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

SRP Analytical Tools and Methods
Session I – Field-Ready Biosensors to Assess Bioavailability and Toxicity
April 17, 1-3 pm EST

Michael Unger
Associate Professor
Aquatic Animal Health
VIMS
munger@vims.edu
804-684-7187

National Institute of Environmental Health Sciences
Superfund Research Program

Virginia Institute of Marine Science
William & Mary

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

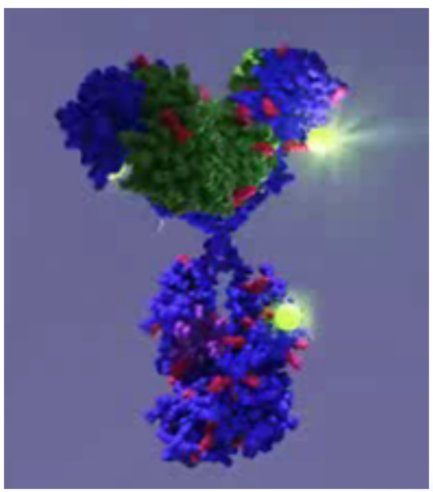
- PAH biosensor, what is it and how do we make it?
- Biosensor applications
  - Elizabeth River, VA: Evaluating PAH transport to assist restoration efforts
  - Baltimore Harbor, MD: Evaluation of contaminated sediment toxicity
  - Future work, possible applications of biosensor technology

Near real-time PAH analysis: VIMS Biosensor

Our Approach

Bio

Monoclonal Antibodies against Contaminants



Sensor

Electronic detection of mAb Binding

KinExA TECHNOLOGY

Sapidyne
Instruments Inc.
Boise, Idaho

NIH
National Institute of Environmental Health Sciences
Superfund Research Program

VIMS | William & Mary
Virginia Institute of Marine Science

Goal: Detect PAH in water rapidly at low concentrations with antibodies

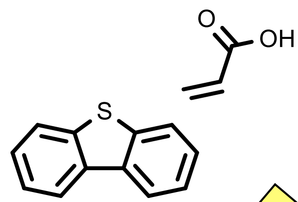
Monoclonal antibodies (mAbs) goals:

1. Unique selected specificity (3-5 ring PAHs)
2. Fast on-rate
3. Affixed with a tag for easy detection
4. High affinity: no sample preparation/ measure directly w/o concentration steps

NIH National Institute of Environmental Health Sciences Superfund Research Program

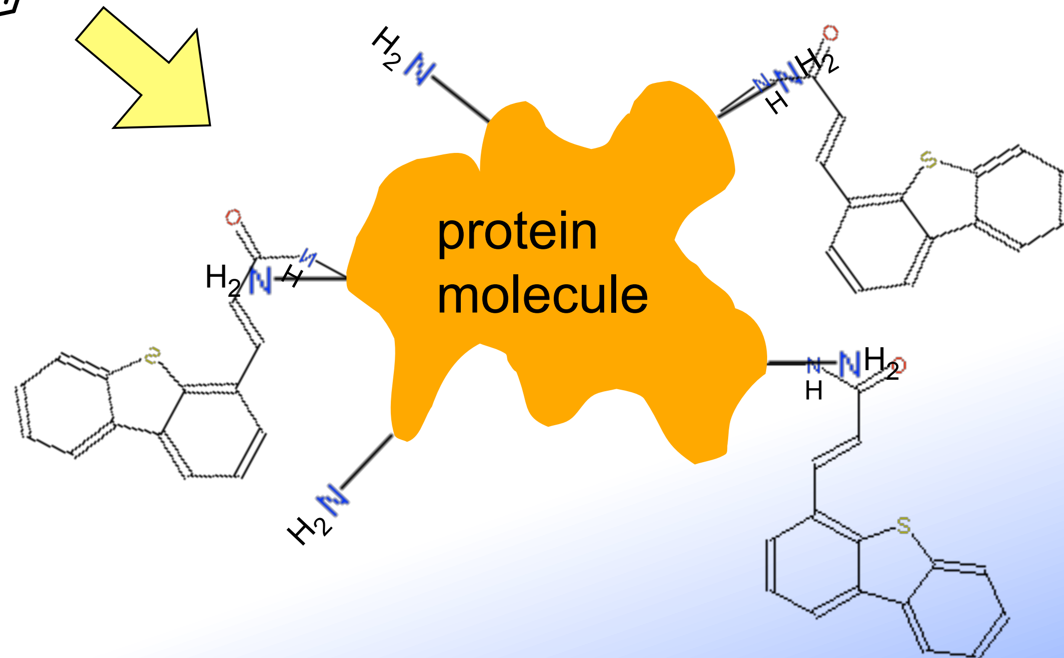
VIMS William & Mary Virginia Institute of Marine Science

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



Hapten- Target surrogate with linking arm

↑ not immunogenic

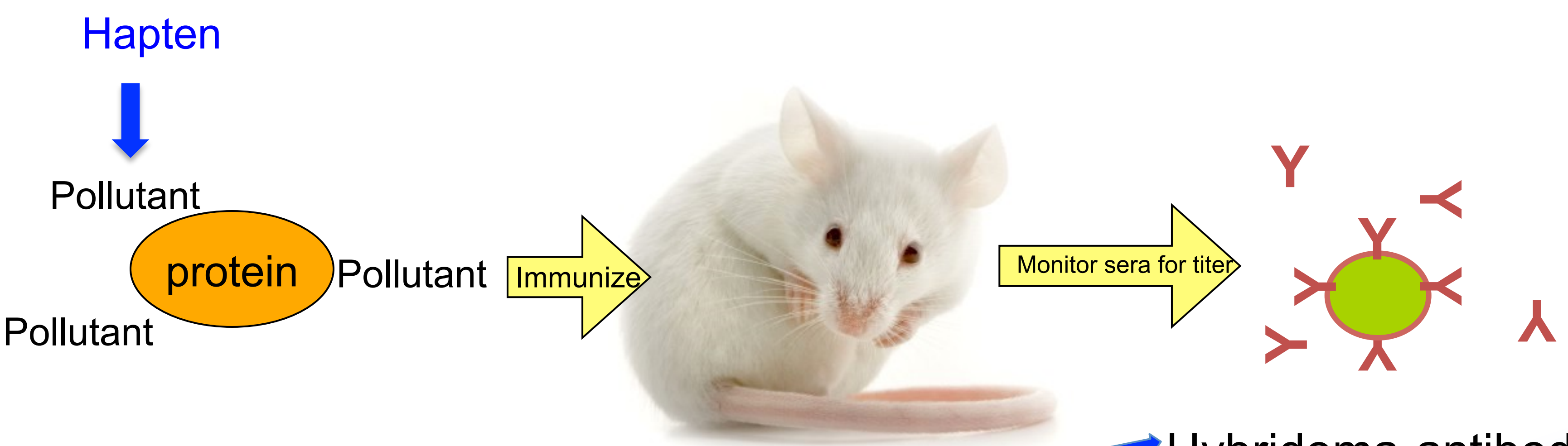


immunogenic →

National Institute of Environmental Health Sciences
Superfund Research Program

VIMS | William & Mary
Virginia Institute of Marine Science

How to make antibodies to pollutants?

