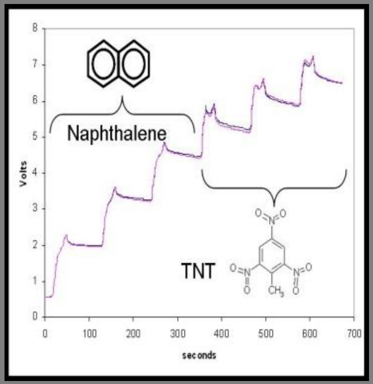
"Biosensors and bioaccumulation: using antibody-based sensor technology to guide PAH remediation strategies "

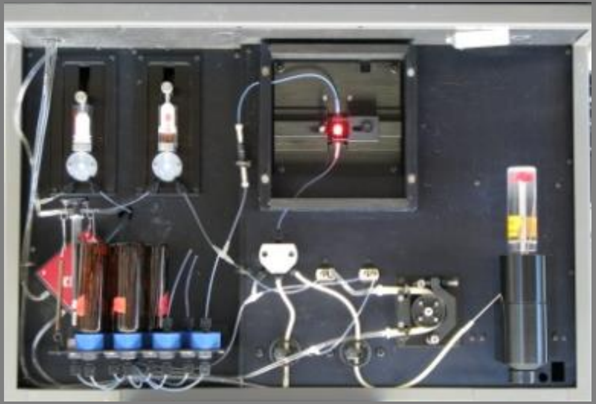
Michael Unger, Associate Professor of Marine Science
Virginia Institute of Marine Science, College of William & Mary,
Gloucester Point, VA
VIMS-Industry Partnership May 23, 2014

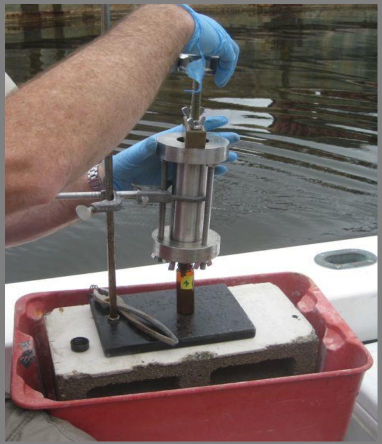


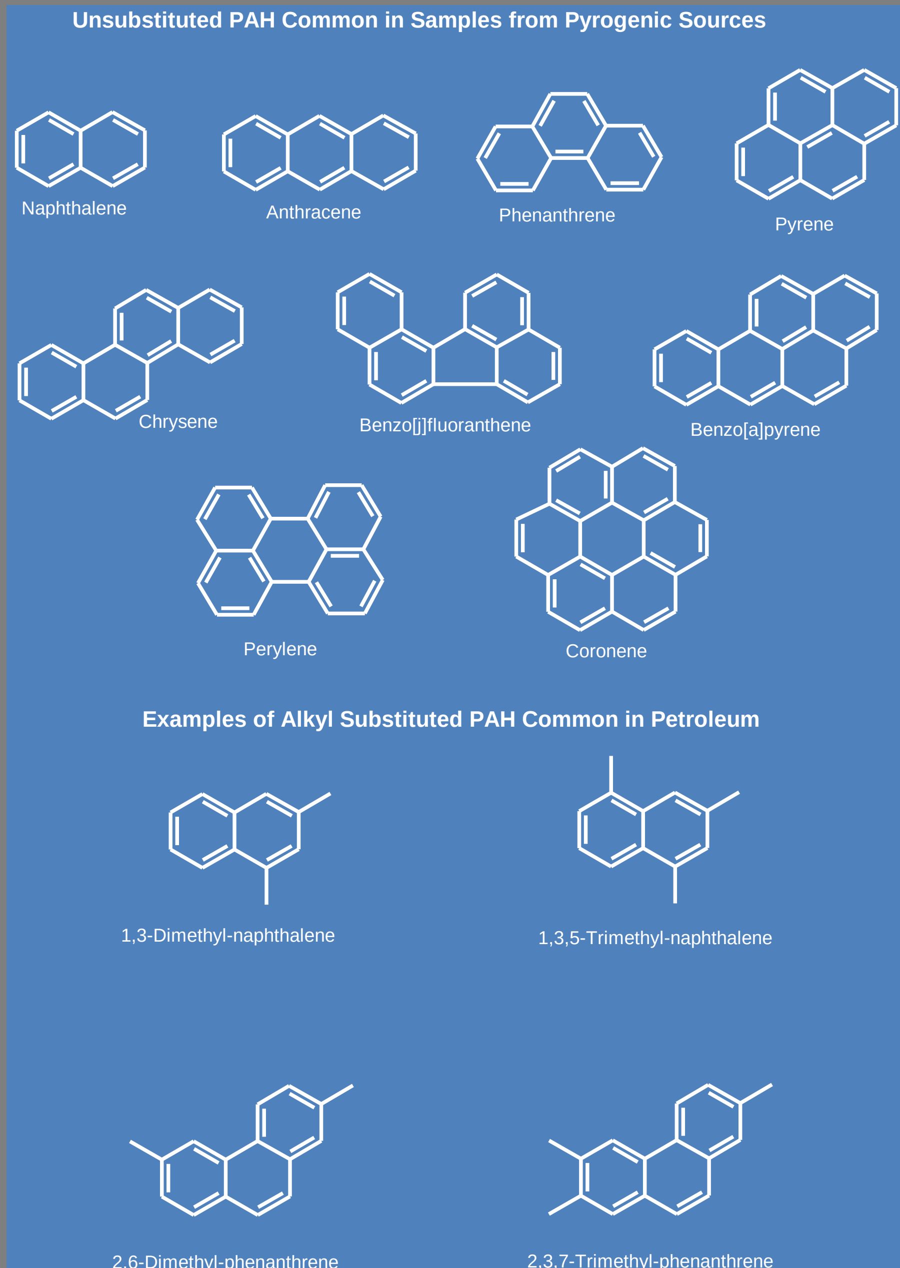
“Biosensors and bioaccumulation: using antibody-based sensor technology to guide PAH remediation strategies ”

- Polycyclic Aromatic Hydrocarbons (PAH)
- The Elizabeth River & remediation plans
- The VIMS biosensor- an overview of development and function
- NIEHS-SRP research- A real-time antibody-based field assay to predict contaminant bioavailability in sediments
    - Results: 2012-2013
- Future research & new areas for collaborations









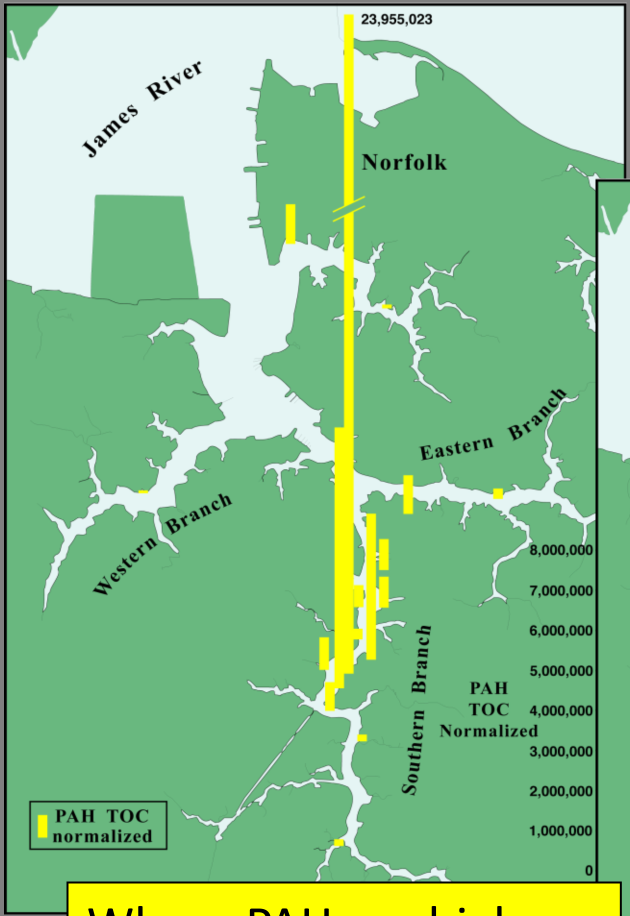
POLYCYCLIC
AROMATIC
HYDROCARBONS
"PAH"

Formed by the Incomplete Combustion of Organic Matter

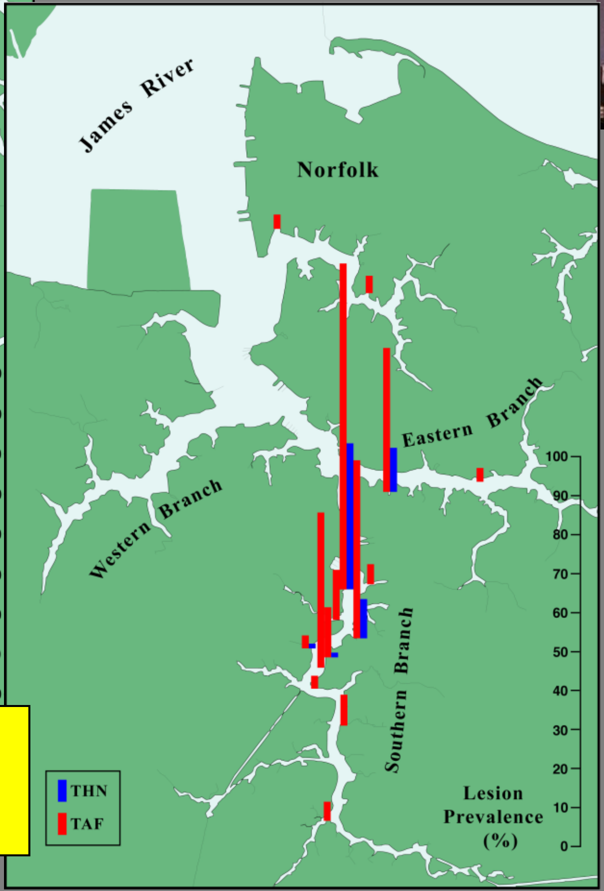
Can be Natural or Anthropogenic (oil & creosote)

