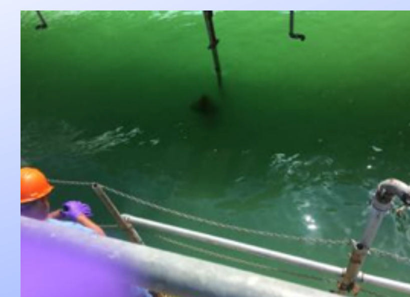
ExxonMobil Collaboration to evaluate PAH plume identification during an oil spill





Lab Study: Water soluble fractions from three different oils at two oil loadings- Model prediction vs. Biosensor measurements

Field Trial: October 2017 Ohmset Leonardo, NJ. Simulated spills PAH fate and transport by Biosensor real time



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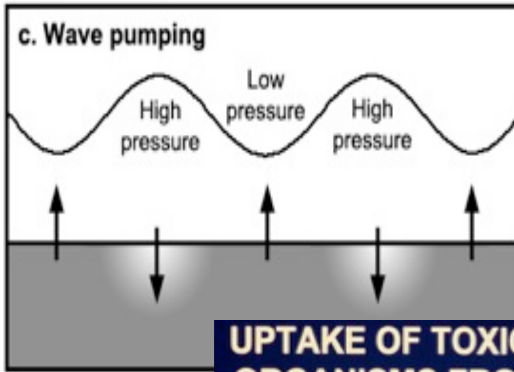
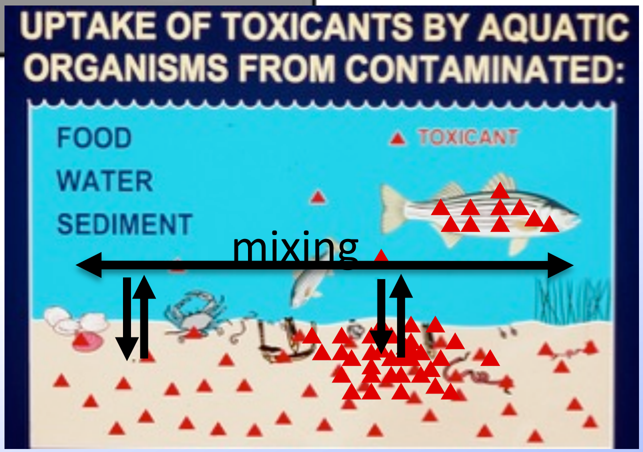
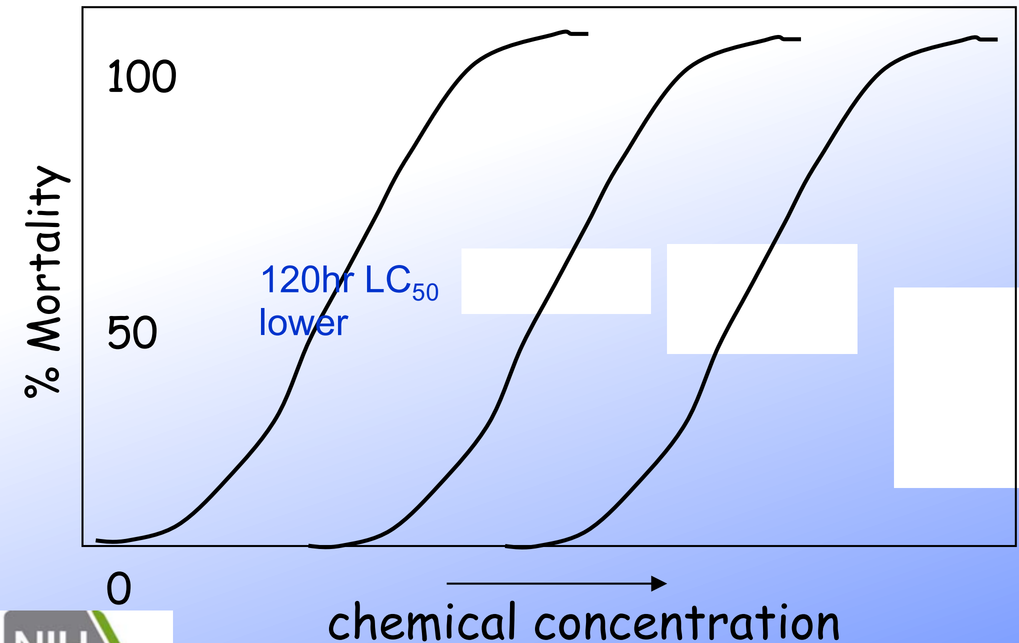
Biosensor analysis of PAH has helped elucidate the mechanisms controlling the fate and transport of PAH in water and sediments







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Paracelsus, Father of Toxicology (1493-1541)

"All substances are poisons; there is none which is not a poison. The right dose differentiates a poison…."

- The dose makes the poison!!!

Simple concept but what is the DOSE in contaminated sediment???