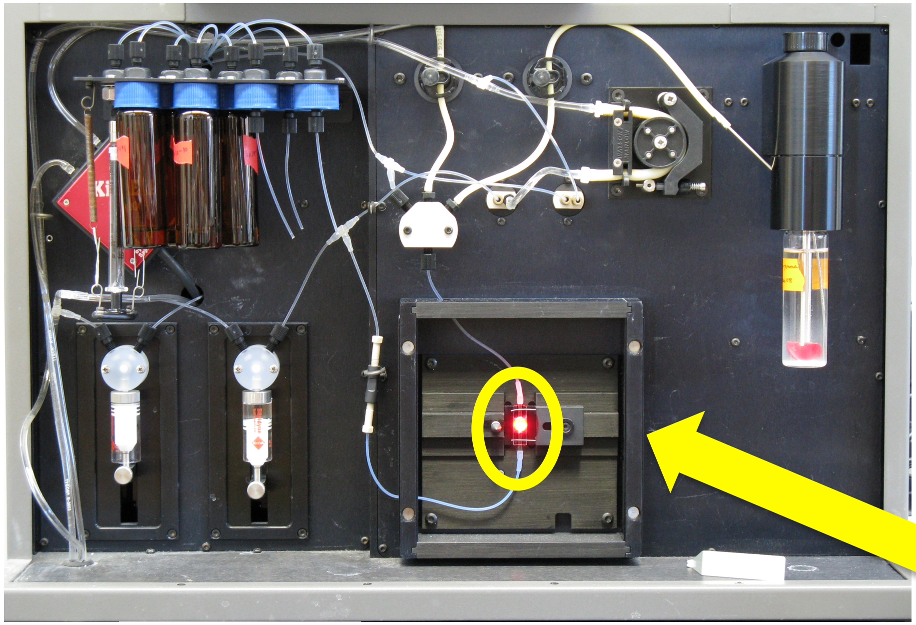


Hybridoma-antibody producing cells

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

Provides an endless supply of antibodies in cell culture

Goal: Quantification of mAb binding



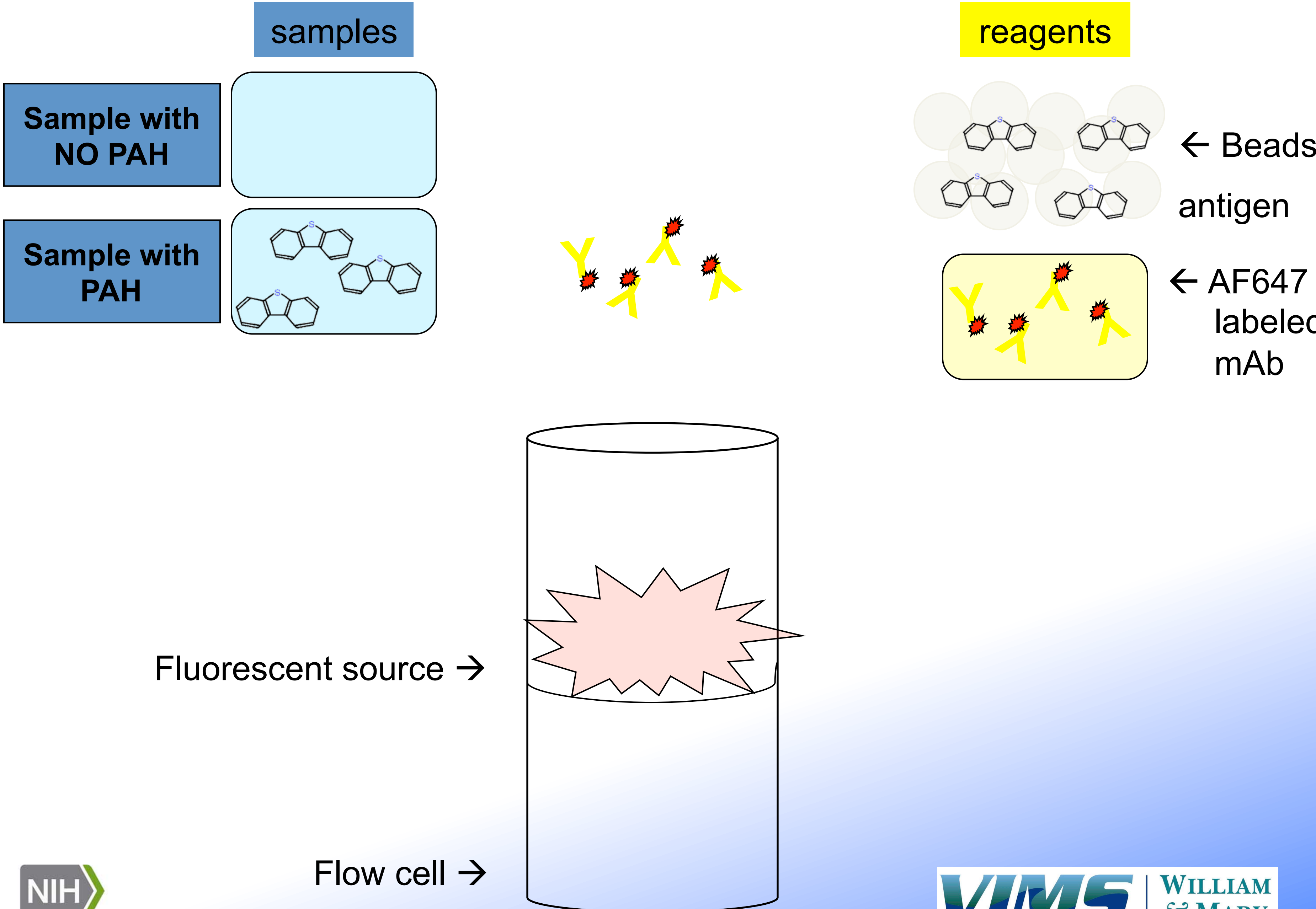
Inline Sensor (Biosensor) features:

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

Sapidyne
Instruments Inc.
Boise, Idaho

National Institute of Environmental Health Sciences
Superfund Research Program

Virginia Institute of Marine Science



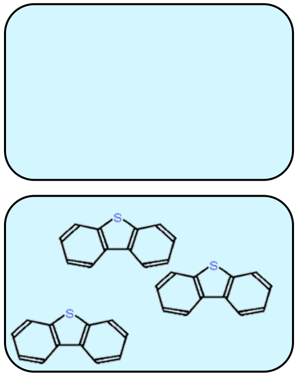
National Institute of Environmental Health Sciences
Superfund Research Program

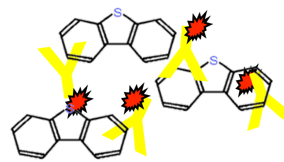
VIMS | William & Mary
Virginia Institute of Marine Science

samples

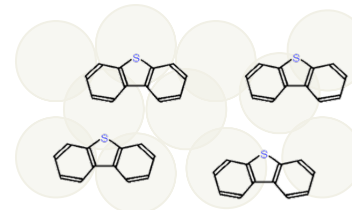
Sample with NO PAH

Sample with PAH



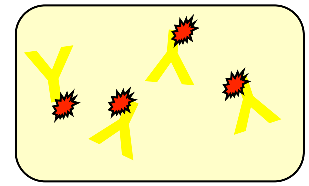


reagents



← Beads

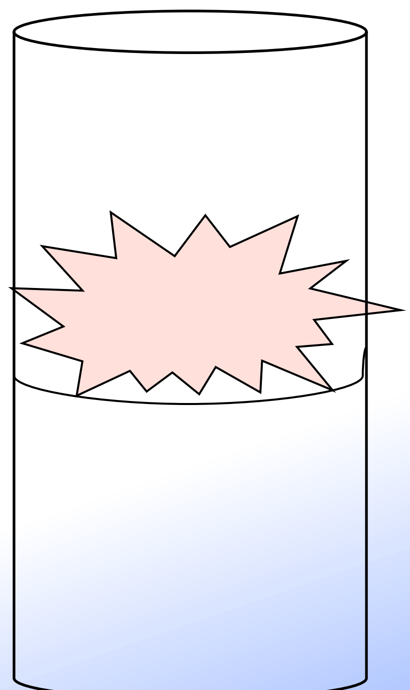
antigen

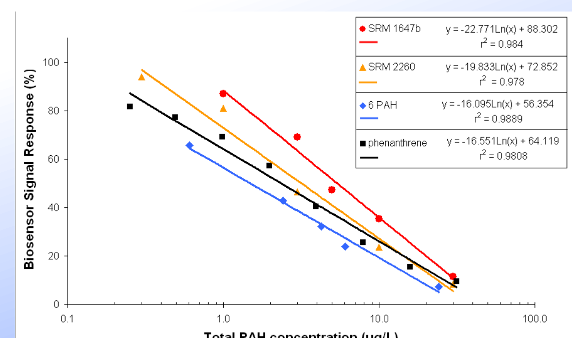


← AF647 labeled mAb

sample with NO PAH
= **high signal**

sample with high PAH
= **low signal**

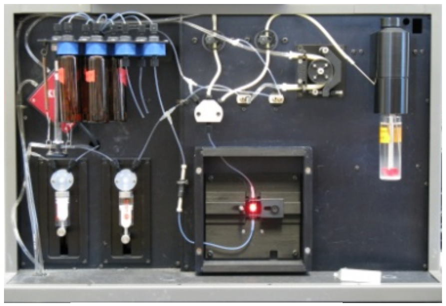




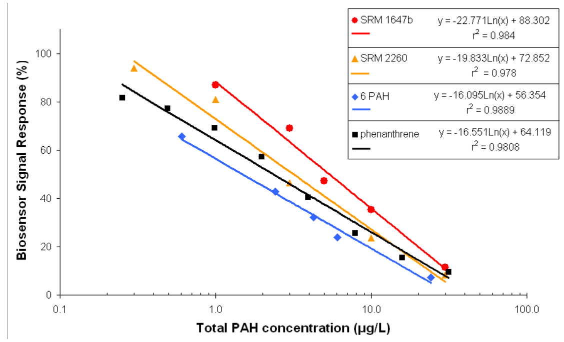
National Institute of Environmental Health Sciences
Superfund Research Program