Toxic and Carcinogenic

PAH induced liver lesions and cancer in *Fundulus heteroclitus*
Ecological impacts



Elizabeth River

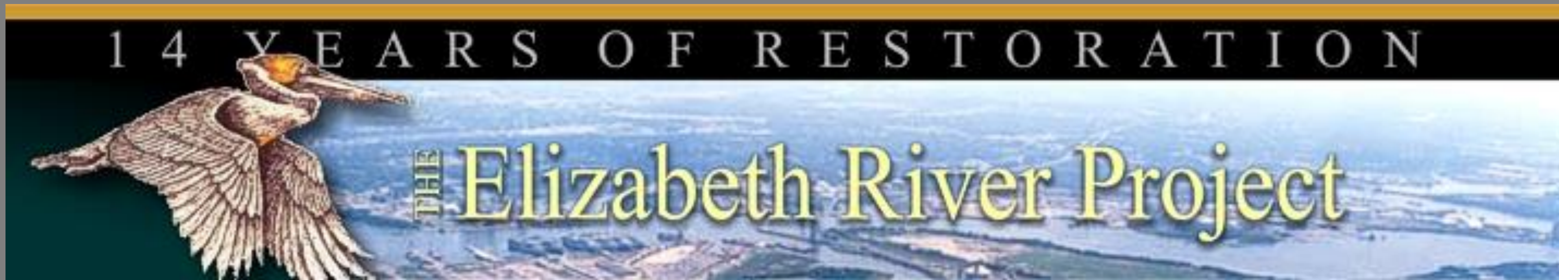


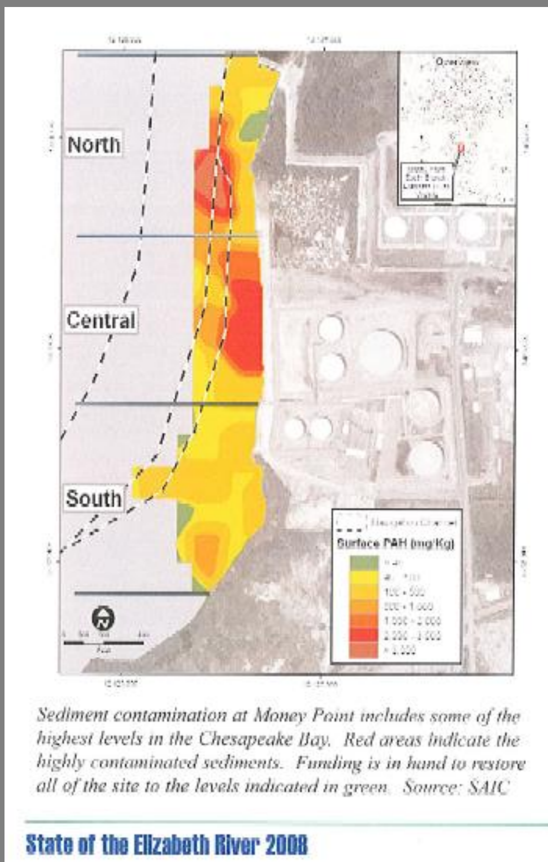
Where PAH are high
Tumors are high

Normal liver

Precancerous lesions

Liver cancer





PAH in sediment

ERP Action plan: THE GOO MUST GO!
Make the Mummichog well again!

Money Point: ERP site targeted for sediment remediation

First voluntary sediment remediation project in Virginia

What are the potential human health risks From PAH contaminated sediments?

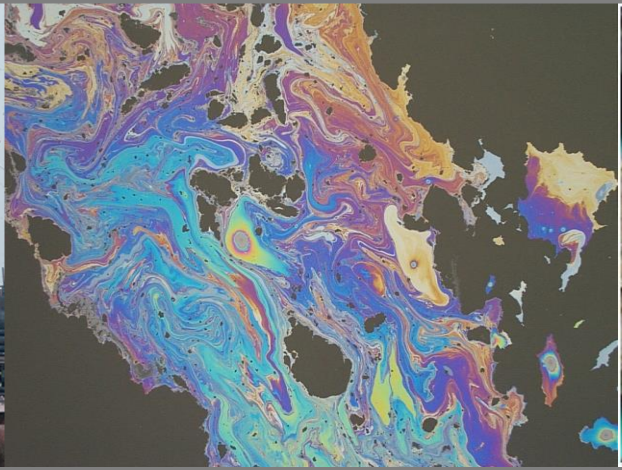
A real-time antibody-based field assay to predict contaminant bioavailability in sediments

Michael Unger, Stephen Kaattari, Wolfgang Vogelbein
Virginia Institute of Marine Science, Gloucester Point, VA
Josef Rieger, The Elizabeth River Project, Portsmouth, VA
NIEHS-Superfund Research Program: 3 year NIEHS-SRP grant (2012-2014) 1R01ES020949

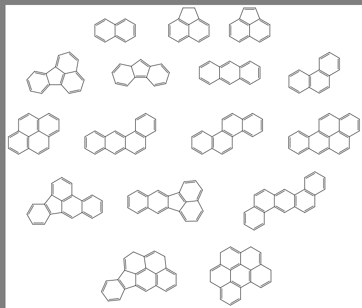
Contaminated sediments → Innovative technology → predict human health risk

OVERALL OBJECTIVE: For this NIEHS-SRP project we will evaluate, refine and validate a quantitative, monoclonal antibody (mAb)-based sensor to measure polycyclic aromatic hydrocarbons (PAH) in sediment-associated water as a rapid predictor of PAH bioaccumulation in the oyster. Can we help managers achieve remediation goals?

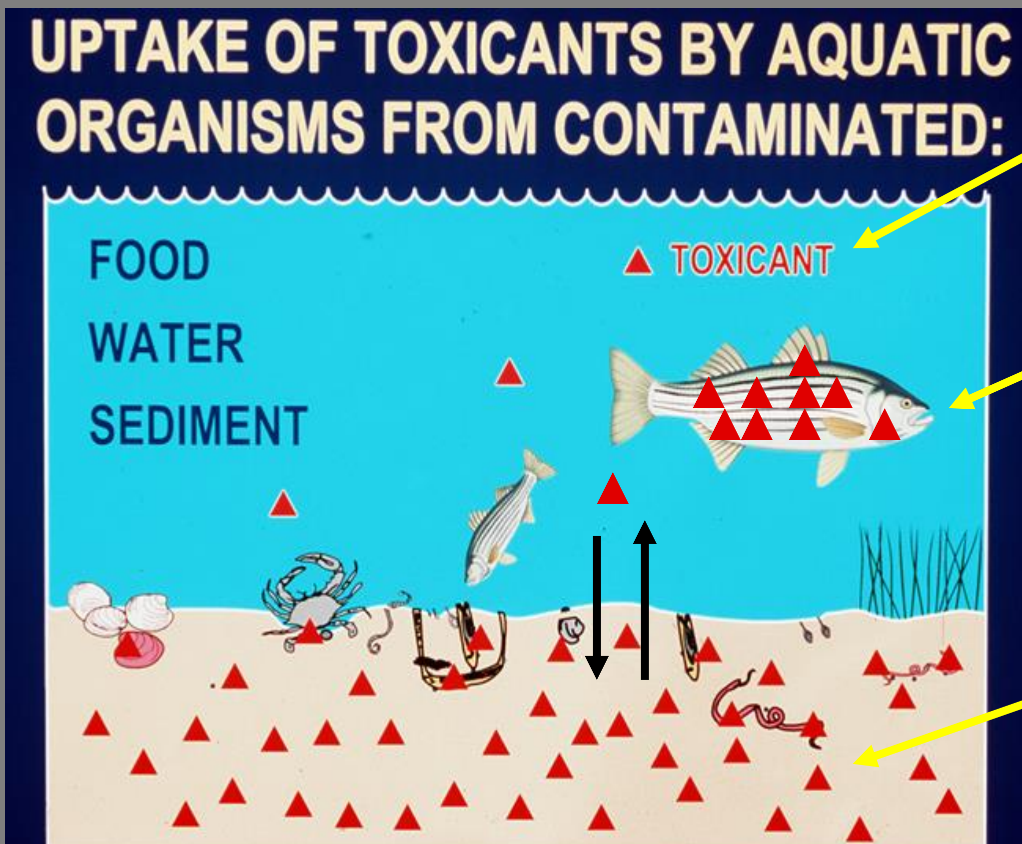
ERP-Swimmable & fishable long-term goals


Background: Bioavailability is governed by partitioning



Polycyclic Aromatic Hydrocarbons (PAH)
Potentially toxic and carcinogenic
Common target of Superfund cleanup (historical contaminants)
Oysters are potential vector for human exposure
Do not metabolize PAH



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

Can we predict how much PAH will accumulate in biota from contaminated sediments?