Environmental Science & Technology

From Bioavailability Science to Regulation of Organic Chemicals

2015 paper, 2017 SETAC Europe: New methods are being proposed to consider more accurate measurements addressing bioavailability in management decisions

Ortega-Calvo et al, ES&T 2015, 49, 10255-10264

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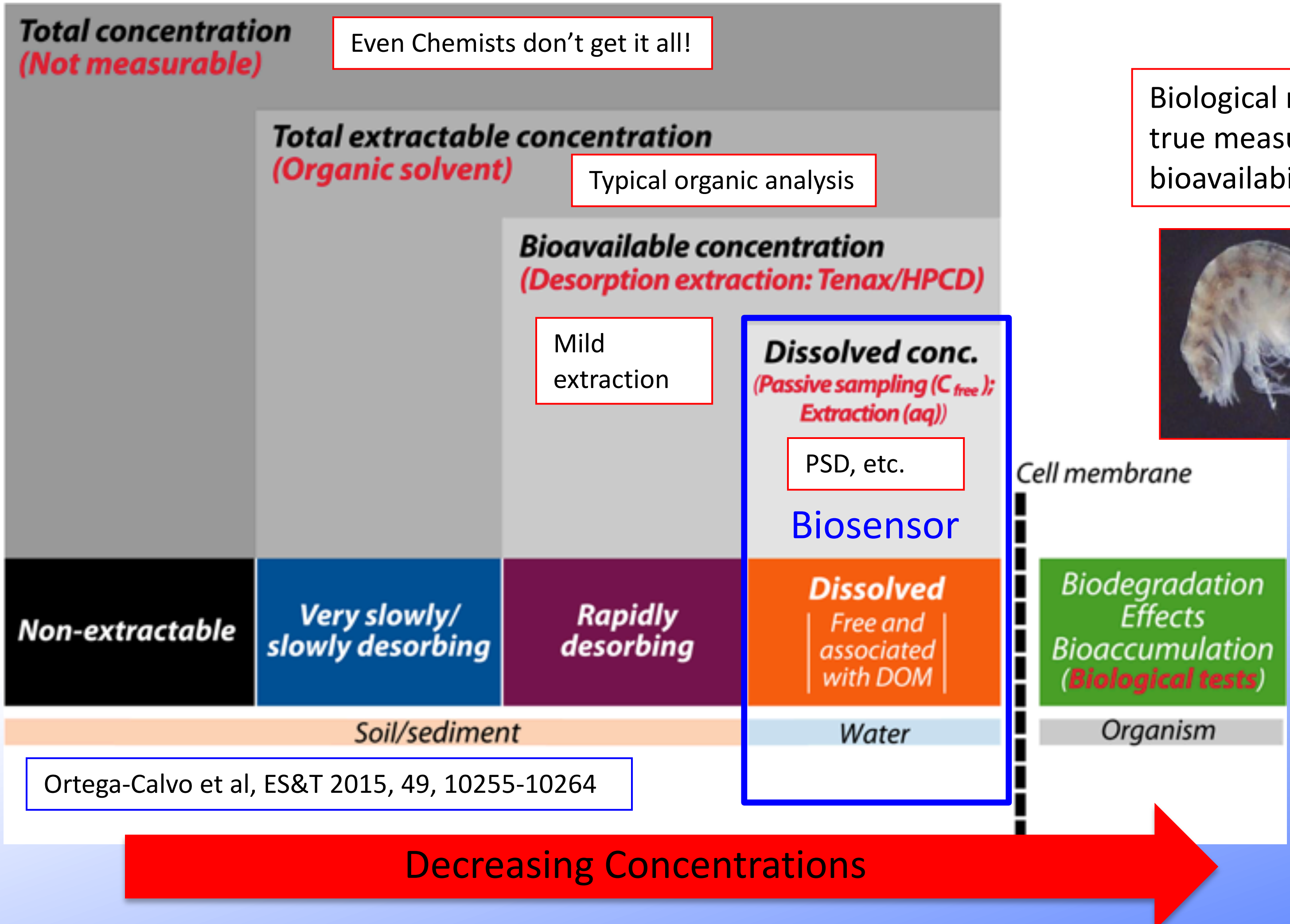
What is the Bioavailable fraction in sediments?



Biological response: true measure of bioavailability

Can we use new antibody based measurement methods to directly analyze the bioavailable/toxic component in porewater?