VIMS | William & Mary
Virginia Institute of Marine Science

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

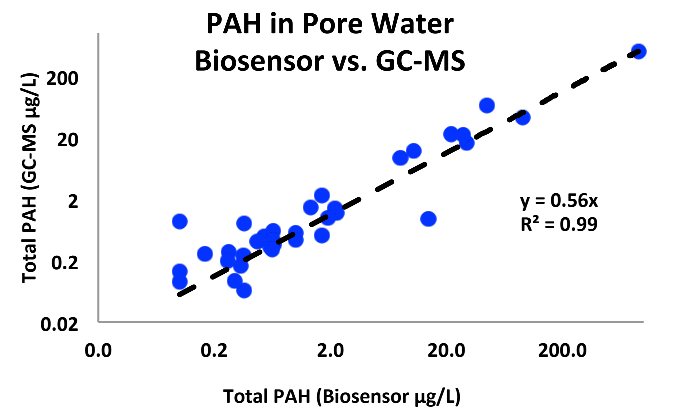


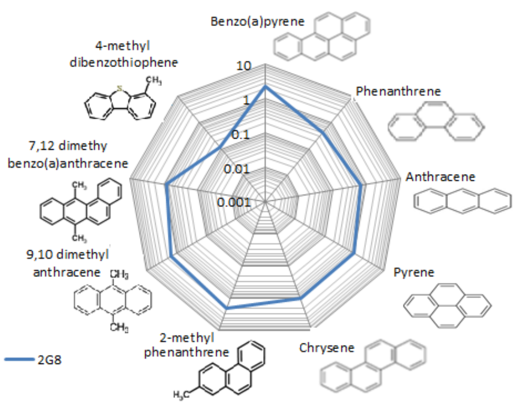
Sāpidyne Instruments Inc.



Sensitive (sub-ppb) and precise

Good correlation to GC-MS





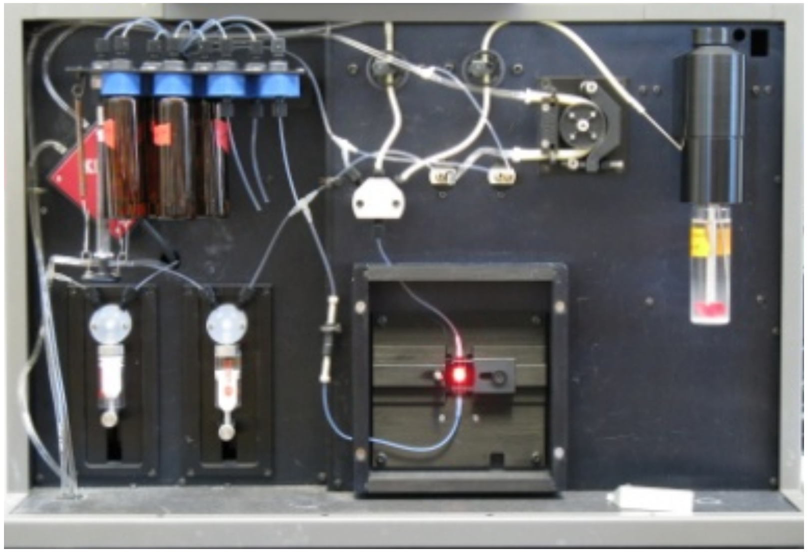
Affinity for a wide range of PAH (3-5 ring)

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

NIH National Institute of Environmental Health Sciences
Superfund Research Program

VIMS | William & Mary
Virginia Institute of Marine Science

How can we exploit this new technology to answer difficult environmental fate and toxicity questions?



Good match to industry and regulatory needs

SMALL **volume samples (1-5 ml)**
FAST **analysis (8 m)**
LOW **concentrations (<0.1 ppb)**

NOT GC-MS!!!
Exploit what it CAN do - porewater

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

NIH
National Institute of Environmental Health Sciences
Superfund Research Program

VIMS | William & Mary
Virginia Institute of Marine Science

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

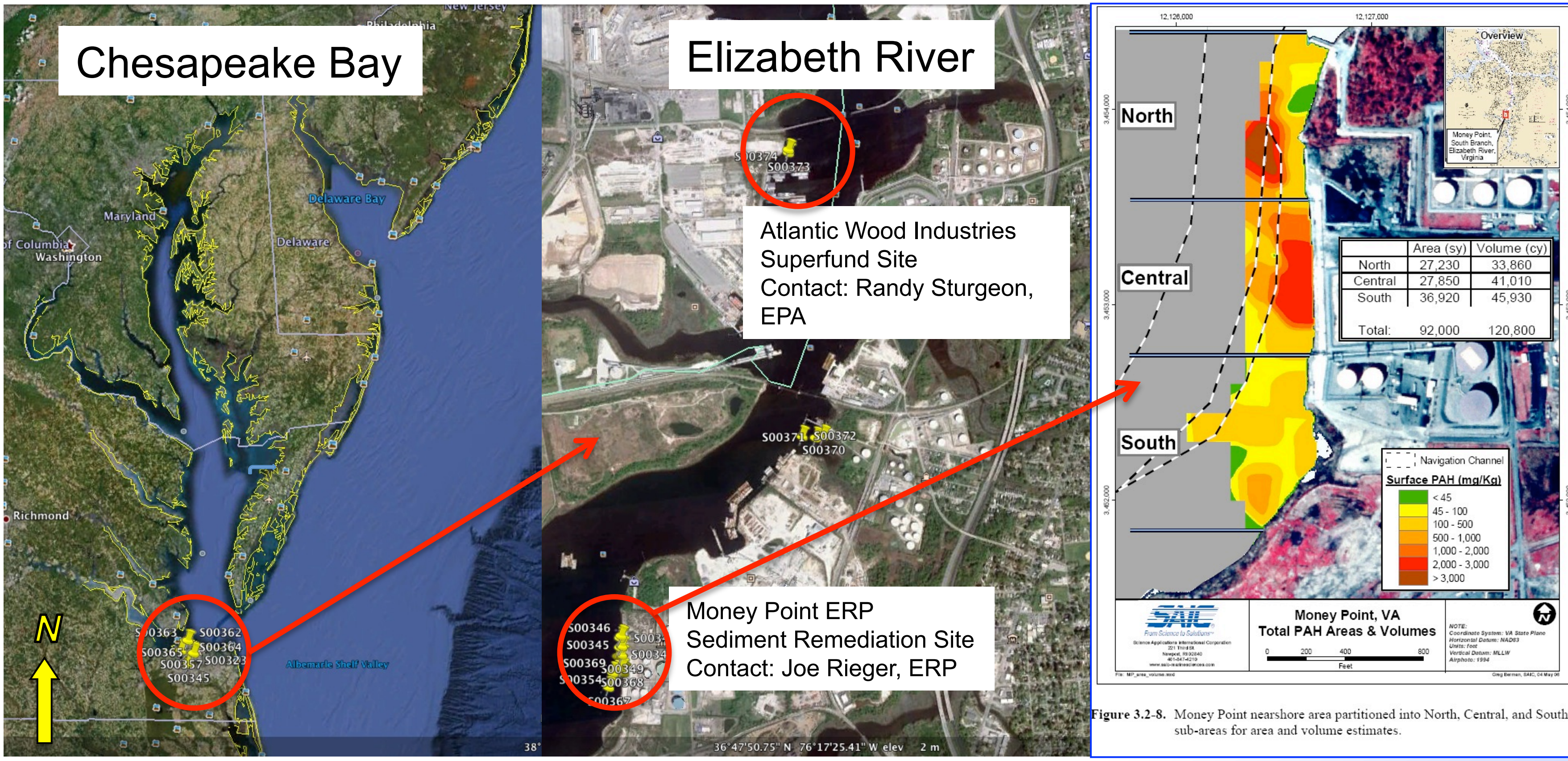


Figure 3.2-8. Money Point nearshore area partitioned into North, Central, and South sub-areas for area and volume estimates.