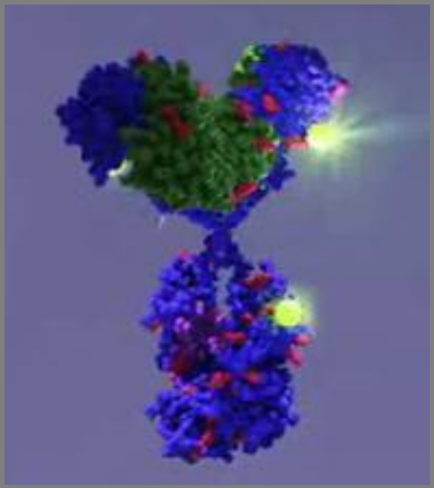
Our Approach

Bio

Monoclonal Antibodies against Contaminants

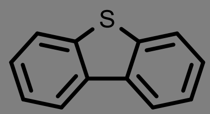
Sensor

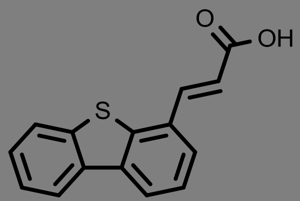
Sensor Evaluation and Development



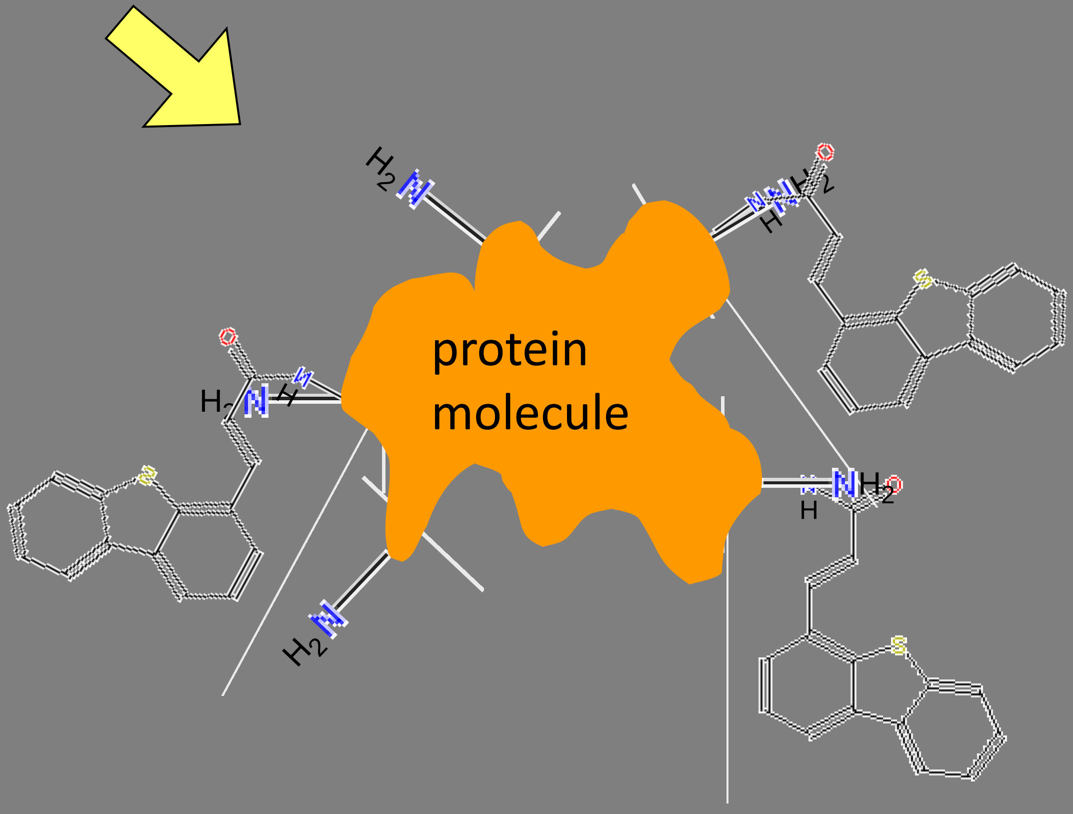
Sapidyne
Instruments Inc.
Boise, Idaho

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



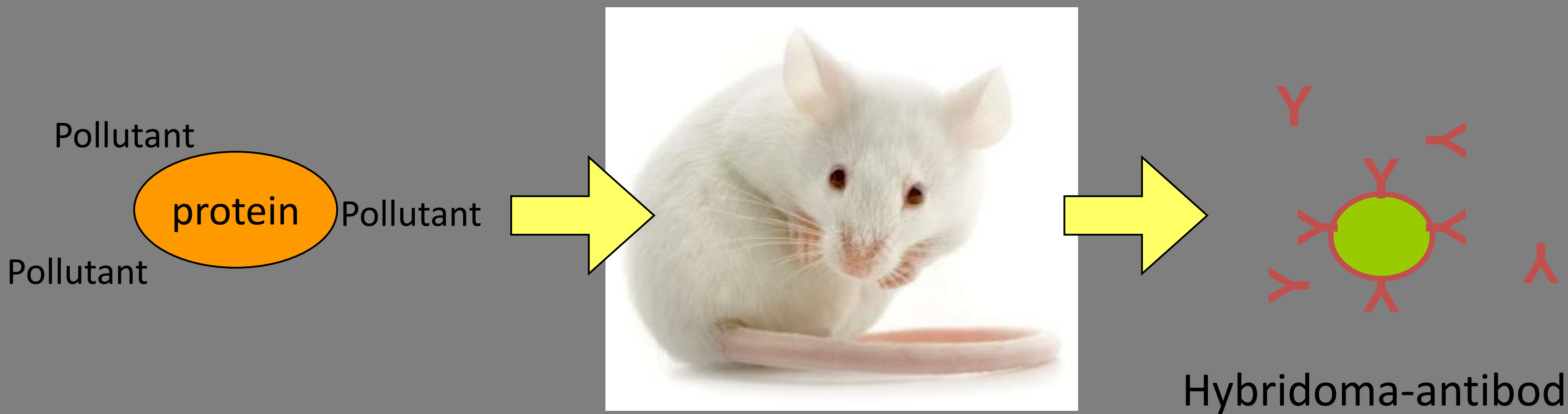


↑ not immunogenic



immunogenic →

How to make antibodies to pollutants?

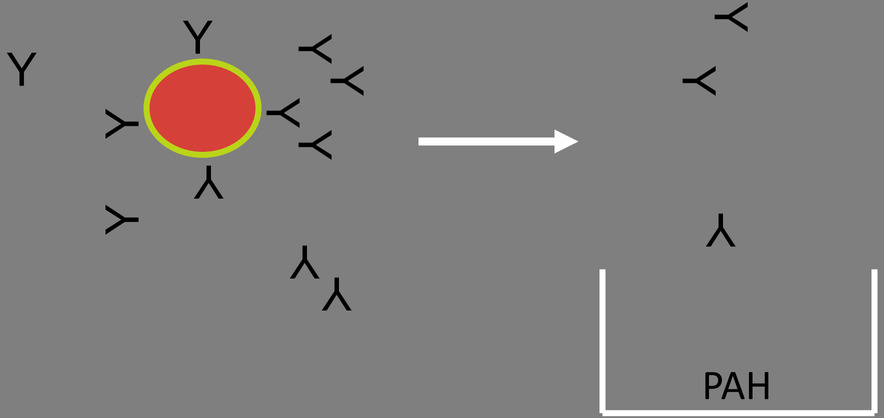


Hybridoma-antibody producing cells

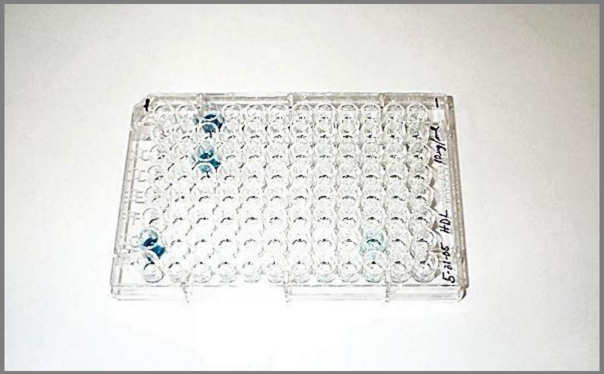
Provides an endless supply of antibodies in cell culture

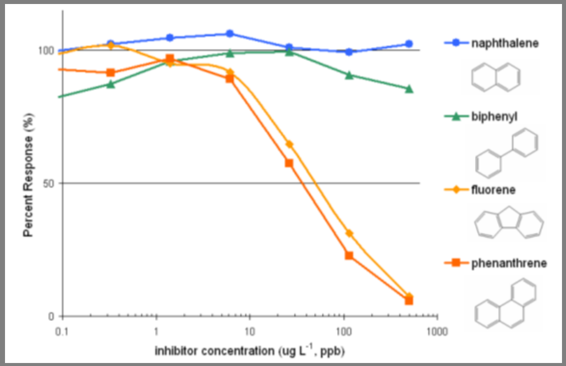
Screening for Anti-PAH Antibodies

Important step for determining the selectivity of the detector



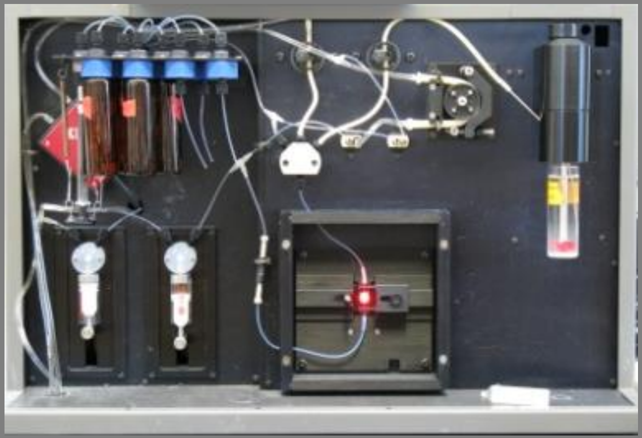
Screen for anti-PAH activity





Our research is showing that this screening process may be more important than hapten development for PAH antibody selection due to the lack of characteristic substitutions (i.e. –OH) to the aromatic ring structure of PAH (Steve Kaattari-future seminar)

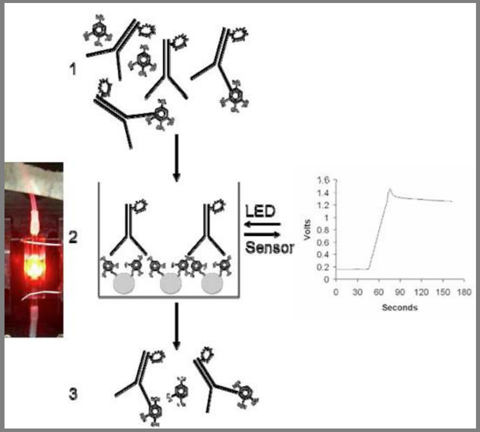
Background: Antibody biosensor technology



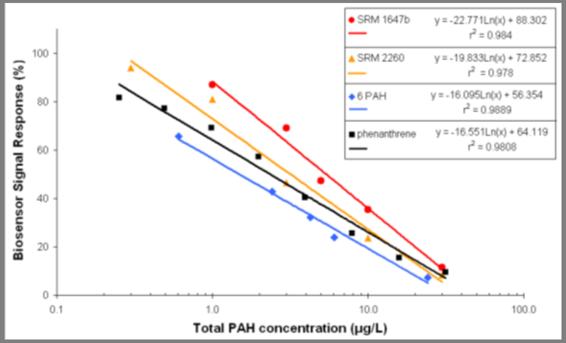
Sāpidyne Instruments Inc.