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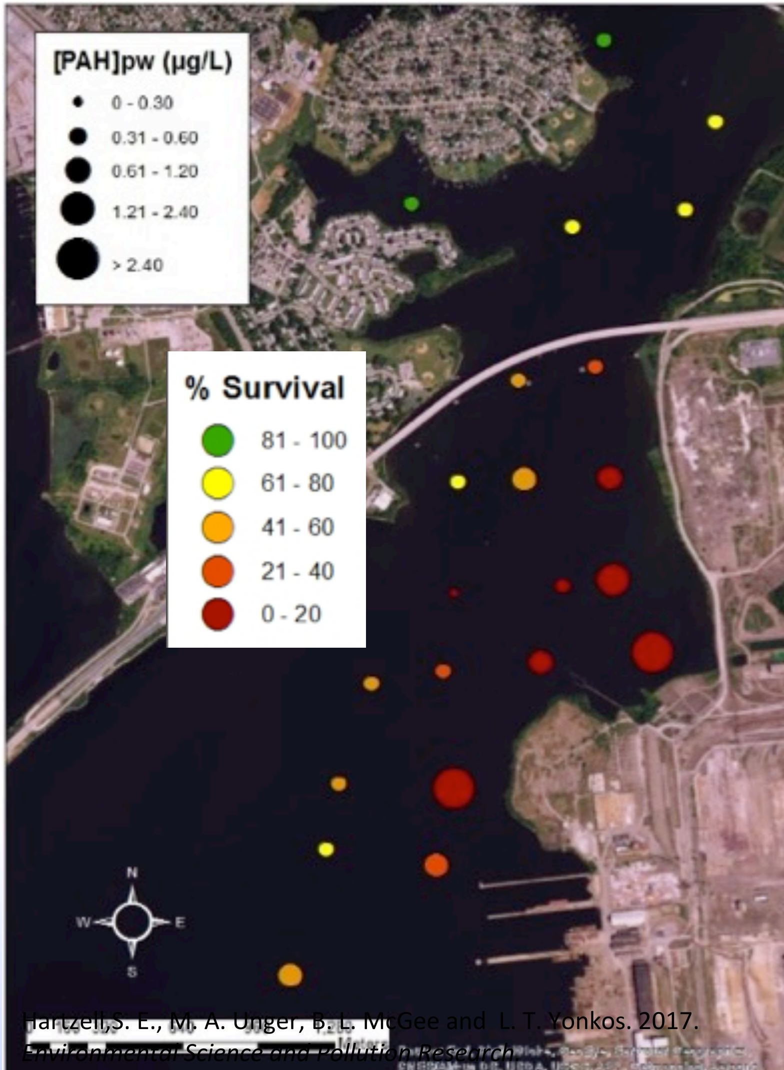
Porewater Toxicity Evaluation via Biosensor

VIMS/University of Maryland Research Collaboration: Sharon Hartzell, Lance Yonkos



Hartzell,S. E., M. A. Unger, B. L. McGee and L. T. Yonkos. 2017. *Environmental Science and Pollution Research*

Baltimore Harbor, MD

Test species – Estuarine *Leptocheirus plumulosus*

Acute 10-d test - Whole sediment collected from field

PAH concentrations in porewater measured by VIMS Biosensor

PAHs in porewater and sediment were strongly correlated with toxicity.

So were: Nickel, Chromium, TPH

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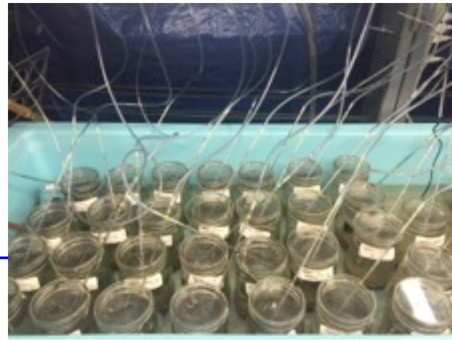