- Sites contain a wide range of PAH contamination and various stages of ongoing sediment remediation
- Some areas contain DNAPL on surface post-remediation (dredging & capping)
- **Methods are needed to better understand and predict PAH transport at sediment remediation sites to assure long-term success**

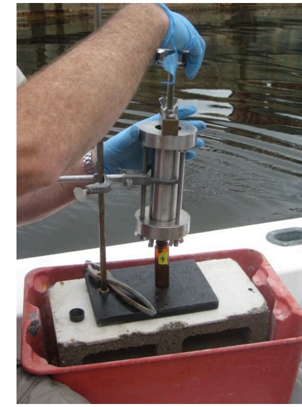
NIH
National Institute of Environmental Health Sciences
Superfund Research Program

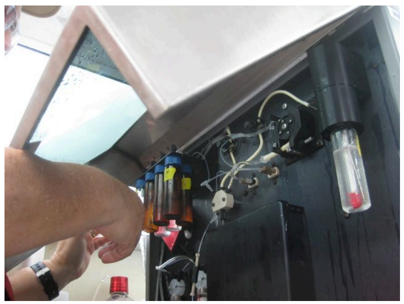
VIMS | William & Mary
Virginia Institute of Marine Science

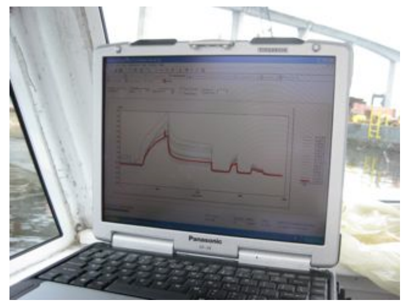
Methods: Porewater sampling surface sediments

- Real-time analysis can be used to map [PAH] in 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

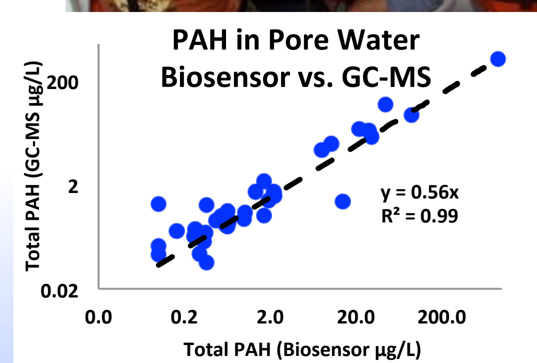






- Small volume samples analyzed on board by biosensor and larger volume samples can be brought back to the lab for GC-MS

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



National Institute of Environmental Health Sciences
Superfund Research Program

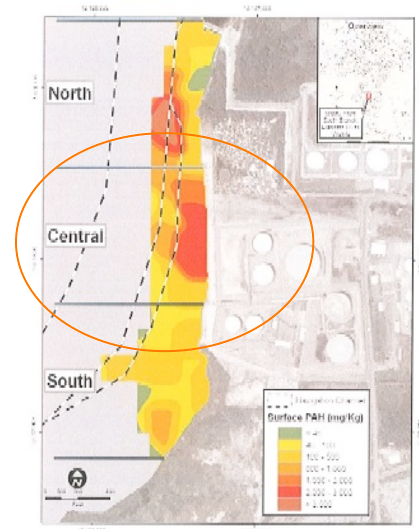
William & Mary
Virginia Institute of Marine Science

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

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

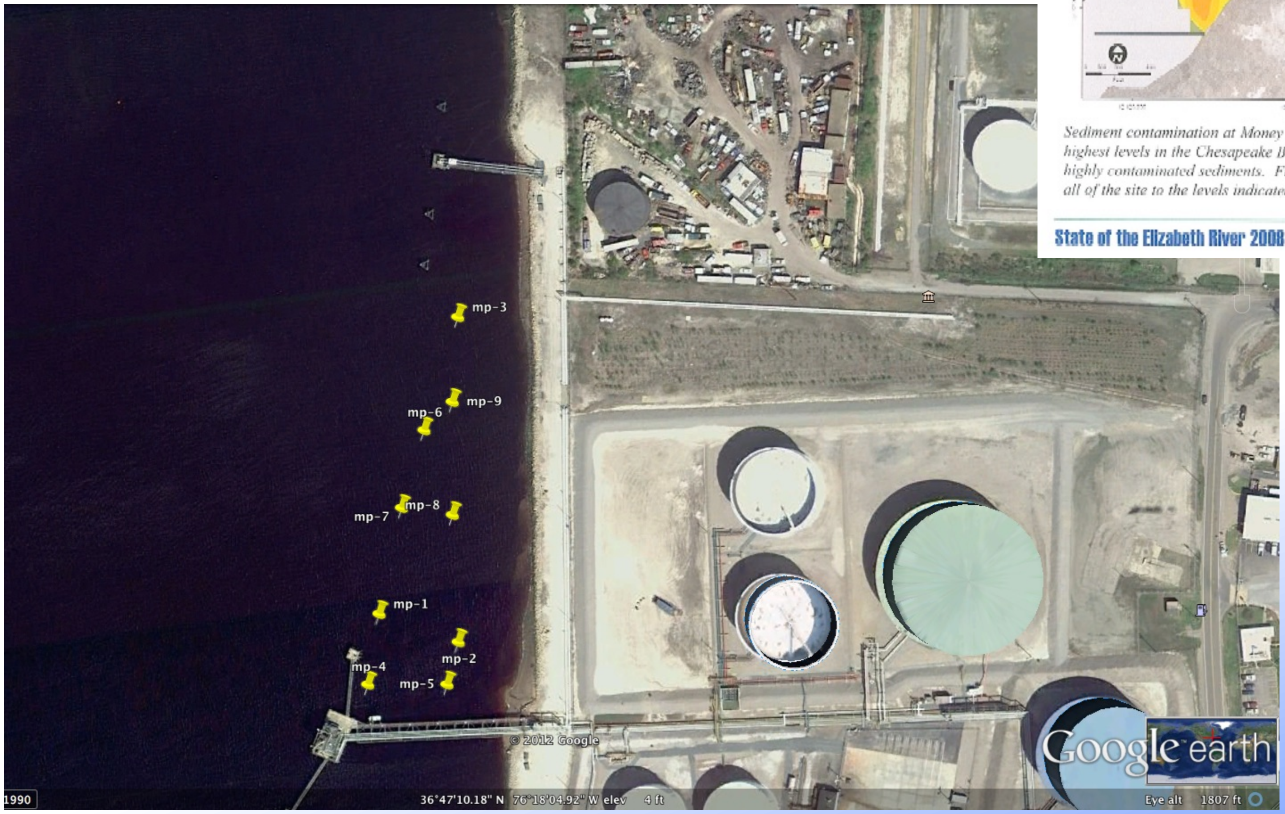
Id	Conc(ug/L)	Station
1	0.08	MP-5 Bot
2	0.12	MP-5 Surf
3	0.25	MP-4 Bot
4	0.2	MP-4 Surf
5	0.11	MP-1 Bot
6	0.19	MP-1 Surf
7	0.3	MP-7 Bot
8	0.13	MP-7 Surf
9	0.1	MP-2 Bot
10	0.15	MP-2 Surf
11	0.1	MP-8 Bot
12	0.07	MP-8 Surf
13	0.07	MP-6 Bot
14	0.09	MP-6 Surf
15	3	MP-9 Bot
16	0.1	MP-9 Surf
17	0.13	MP-3 Bot
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
Phase 3 North future 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



NIH
National Institute of Environmental Health Sciences
Superfund Research Program