Features:

- Reagent reservoirs
- Automated sample handling
- Precise fluidics for transferring small volumes accurately
- Quantitative/sensitive fluorescence detection with antibody "specificity"



KinExA Inline Sensor
Fluorescence detection, rapid (minutes), small sample volume (1mL for 0.1 ppb)



PAH selective antibody (Spier et al, 2009, Anal. Biochem.)
Sensitive (sub-ppb in mls of sample) and precise

What about accuracy? Antibodies can be selected for various targets (ie. 3-5 rings)

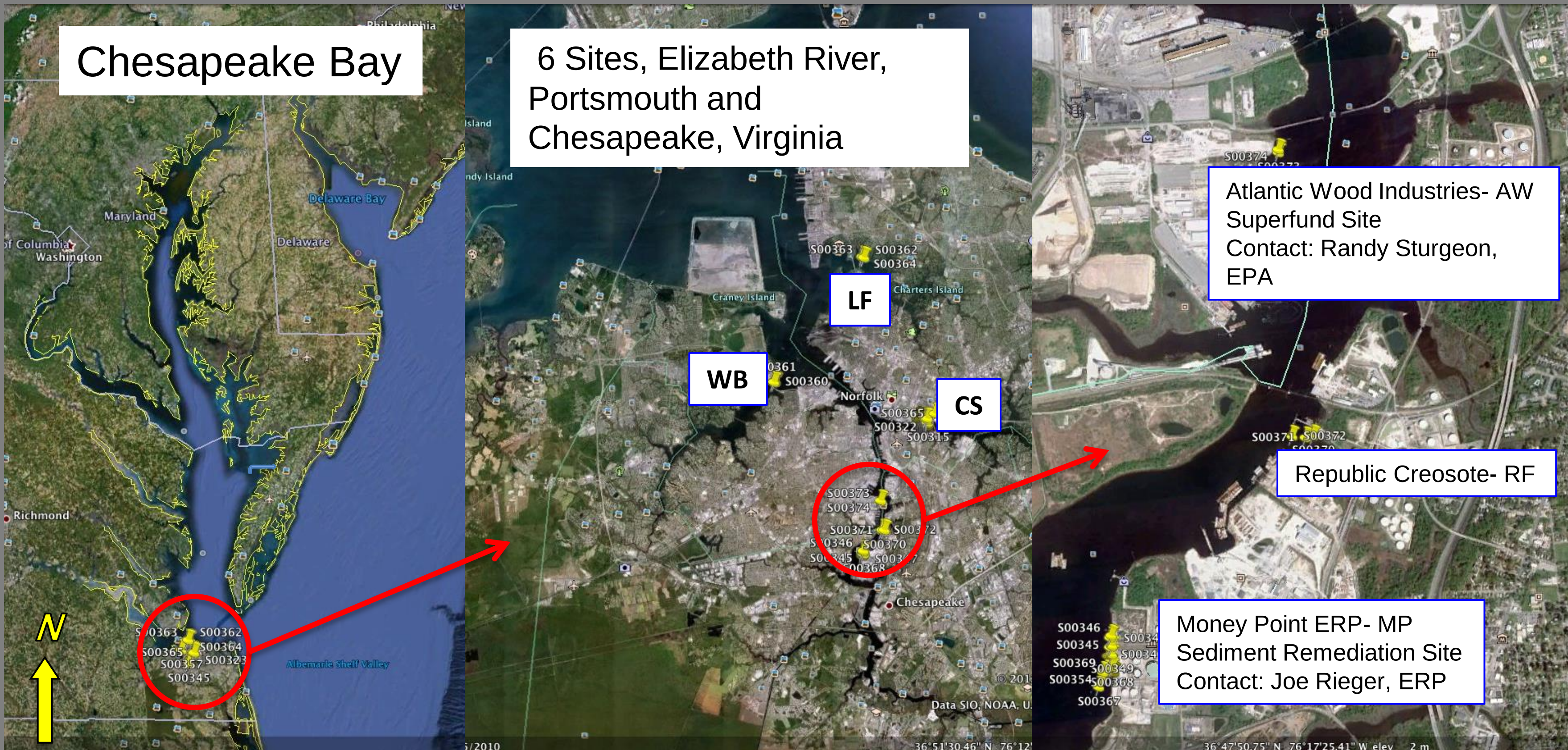
Background: Three aims of our 3 year project

Aim 1. Conduct on-site PAH (polycyclic aromatic hydrocarbons) measurements of water using a rapid, cost-effective real-time biosensor and test its ability to predict tissue burdens in oysters from PAH-contaminated environments. *Hypothesis: Real-time biosensor estimates of PAH concentration in aqueous samples (sediment pore water, surface water) rapidly and specifically predict lipid-normalized PAH concentrations in the tissues of native oysters inhabiting PAH-contaminated sites*.
Field study

Aim 2. Conduct controlled laboratory dosing of oysters to validate the biosensor as an effective predictor of oyster tissue burdens as a strict function of dose (concentration, time). *Hypothesis: Biosensor measurements of aqueous PAH concentrations are specific, dose-responsive, correlate directly with tissue concentrations of PAH in dosed oysters and are therefore predictive surrogates of tissue bioaccumulation.*
Laboratory study

Aim 3. Extend current Biosensor capabilities via development of a bi- or multi-analyte biosensor that permits simultaneous monitoring of different PAH classes within a single site. *Hypothesis: Incorporation of mixed analyte beds with differentative antibody specificities for different PAH classes will provide for more accurate discernment of the relative contribution of these different PAHs in the field and laboratory.*
Antibody Development

Methods. 2012 Study Location: 6 sites, wide range of PAH contamination

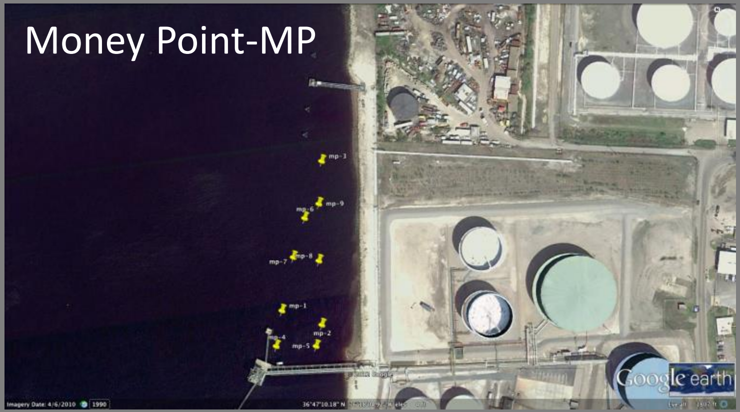


2012

- 2 Reference sites: WB, LF
- 1 Superfund creosote clean-up site: AW
- 3 other creosote contaminated sites: CS, RS, MP
- 2 active sediment remediation sites: AW, MP

Methods: Water, pore water, sediments and oysters in the E. River

Six sites – 9 stations/site for water and pore water (approx. 50 m apart)
Biosensor: water column top and bottom -18 samples/site
porewater-9 samples each site
GC-MS on 3/9 stations per site (1L split samples)
water surface and bottom- 6 samples
pore water-3 samples (100 ml)
sediment-3 composite grab samples/3 stations
oysters July-August 2012 (28 days)
48 individuals/1 station + natives

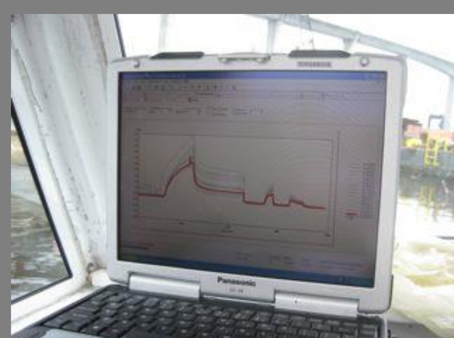


Methods: Pore water sampling and analysis

Real-time analysis required new sampling techniques for the field.