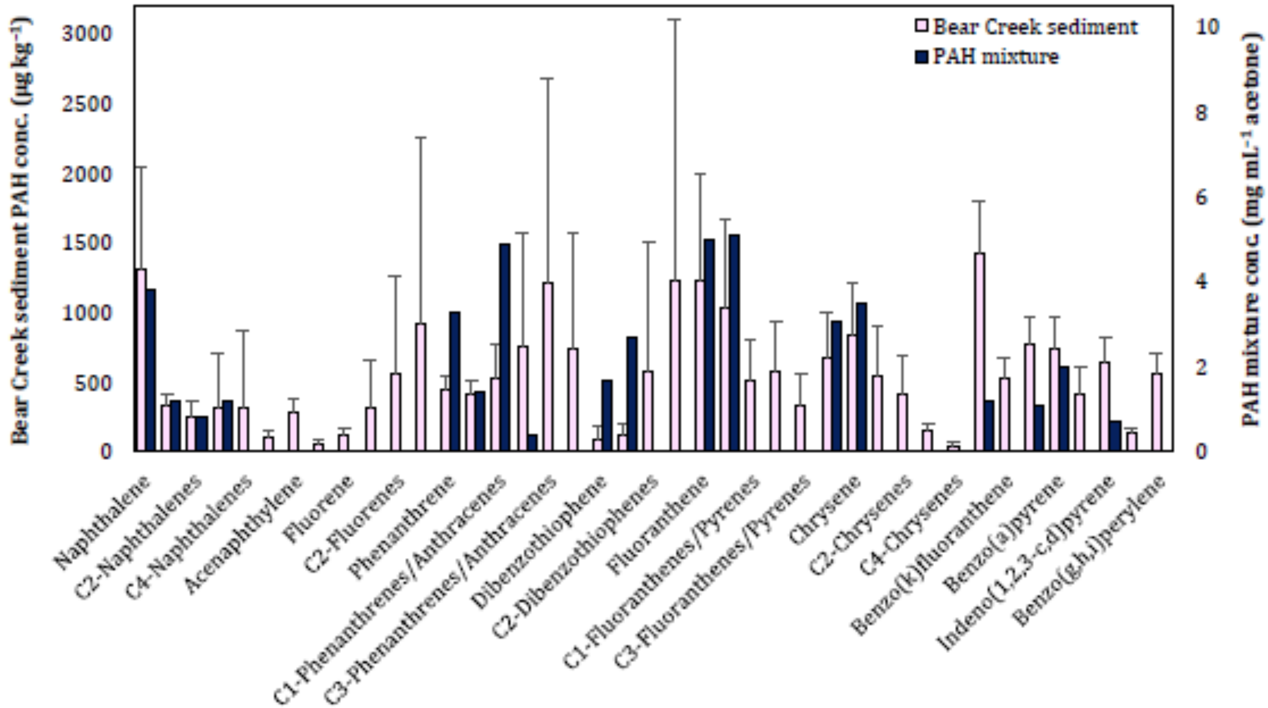

Figure 2

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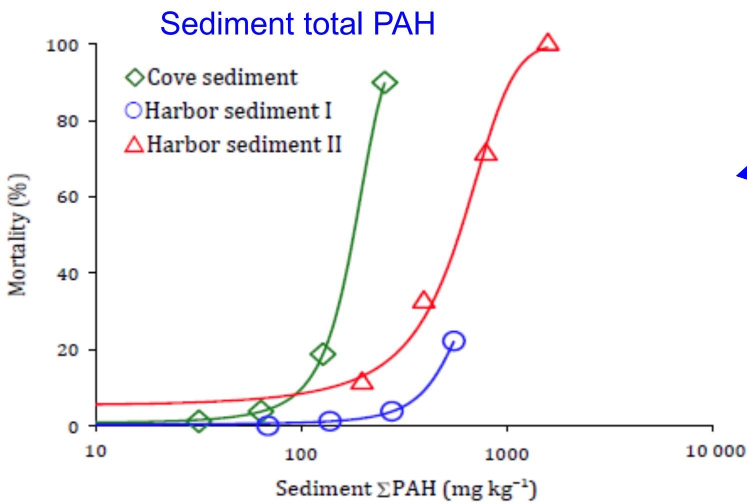
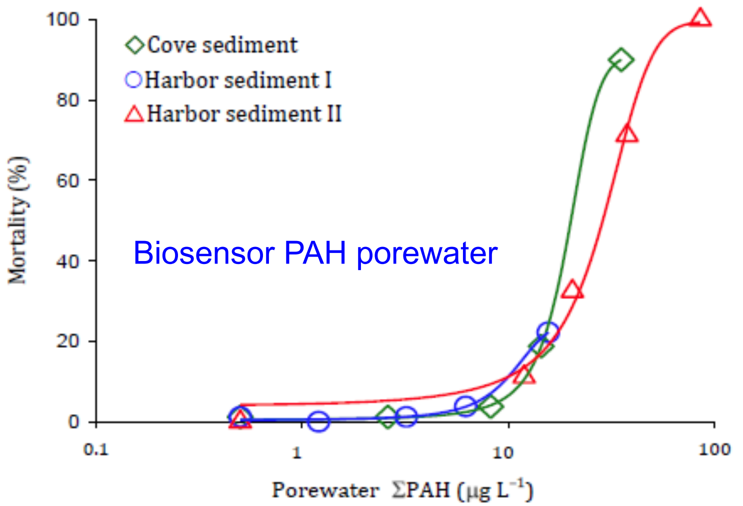
Results-Spiked Control sediment from Baltimore Harbor

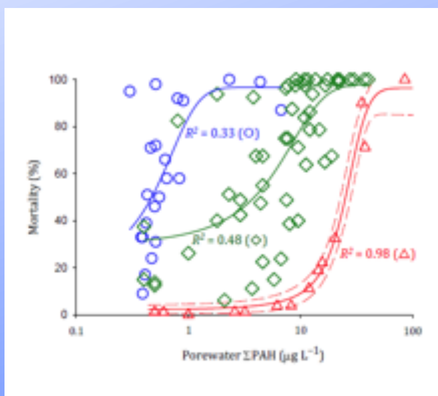


PAH concentrations in whole sediments aren't very good predictors of toxicity

Biosensor measurement of PAH porewater concentrations predicts toxicity

Porewater analysis by Biosensor can be used to rapidly identify toxicity in field sediments





PAH & Metals

Hartzell, S. E. M.A. Unger, G. G. Vadas , and L. T. Yonkos 2018. Evaluating porewater PAH-related toxicity at a contaminated sediment site using a spiked field-sediment approach. *Environ. Toxicol. Chem.* DOI: 10.1002/etc.4023

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New Research: Kristen Prossner SRP Trainee at VIMS

WHY?—Current state of the science for seafood PAH contamination
Public distrust from inaccurate or slow response during spills or floods

After *Deepwater Horizon*:

NOAA

SNIFF TEST Steven Wilson, chief quality officer for NOAA's Seafood Inspection Program, demonstrates sensory analysis of a sample of shrimp.