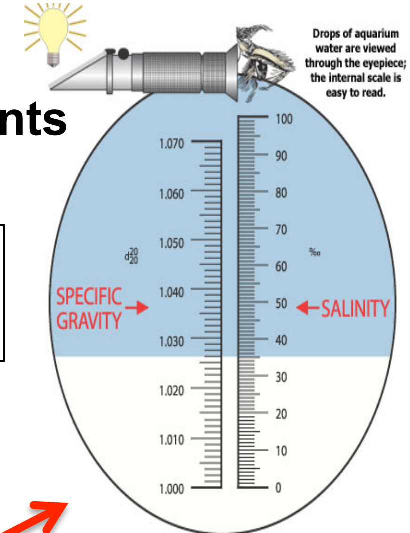
VIMS | William & Mary
Virginia Institute of Marine Science

PAH Transport within sediment : Methods

Drive-point Piezometer

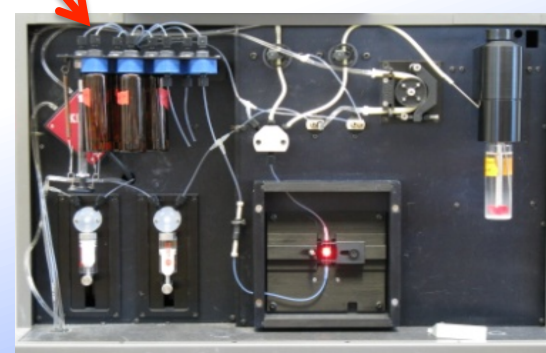
In-situ pore water measurements

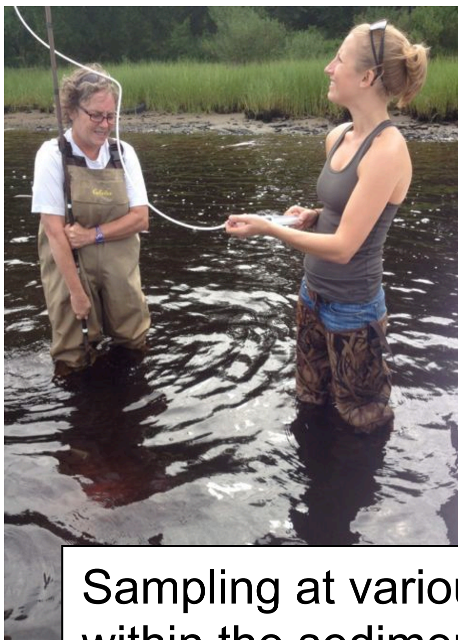
Small volume sample
0.45 µm filtered



Salinity by refractometer

Total PAH by biosensor



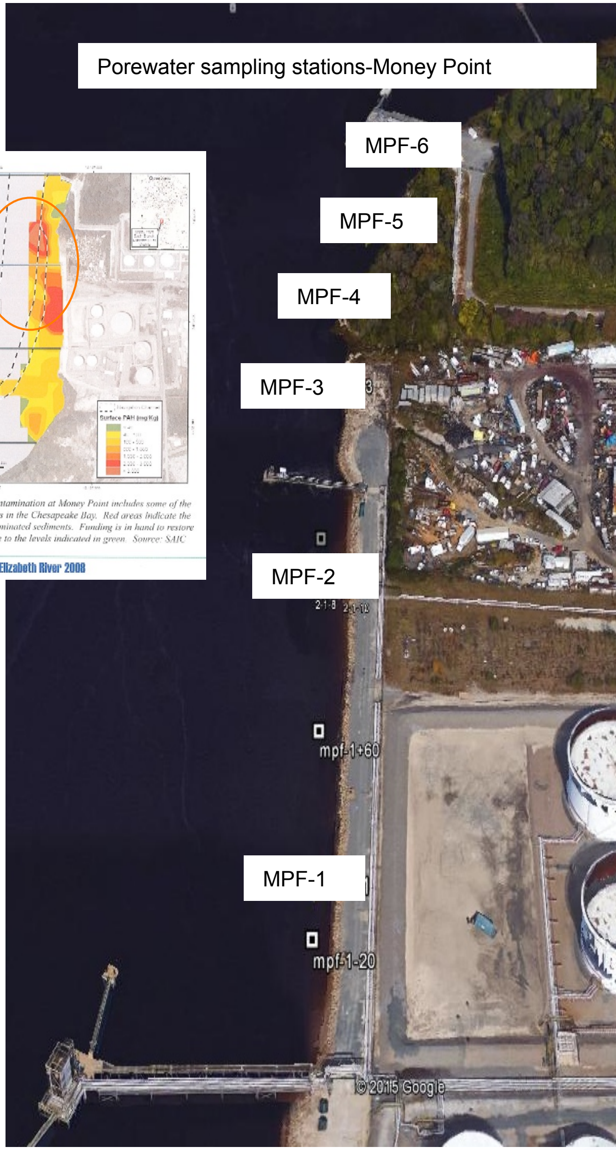


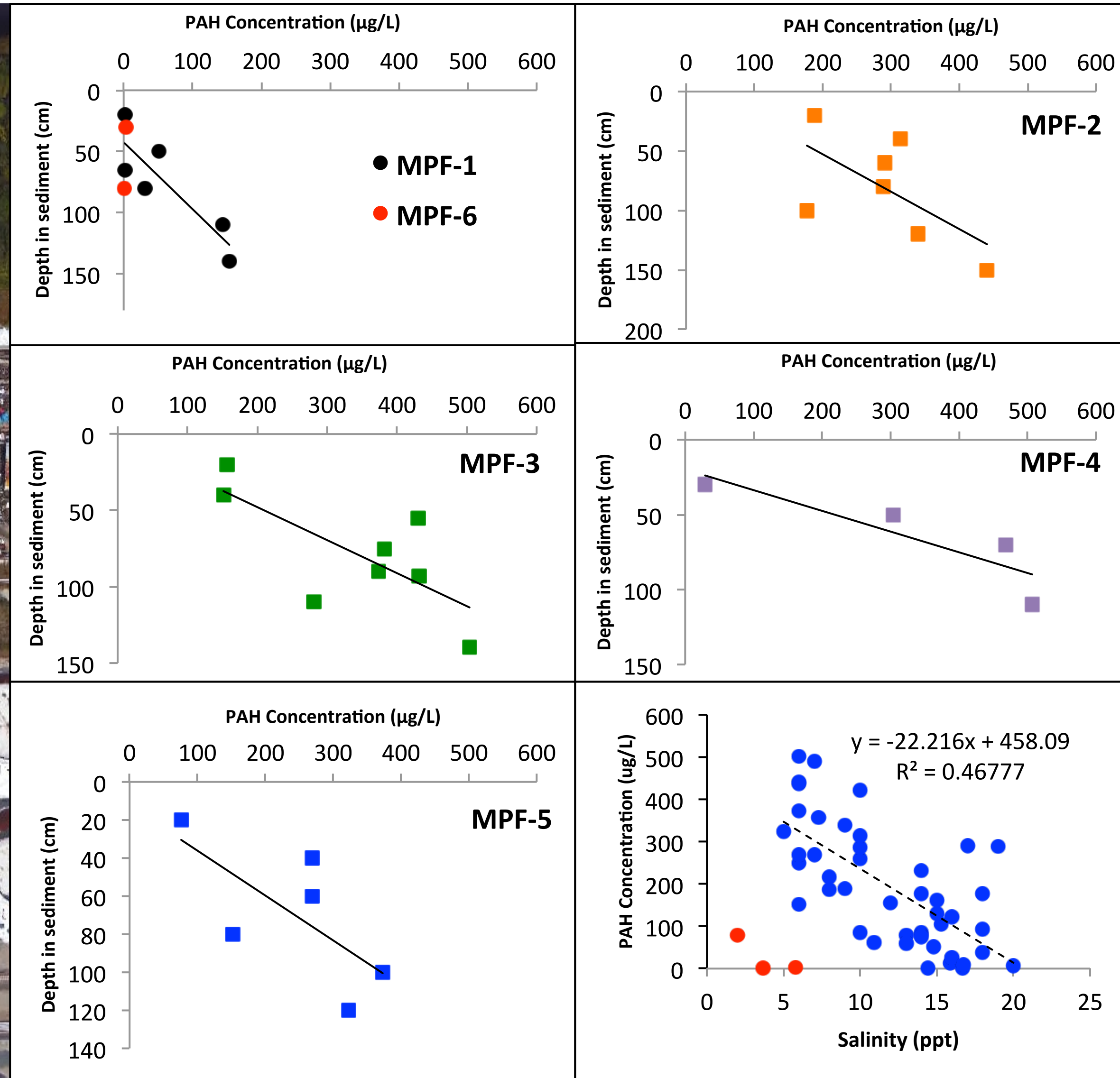
Sampling at various depths
within the sediment