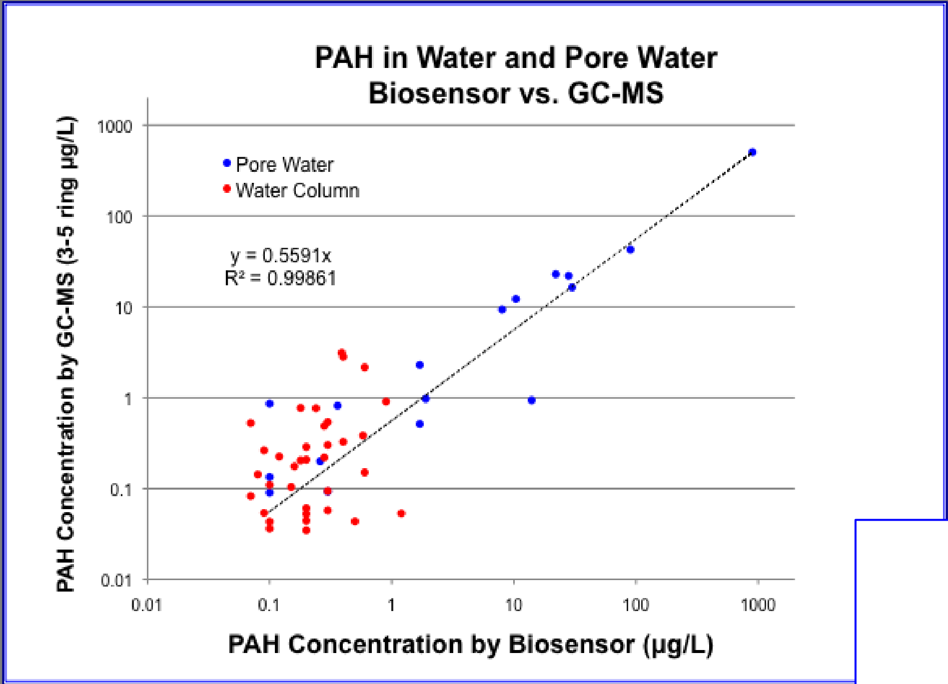
Dissolved phase (0.47 µm) pore water samples were required in minutes for analysis.

Each study site, 9 stations, 27 samples can be surveyed in 1 day



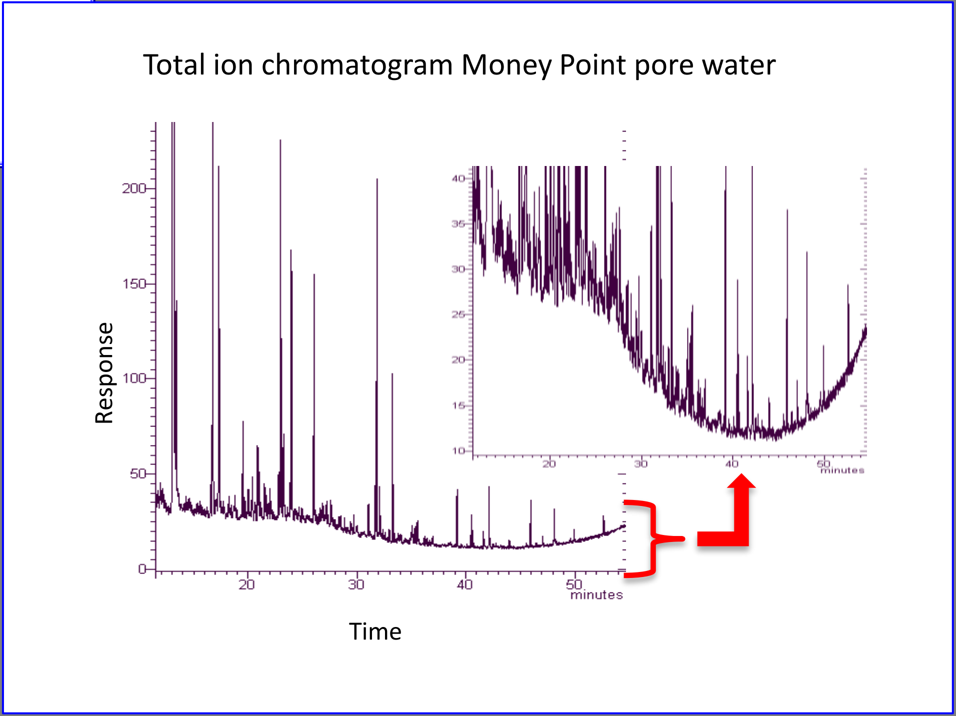
Samples were analyzed on board by biosensor and larger volume samples brought back to the lab for GC-MS

Results- Water Analyses by Biosensor and GC-MS

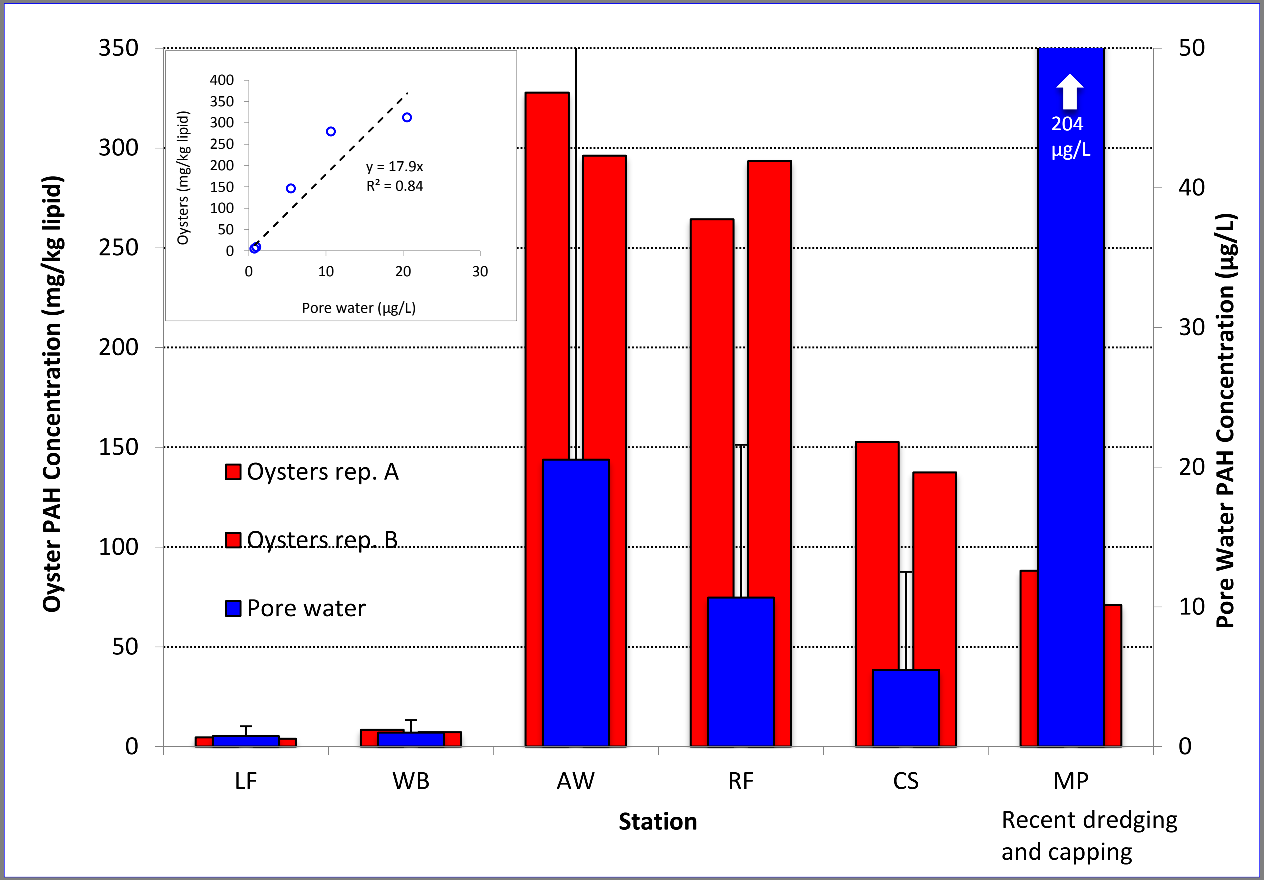


- Total PAH ranged from <0.1-1000 µg/L in pore water samples
- Water column below 1 µg/L
- Good correlation btw biosensor and GC-MS for split pore water samples

- Biosensor 1.8X reported GC-MS ΣPAH
- GC-MS typically Σ20-40 compounds based on list and detection limit
- Hundreds of compounds present and summed by biosensor antibody



Results 2012- PAH Accumulation in Oysters after 28 days



- ΣPAH in oysters ranged from 3 – 300 mg/kg lipid
- •Trend between mean (n=9) pore water [PAH] and oyster tissue [PAH] at five historically contaminated sites
- •Pore water [PAH] are variable on small (cm-100m) and large (km) scales
- •High variability in pore water at some sites (AW, MP)
- •Site of recent dredging and capping has elevated pore water concentrations but not corresponding increased PAH accumulation in oysters

•[PAH] in oysters are highly correlated with pore water concentrations at historically contaminated sites
•Newly remediated (dredged and capped) site has high pore water [PAH] relative to sediment and oysters

•Are transplants representative of bioaccumulation at sites?
•PAH toxicity (narcosis?) → decrease in bioaccumulation by oysters?
•Coarse capping material & DNAPL, local non-equilibrium conditions or spatial variability/sampling issues?