AND

Rapid Sniff Testing

Slow GC-MS Tissue Analysis

From policy standpoint: Fast, quantitative analysis allows quicker turnaround time to get data on seafood status back to stakeholders, build trust

From science standpoint: Allows analysis of PAH dynamics within <u>individual oysters</u> on temporal scales not possible with GC-MS

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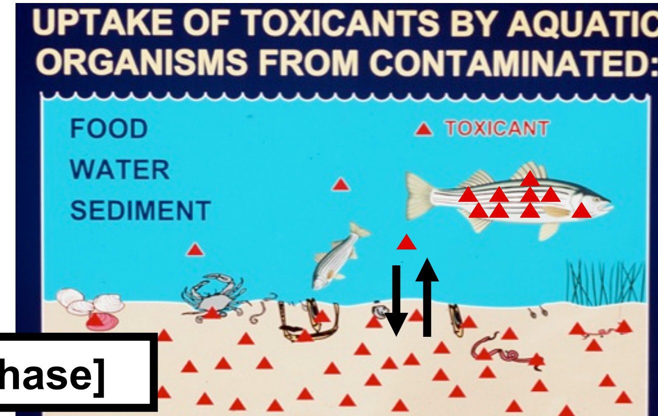
Shift the scales of equilibrium partitioning

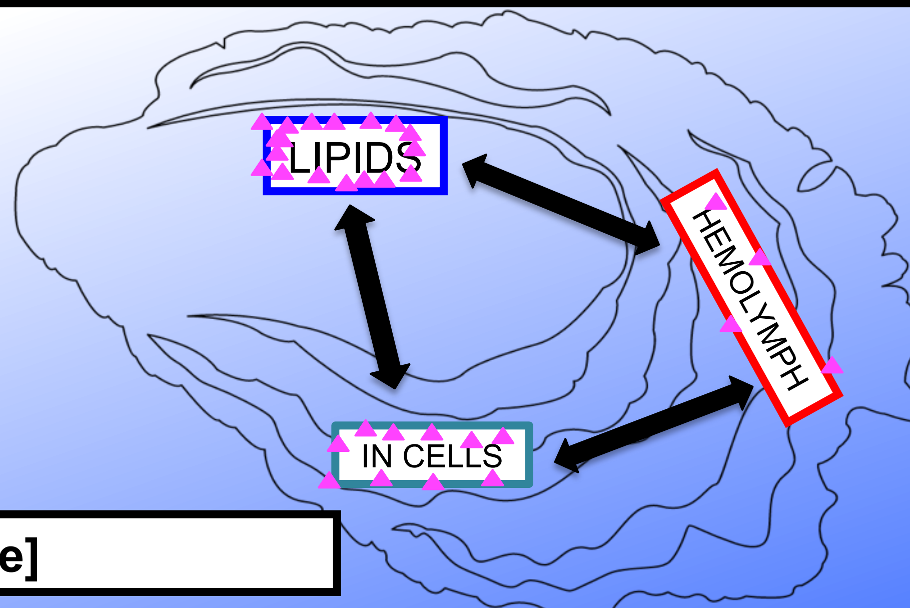
K_p predicts distribution of PAHs in the environment



K_P = [PSD]/[Aqueous phase]

K_P = [Sediment]/[Aqueous phase]

Does it predict distribution of PAHs in a bivalve?



$K_{PAHoyster}$ = [lipid tissue]/[oyster aq. phase]

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Methods

Collect mantle fluid-
Aqueous phase

(n=6)

6 individuals per
homogenate

~1g-7g
dry wt.

Freeze-dry homogenate

ASE extraction

Gel permeation
chromatography

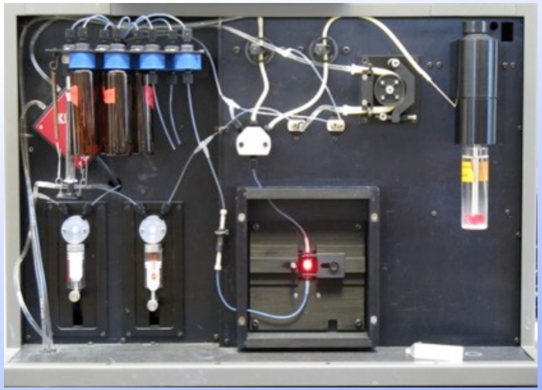
Silica gel column
chromatography

-Field oysters from
contaminated sites in
Elizabeth River

-28-day lab exposure
oysters

0.45µm PTFE
syringe filter

Biosensor (Li et al. 2016)