NIH
National Institute of
Environmental Health Sciences
Superfund Research Program

VIMS | William & Mary
Virginia Institute of Marine Science

Pore Water PAH Concentration Profiles within the Sediment at Money Point





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

National Institute of Environmental Health Sciences
Superfund Research Program

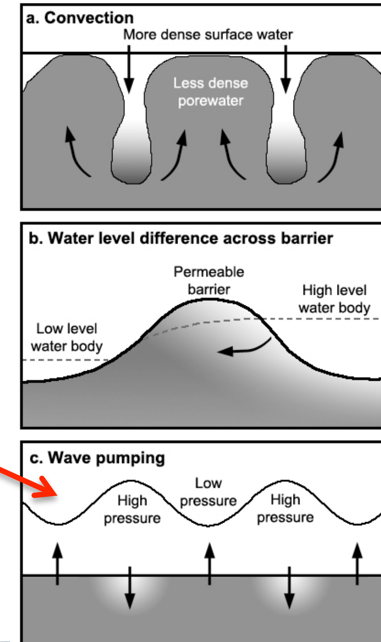
VIMS William & Mary
Virginia Institute of Marine Science

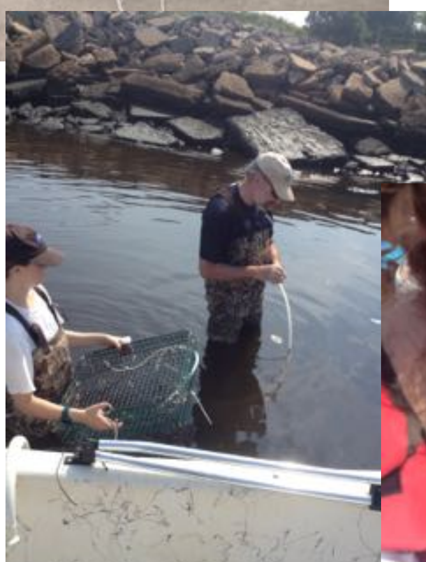
PAH transport from the sediments to the water column

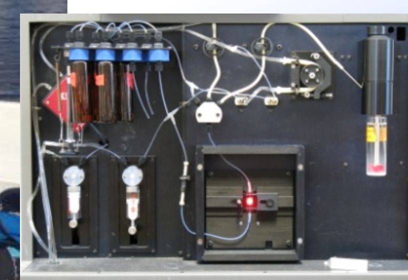
Seepage Meters
Hourly flow measurements
Analysis of salinity, PAH
Concentrations by biosensor

PAH flux may be more important than concentration when evaluating remediation strategies.

Do non-traditional groundwater transport mechanisms drive contaminant flux?





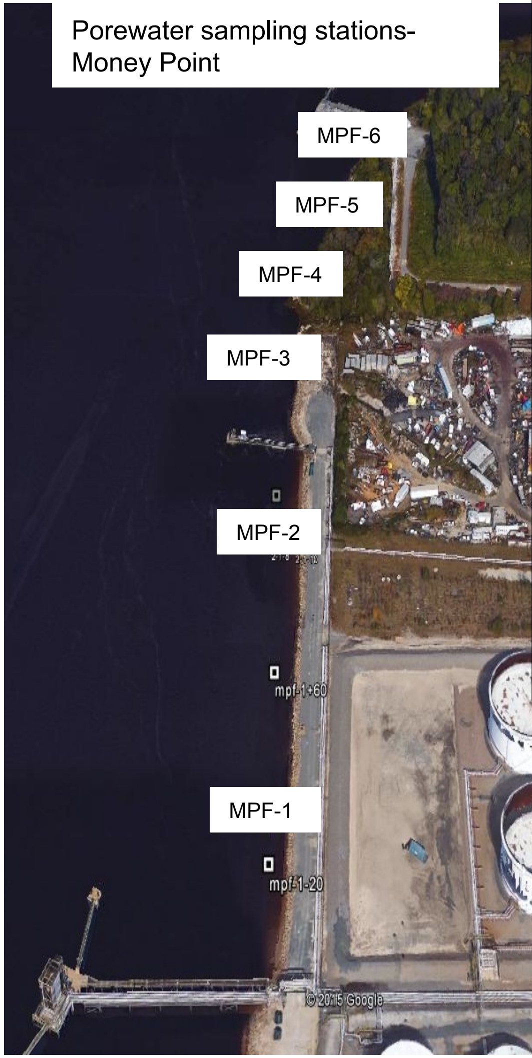


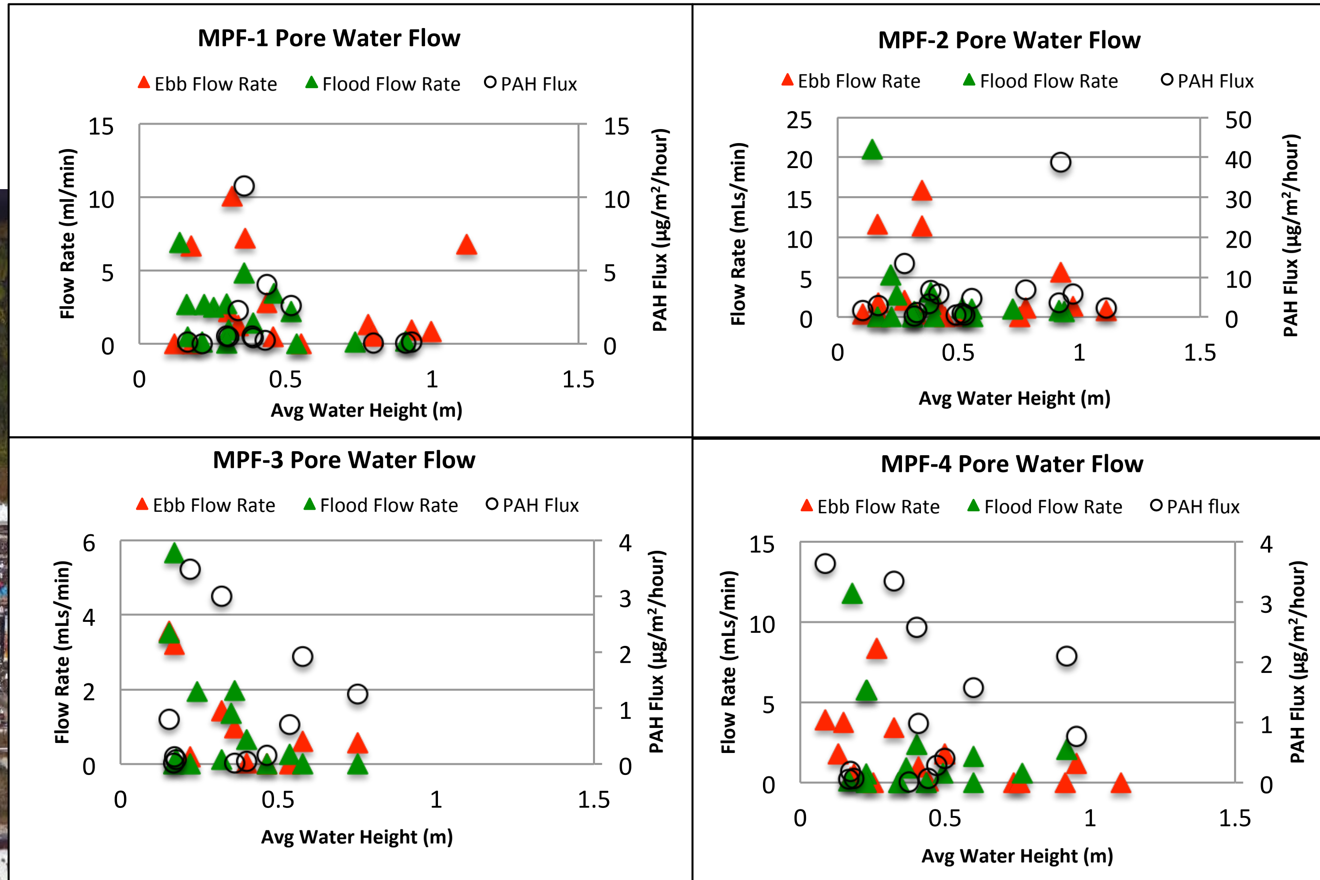
NIH
National Institute of Environmental Health Sciences
Superfund Research Program