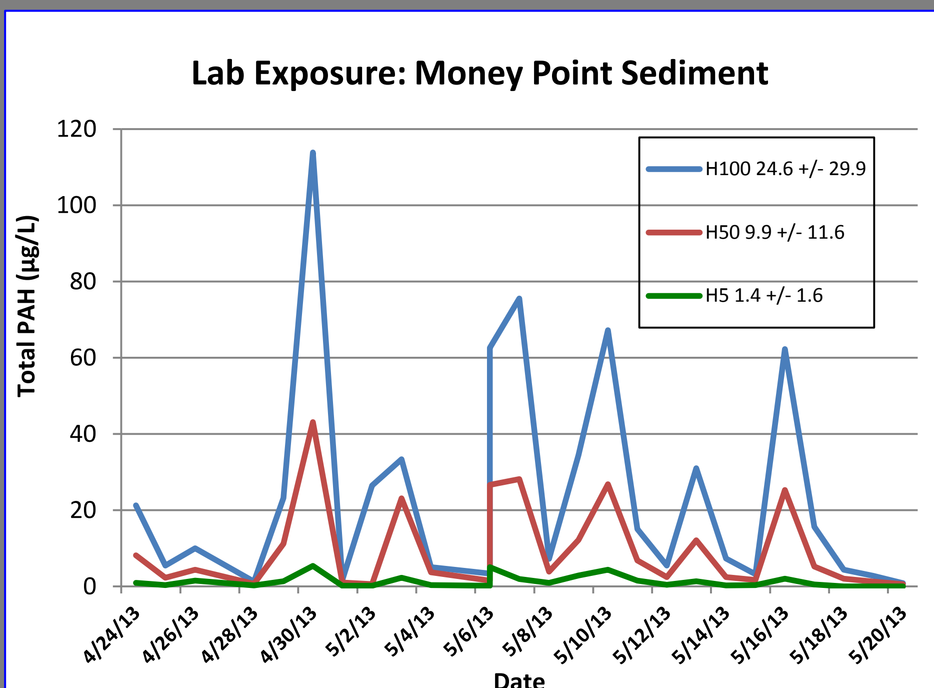
•PAH toxicity (narcosis) = decrease in bioaccumulation by oysters?

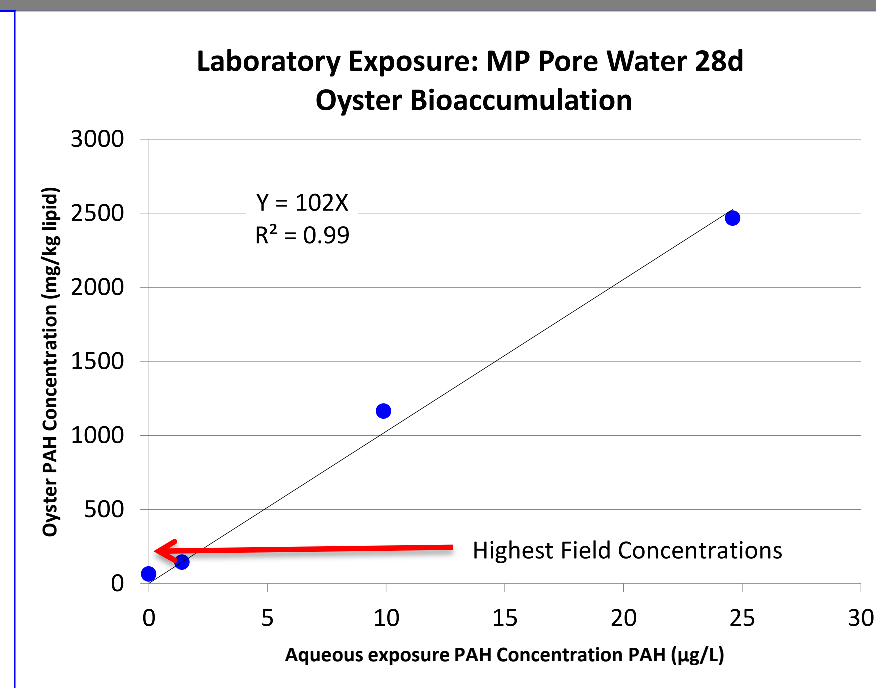
Aim 2. Conduct controlled laboratory dosing of oysters to validate the biosensor as an effective predictor of oyster tissue burdens as a strict function of dose (concentration, time). *Hypothesis: Biosensor measurements of aqueous PAH concentrations are specific, dose-responsive, correlate directly with tissue concentrations of PAH in dosed oysters and are therefore predictive surrogates of tissue bioaccumulation.*

PAH Accumulation in Oysters after 28 day Lab Exposure- Dose Response

Natural sediment from Money Point in generator columns

Diluter system to provide range of PAH concentrations





•Coarse capping material but DNAPL, local non-equilibrium conditions or spatial variability?

Methods 2013. Study Location: Money Point 6 sites, wide range of PAH contamination

Aim 1. Conduct on-site PAH (polycyclic aromatic hydrocarbons) measurements of water using a rapid, cost-effective real-time biosensor and test its ability to predict tissue burdens in oysters from PAH-contaminated environments. *Hypothesis: Real-time biosensor estimates of PAH concentration in aqueous samples (sediment pore water, surface water) rapidly and specifically predict lipid-normalized PAH concentrations in the tissues of native oysters inhabiting PAH-contaminated sites*.

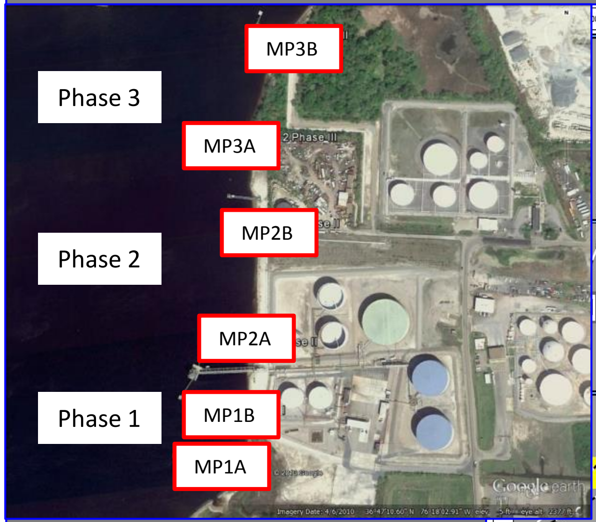
June-July 2013 (≈1 km) Money Point

Six sites for oysters-28d deployment
9 pore water stations/site-54 total
3 composite transects/site

Evaluate:

Spatial variability on various scales, pore water and oysters

Pre and post remediated sites
Phase 1, dredged and capped- 2009
Phase 2, dredged and capped- 2011
Phase 3, pre-remediation



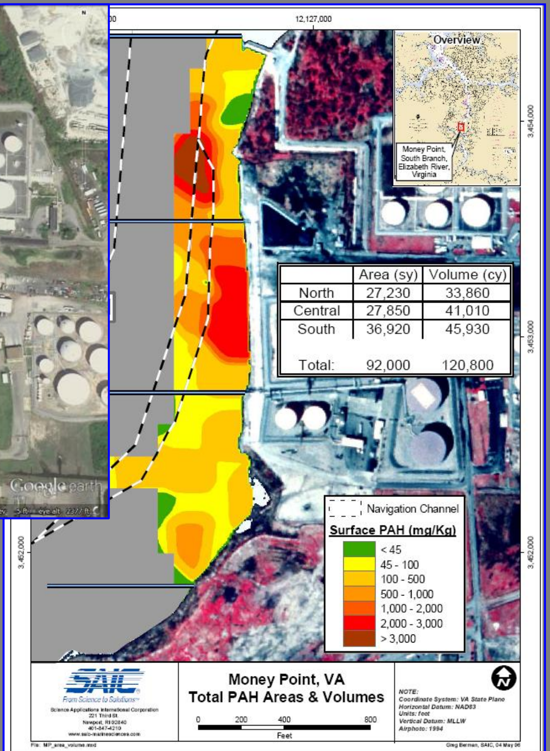
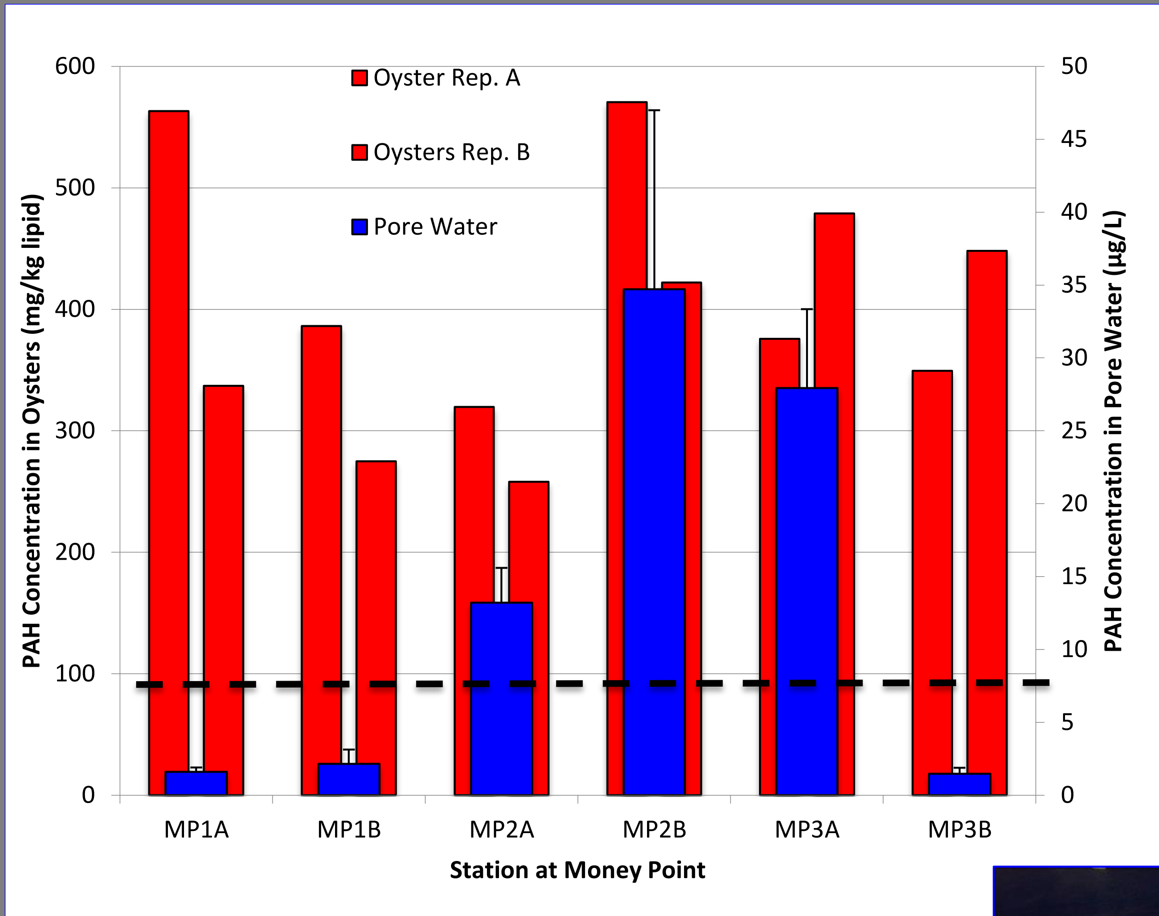


Figure 3.2-8. Money Point nearshore area partitioned into North, Central, and South sub-areas for area and volume estimates.