GC-MS

Sapidyne Instruments
Boise, Idaho

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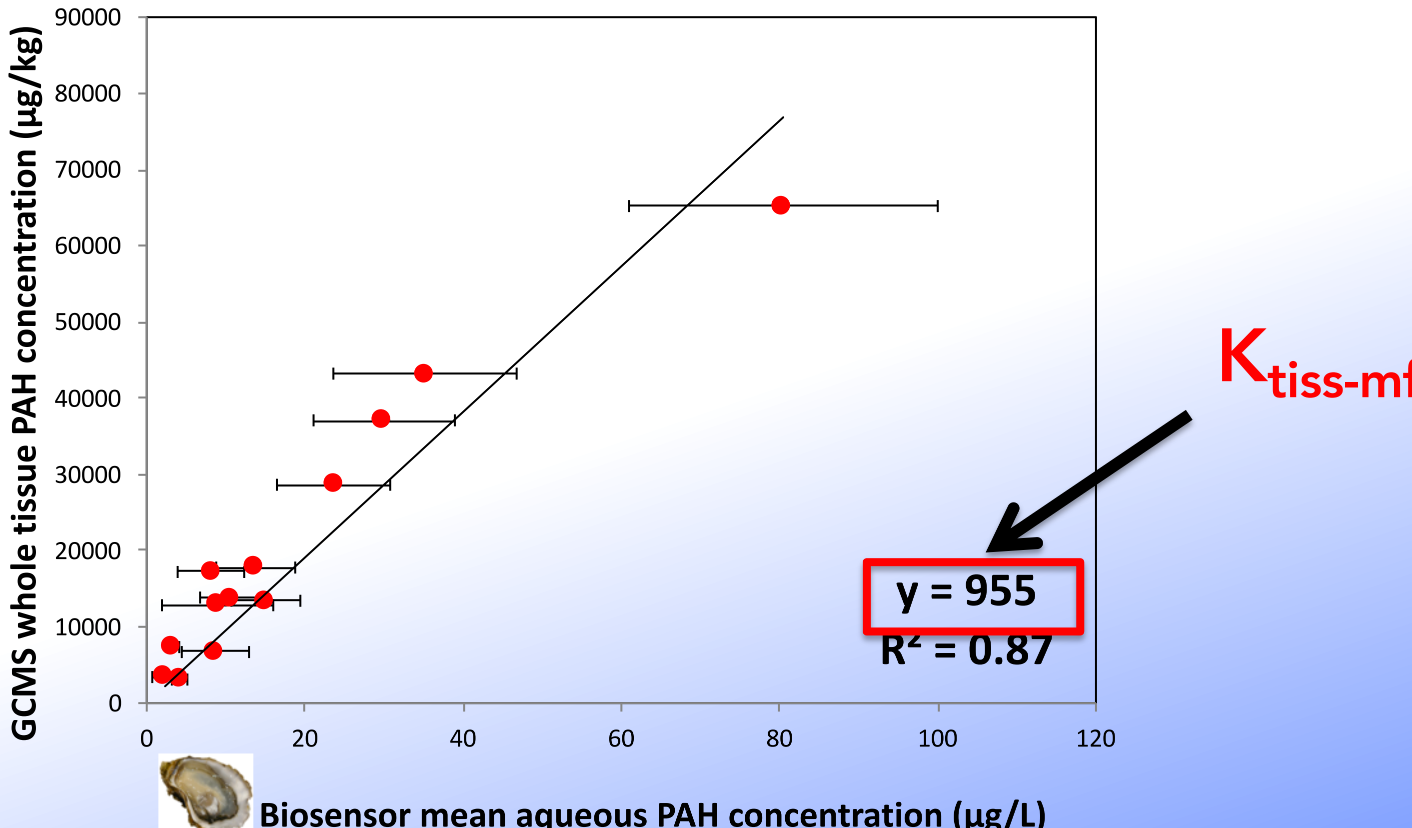
Results—Biosensor vs. GC-MS

Weeks



Minutes

[whole tissue] = [oyster mantle fluid] * $K_{tiss\text{-}mf}$

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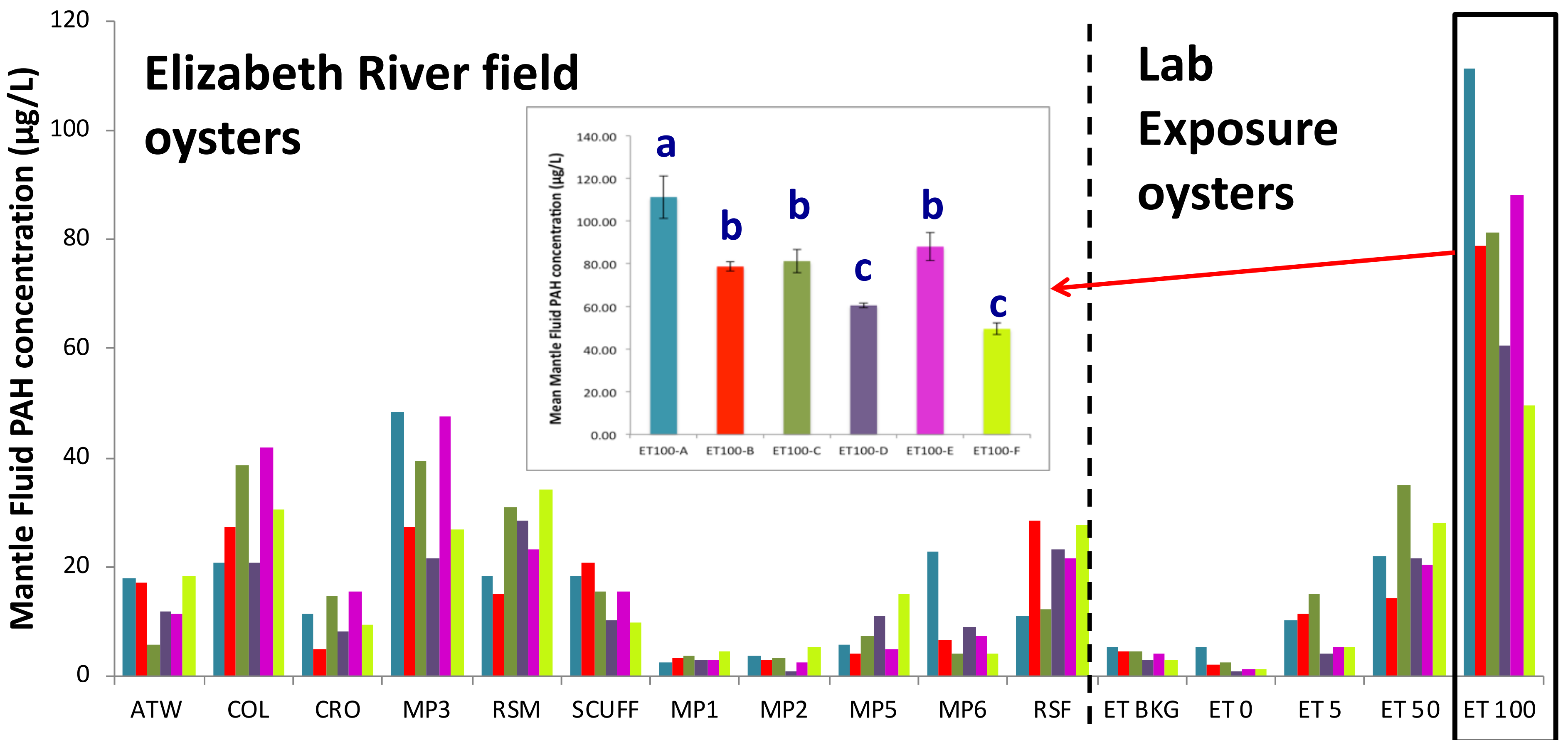
RESULTS—Variability among individual oysters