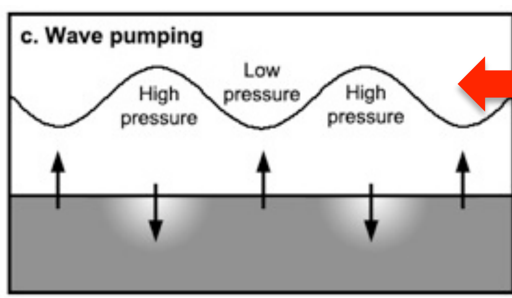
VIMS | William & Mary
Virginia Institute of Marine Science

PAH Flux

Transport to the water column: seepage meter data







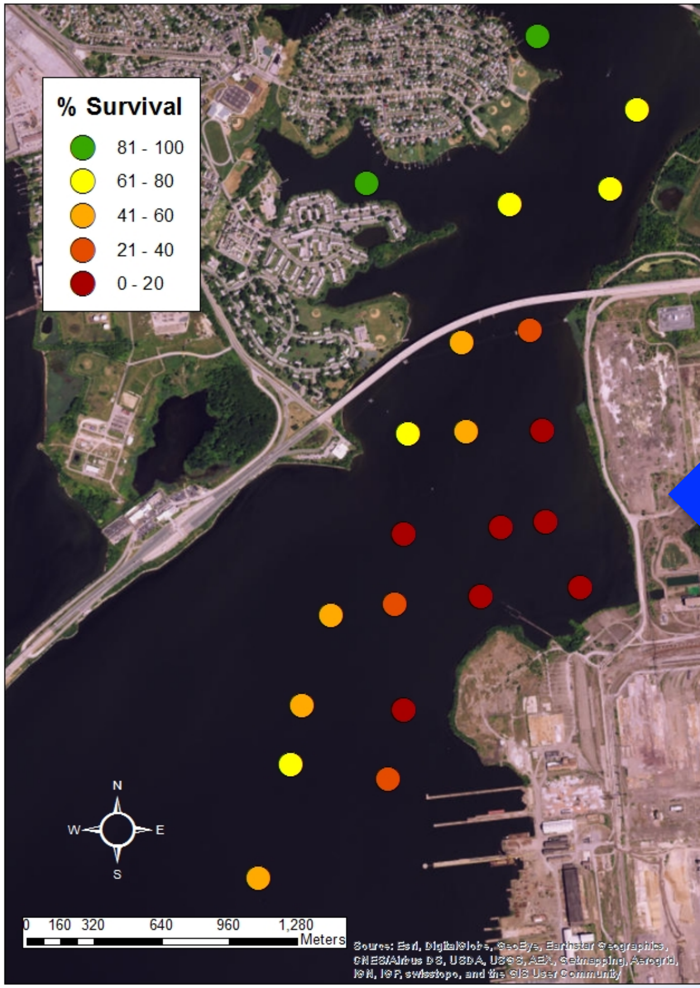
Tidal cycle driven advection within the sediment

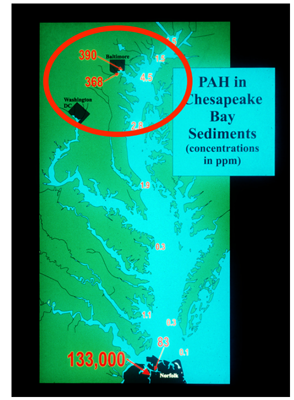
National Institute of Environmental Health Sciences
Superfund Research Program

VIMS William & Mary
Virginia Institute of Marine Science

Pore Water Toxicity Evaluation

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





Baltimore Harbor, MD

Baltimore Harbor sediments
Highly industrialized
Metals and organic contaminants

Previous research has shown toxicity in surface sediments near Sparrow's Point

Are the PAH responsible for the toxicity?
Can we predict toxicity with the Biosensor measurements?

National Institute of Environmental Health Sciences
Superfund Research Program

VIMS | William & Mary
Virginia Institute of Marine Science

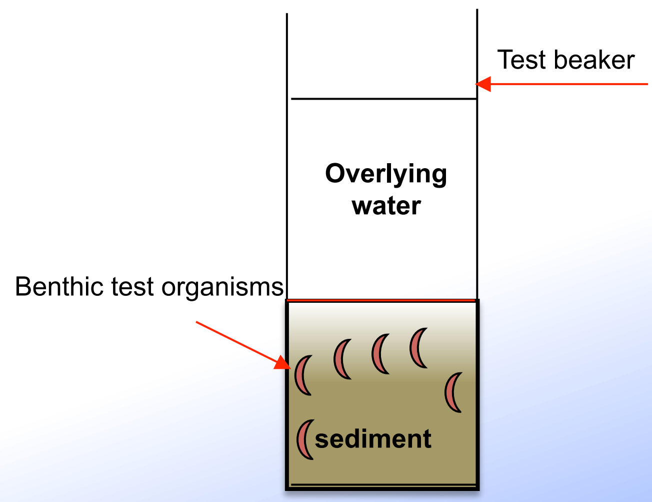
Sediment Test Methods

Test species – Estuarine infaunal amphipod *Leptocheirus plumulosus* cultured in the laboratory

Acute 10-d test - Whole sediment collected from field

PAH concentrations in pore water measured by Biosensor

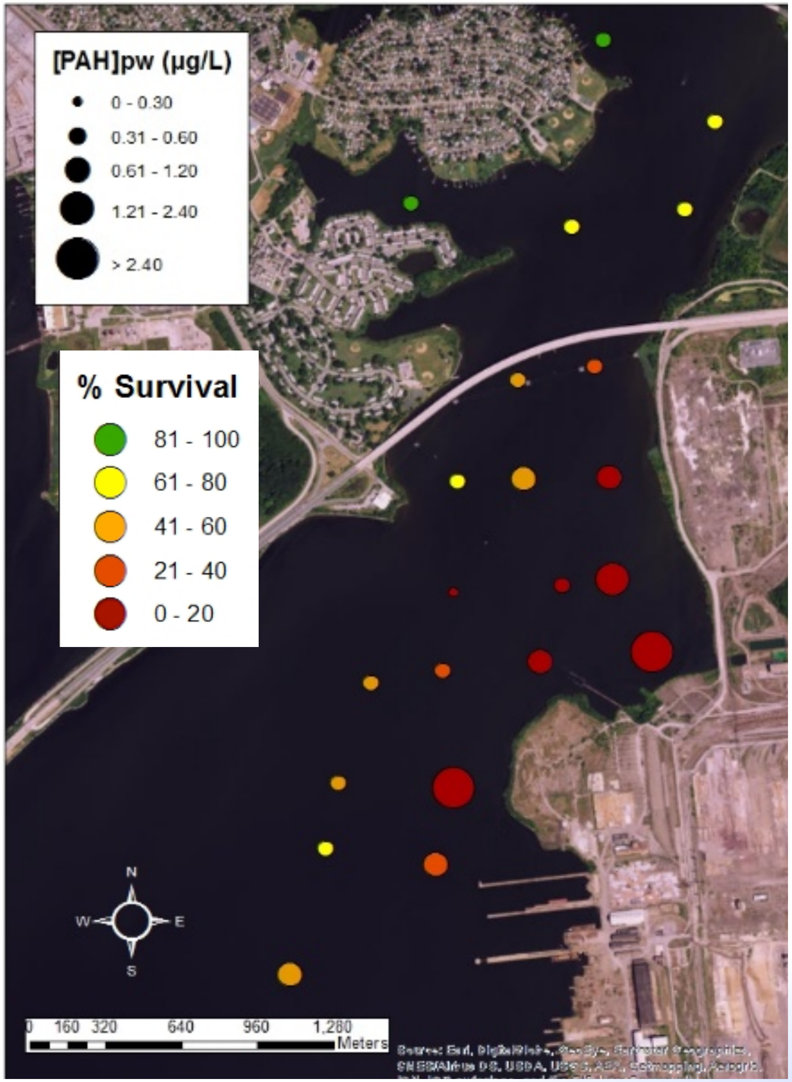
- Temperature - 25°C, Static
- 16h light: 8h dark light cycle
- Overlying water – Filtered River water adjusted to 10‰
- I L glass beakers
- Amphipod size at start - 2.0 - 4.0 mm, sub-adults
- 5 replicates/site and 20 organisms per replicate
- No Feeding
- Endpoint - Survival