Results 2013- PAH Accumulation in MP Oysters after 28 days

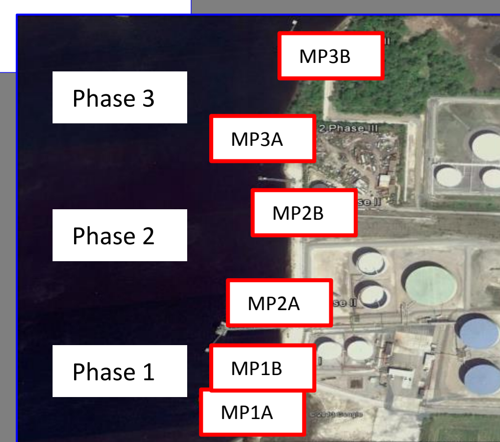


Oyster PAH concentrations are similar and uncorrelated to localized pore water concentrations

Pore water concentrations are variable due to remediation history and the presence of DNAPL

Over the scale of 1 km oysters are integrating the exposure from contaminated sediments in the area

Average pore water concentrations on a large scale are a potentially better estimate of oyster exposure over 28d due to tidal mixing and transport



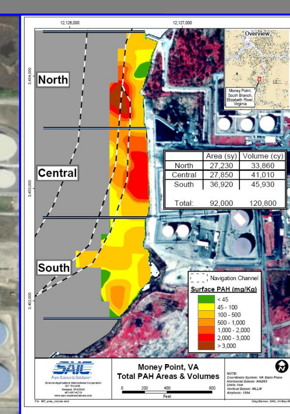
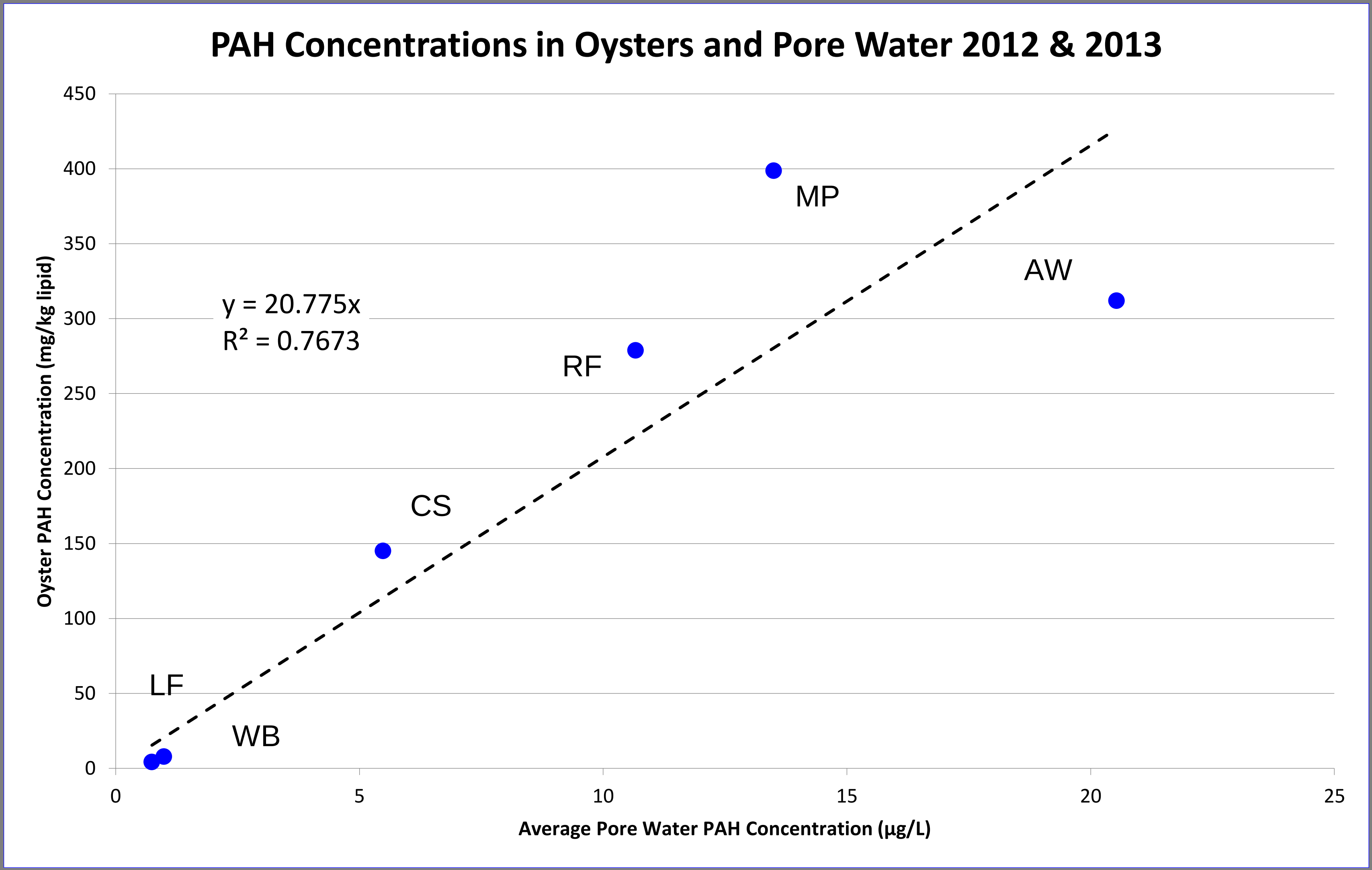
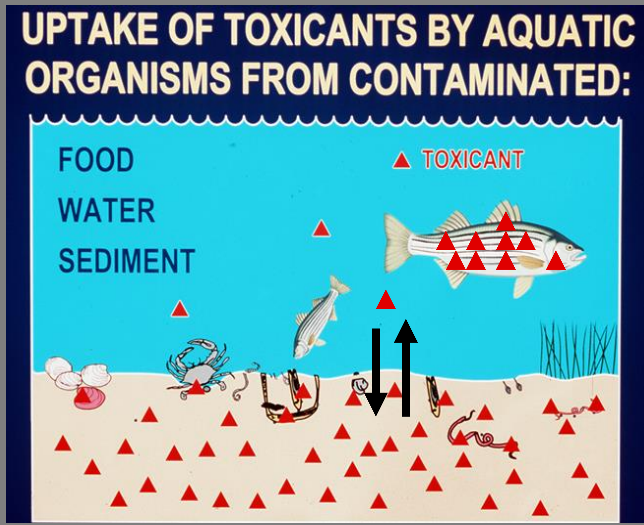


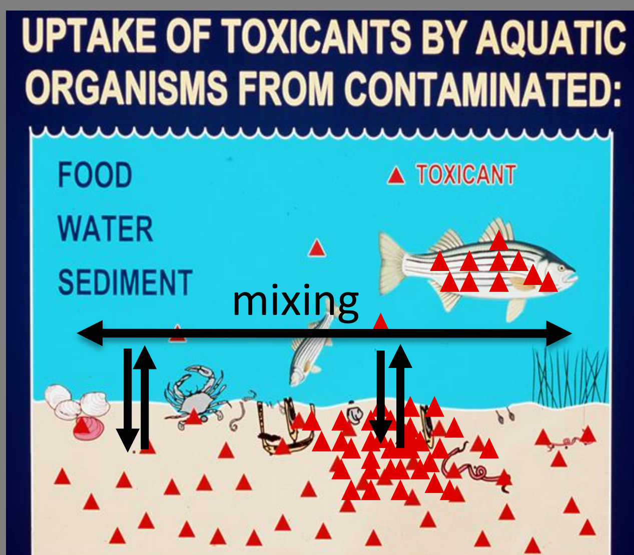
Figure 3.2-8. Money Point nearshore area partitioned into North, Central, and South sub-areas for area and volume estimates.

Results 2012& 2013- Predicting PAH Accumulation in E. River Oysters



Oysters [PAH] correlated to pore water when averaging over large (km) scales at locations with high localized variability (cm-m scales)





- Heterogeneity is high at PAH contaminated sites so scale is important depending on the target biota.

- Benthic infauna (localized exposure) vs. oysters (integrate over large area)

- Are equilibrium based sampling techniques and estimates the best predictors?

- Heterogeneity is high at PAH contaminated sites so sediment pore water flux and exchange with the water column needs to be considered on multiple scales when predicting bioaccumulation or remediation strategies on a larger scale

New research focus-Can we couple pore water flux measurements with contaminant concentrations to determine contaminant flux?