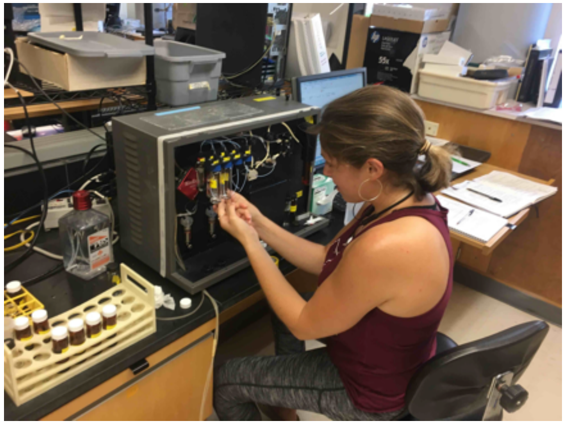
n=6 individual oysters per site/exposure treatment

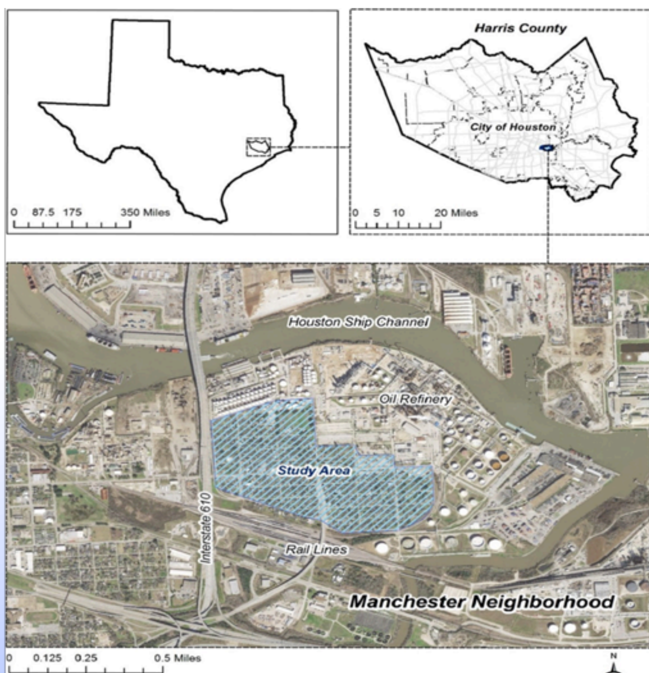
Sensitivity of biosensor for small volume samples allows for total 3-5 ring PAH concentration measurements at an **INDIVIDUAL** level—**GC-MS analysis usually requires composite samples**

Better understanding of individual variability



New Research: Collaboration with TAMU SRP Center
Tony Knap and Krisa Camargo (SRP trainee and KC Donnelly Fellow)





Working on a Biosensor based method for rapid screening of PAH in soil and sediments

- **Use Biosensor data to guide future sampling in the field for compound specific analysis by GC-MS to delineate sources**
- **Map potential PAH gradients during flood events**
- **Scheduling for summer/fall 2019 to map PAH in near real time in Houston to guide future areas of focus**
- **Lessons learned in Houston area have potential to advise flood prone areas like Chesapeake Bay**

Source: City of Houston GIS Open Data, Texas Natural Resources Information System Study Area 25)