NIH
National Institute of Environmental Health Sciences
Superfund Research Program

VIMS | William & Mary
Virginia Institute of Marine Science

Spatial Contamination Patterns



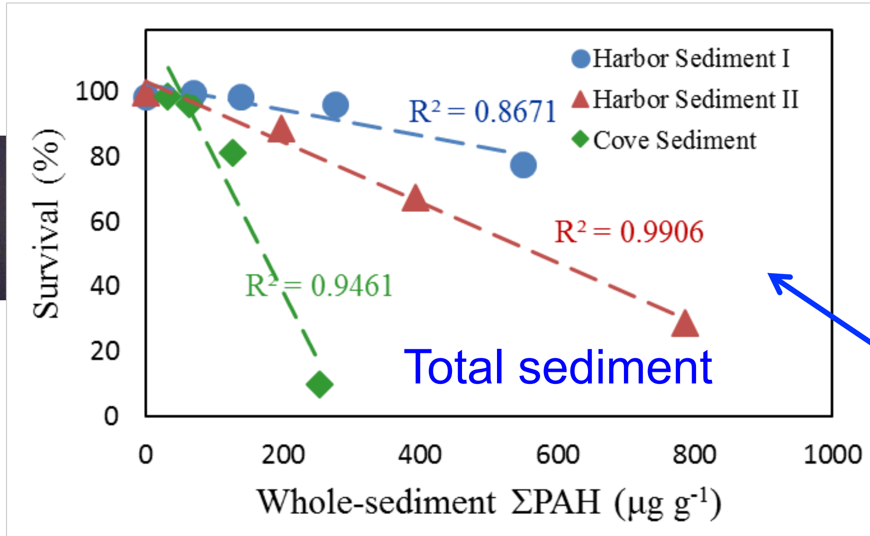
- PAHs in porewater and sediment were strongly correlated with toxicity
- So were
  - Nickel: R = 0.53, p = 0.01
  - Chromium: R = 0.58, p = 0.003
  - TPH (Total Petroleum Hydrocarbon): R=0.64, p = 0.001
- Agrees with previous studies that found multiple contaminants and no clear causation (Klosterhaus et al., 2006; McGee et al., 1999, 2004)

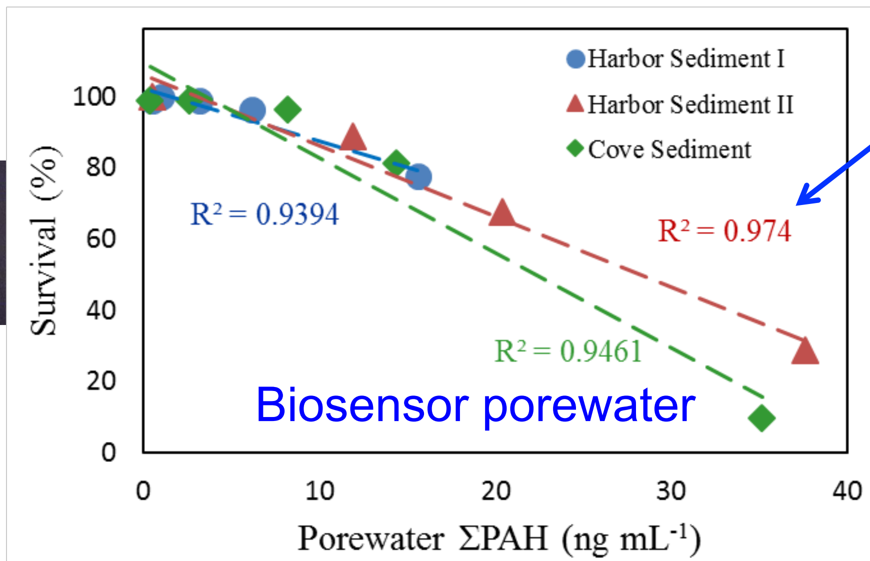
Are PAH contributing to the toxicity?
Spiking experiment-Add the PAH alone

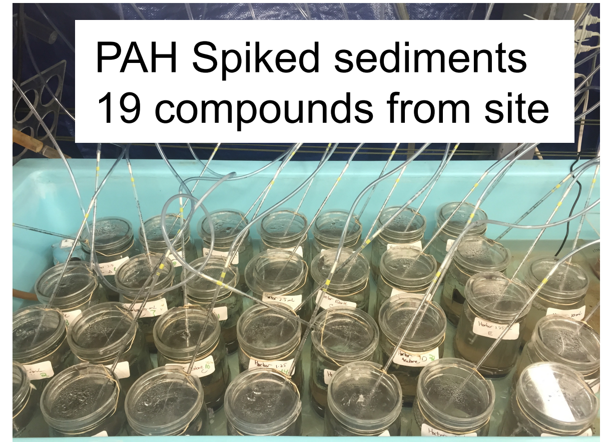
NIH National Institute of Environmental Health Sciences
Superfund Research Program

VIMS William & Mary
Virginia Institute of Marine Science

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

1) Porewater analysis can be used to identify toxicity in field sediments rapidly

2) Baltimore harbor toxicity from PAH + other contaminants

National Institute of Environmental Health Sciences
Superfund Research Program

William & Mary
Virginia Institute of Marine Science

Summary

- **Total PAH concentrations (3-5 ring) in minutes from small volume samples allows 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 sources and transport mechanisms**
- **Direct measurement of contaminant flux at sediment/water interface**
- **Prioritize samples for compound specific GC-MS based on total PAH (don't pay for non-detects!)**
- **Measurement of the bioavailable or toxic fraction in sediments is possible in near real-time**

National Institute of Environmental Health Sciences
Superfund Research Program

VIMS | William & Mary
Virginia Institute of Marine Science

Future Biosensor work

- Advection mechanisms driving PAH transport in sediments- continuing NIEHS-SRP work that is helping to guide remediation efforts
- Biosensor detection of oil-derived PAH to trace oil spill fate and toxicity- Summer 2017
- Flux measurements: evaluating biosensor measurements vs. passive sampling and Ra/Th measurements- Summer 2017
- Biosensor measurements vs. new toxicological endpoints, cardiac abnormalities, inflammation
- Biosensor hardware development, smaller, more portable - Sapidyne Instruments-ongoing
- New antibodies for PCBs, HABs or ???

Acknowledgements

NIEHS-SRP Grant #R01ES024245

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

PIs: Michael Unger, Aaron Beck, Collaborator/RTC: Josef Rieger, The Elizabeth River Project, Portsmouth, VA

Steve Kaattari, Mary Ann Vogelbein, George Vadas, Kristen Prossner, Aaron Beck, Michele Cochran, Xin Li, Jonathan Ricks, Ellen Harvey, Matt Mainor

Joe Rieger, Dave Koubsky

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

Terrance Lackey

Steve Kaattari

Questions?

Relevant PAH Biosensor Publications

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.

Spier, C. S., M. A. Unger and S. L. Kaattari. 2012. Antibody-Based Biosensors for Small Environmental Pollutants: Focusing on PAHs *In: Biosensors and Environmental Health.* (Eds. Preedy, V.R. and Patel, V.B.). CRC Press, Boca Raton, FL. P. 273-295.

Spier, C. S., G. G. Vadas, S. L. Kaattari and M. A. Unger. 2011. Near-real-time, on-site, quantitative analysis of PAHs in the aqueous environment using an antibody-based biosensor. *Environ. Toxicol. Chem.* Vol. 30, No.7, pp. 1557-1563.