New Proposals to NIEHS-SRP and SERDP: Beck and Unger

The role of pore water–surface water dynamics in controlling PAH bioavailability

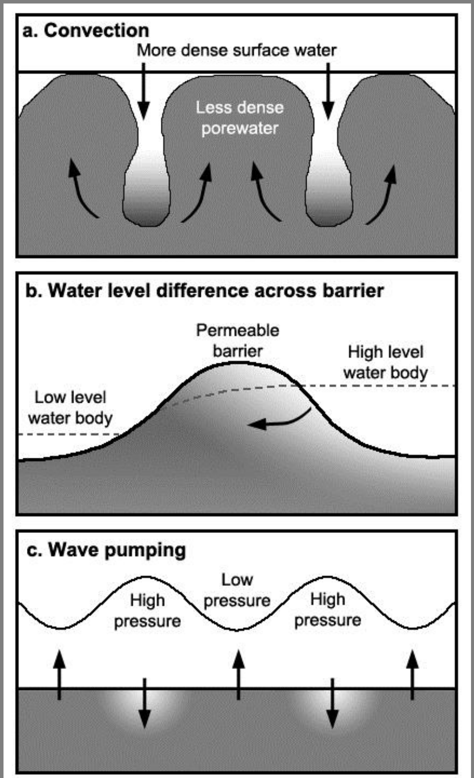


Figure 1. Illustration of three non-canonical porewater advection mechanisms. (a) Convection driven by temperature or salinity gradients, (b) Flow through permeable barrier due to surrounding water level differences, (c) Pumping of surficial porewater due to wave-induced pressure gradients. (Redrawn after Ref. 10.)

Can we couple new innovative measurement techniques to quantify ground water exchange with contaminant concentrations to quantify contaminant flux?

Th-Ra-Rn system + Biosensor PAH in same sample
DNAPL effects on PAH transport in sediments (Phase 2 MP)
Laboratory (mechanistic) and field studies combined

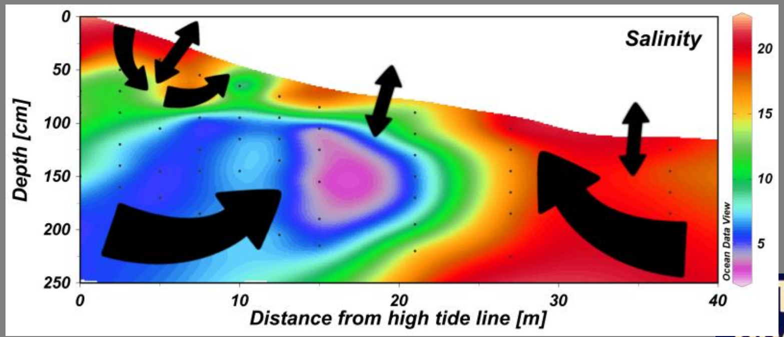
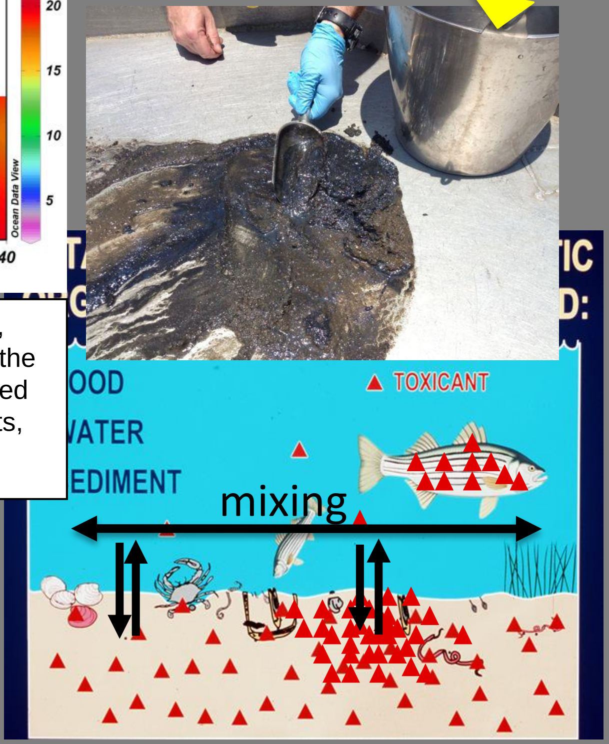
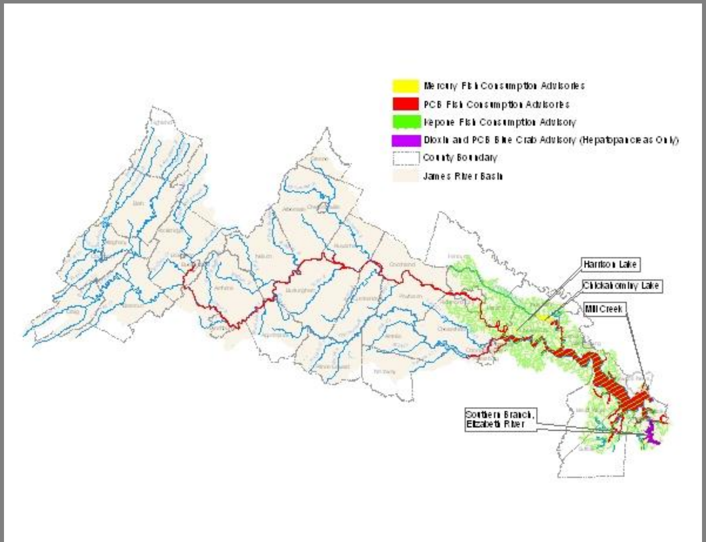


Figure 2. Porewater salinity section from Gloucester Point, VA. This site is similar in surface aquifer characteristics to the proposed study sites. Inferred advection flowpaths are based on evidence from chemical distributions, hydraulic gradients, and seepage measurements.



Pore water/contaminant flux may determine the success of long-term sediment remediation efforts

New research with HRSD on PCB analysis by biosensor



Fish Advisory James River I-95 south

PCBs	Gizzard Shad	DO NOT EAT
PCBs	Carp	DO NOT EAT
PCBs	Blue Catfish ≥ 32 inches	DO NOT EAT
PCBs	Flathead Catfish ≥ 32 inches	DO NOT EAT
PCBs	Blue Catfish < 32 inches & everything else	No more than two meals/month

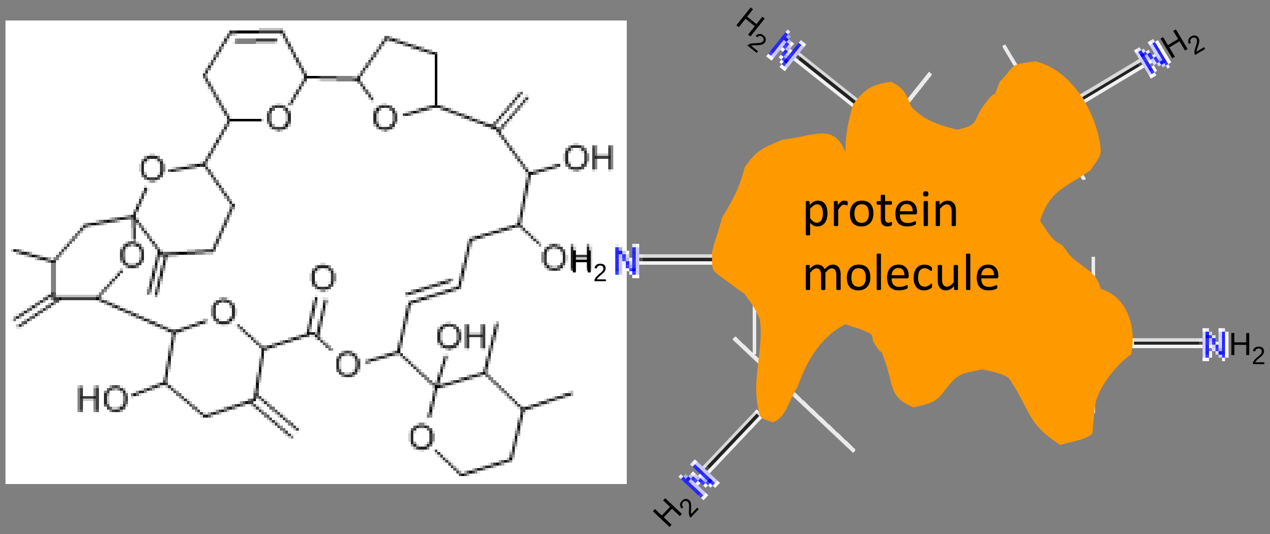
TMDL-PCBs in the James River
Industry needs to evaluate effluent &
Develop a plan for minimizing PCBs

Jonathan Ricks VIMS PhD student/HRSD Employee

Biosensor analysis of PCB in wastewater-GCMS difficult
< pptr detection limit needed-current antibodies???
Antibody affinity and selectivity (congeners vs. total)
Sample concentration/clean up steps (affinity columns)
Goal identify PCB sources to WWTP
- temporal and spatial variability

Future research-Algal Toxins, BDEs or ?

Analysis in minutes on small sample volumes with Inline Biosensor
Toxins have a complex structure that encourages specificity in mAb development
Use surrogate or part of molecule to develop hapten conjugate to protein?
Also need antigen for stationary phase in biosensor



BDE Detection by Antibodies

Superfund Research Program Annual Meeting Baton Rouge, Louisiana October 2013

Immunoanalytical Method for the Sensitive and Specific Detection of BDE-47
Candace Bever, Majkova Z, Wang Y, Dechant J, Gee S, Hammock B University of California, Davis

Gulf of Mexico : Deep Water Horizon Oil Spill Effects

Where is the oil now? Is it in the sediment?

Are there long-term effects on fish health?
“FISHNET” FISH HEALTH NETWORK
GOMRI proposal, Auburn, VIMS, LSU, USM-GCRL

Can the biosensor identify dose and help establish cause and effect relationships?

New prototype biosensors for the Gulf!

I thank NIEHS-SRP for funding this study and the opportunity to work with my co-PIs Steve Kaattari, Wolf Vogelbein and Joe Rieger. Also, we thank George Vadas, Patrice Mason, Mary Ann Volgelbein, Nicol Parker, Jenna Luek, Drew Luellen, Matt Mainor, Dave Koubsky, Ellen Harvey and Ellen Travelstead for assistance in the field and lab.

Grant RO1-ES2094