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Summary Biosensor Technology

- **Total PAH concentrations (3-5 ring) in minutes from small volume samples allows spatial and temporal measurements not possible by conventional methods: good correlation to GC-MS analysis in split samples**

- **Mapping of concentration gradients in the water column and within sediments is possible to identify contaminant sources, transport and flux. It can provide a measure of the toxic or bioavailable fraction.**

- **Similar initial instrumentation costs but a few dollars/analysis vs. 100s dollars for GC-MS, data in minutes, green technology: no solvents**

- **Prioritize samples for compound specific GC-MS based on total PAH measurements by biosensor (don't pay for non-detects!)**

Summary Sediment Remediation Needs

- **Bioavailability is governed by contaminant partitioning and transport- whole sediment measurements alone are not good for assessing remediation effectiveness for reducing exposure to biota/humans.**
  - **Reducing contaminant bioavailability and flux to the water column should be the metrics for success- We are now advising environmental managers on the need for redefining regulatory goals to reflect bioavailability**
- **Future remediation strategies should consider ways to mitigate porewater transport. (i.e. barriers, sorptive amendments, etc.)**
  - **Can we convince regulators that remediation may involve leaving contaminated sediments in place? Change the partitioning and you change the bioavailability/toxicity. Funds will potentially go farther to improve greater areas of the watershed**

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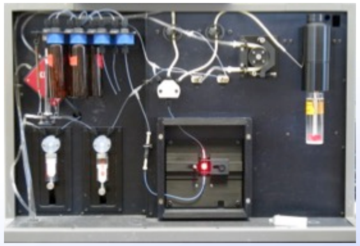
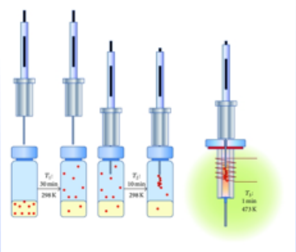
Current and Future Biosensor Work

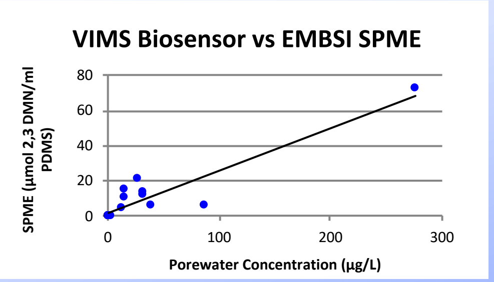
- **Biosensor hardware development, smaller, more portable - Sapidyne Instruments & commercialization of current mAbs**

Portable, battery powered easy to operate

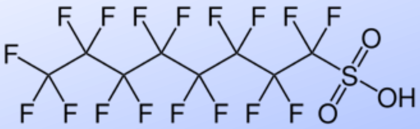
- **Detection of oil spills and sediment toxicity**
  - **ExxonMobil-water soluble PAH, porewater, SPME & toxicity**

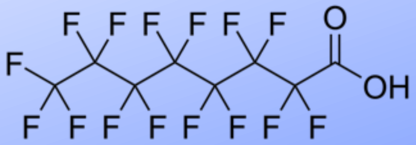


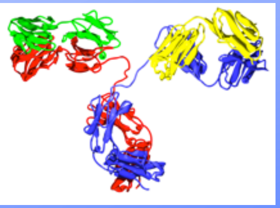




- **New antibodies for other new hydrocarbons, PFAS, HAB toxins or ???**







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Acknowledgements

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Impact of groundwater-surface water dynamics on in situ remediation efficacy and bioavailability of NAPL contaminants

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Sharon Hartzel, Lance Yonkos, Yonkos lab members: Wenqi Hou, Amy Wherry and Shannon Edmonds

Terrance Lackey

Dave Marsell

Chris Prosser, Tom Parkerton

Paracelsus

Questions?

Relevant PAH Biosensor Publications

Hartzell, S. E. M.A. Unger, G. G. Vadas , and L. T. Yonkos 2018. Evaluating porewater PAH-related toxicity at a contaminated sediment site using a spiked field-sediment approach. *Environ. Toxicol. Chem.* Vol. 37, no. 3, pp 893-902. DOI: 10.1002/etc.4023

Hartzell, S. E., M. A. Unger, B. L. McGee and L. T. Yonkos. 2017. Effects-based spatial assessment of contaminated estuarine sediments from Bear Creek, Baltimore Harbor, MD, USA. *Environmental Science and Pollution Research.* http://dx.doi.org/10.1007/s11356-017-9667-0

Li, X., S. L. Kaattari, M. A. Vogelbein, and M. A. Unger. 2016. Evaluation of a time efficient immunization strategy for anti-PAH antibody development. *Journal of Immunoassay and Immunochemistry.* Vol. 37, Issue 6, 671-683.

Li, X., S. L. Kaattari, M. A. Vogelbein, G. G. Vadas and M. A. Unger. 2016. A highly sensitive monoclonal antibody based biosensor for quantifying 3-5 ring polycyclic aromatic hydrocarbons (PAHs) in aqueous environmental samples. *Sensing and Bio-sensing Research.* 7:115-120.