Welcome to the CLU-IN Internet Seminar

Using Ecological-Based Tools and Approaches to Assess Bioavailability

Sponsored by:National Institute of Environmental Health Sciences, Superfund Research Program

Delivered: June 30, 2010, 1:30 PM - 3:30 PM, EDT (17:30-19:30 GMT)

Instructor(s):
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Moderator(s):

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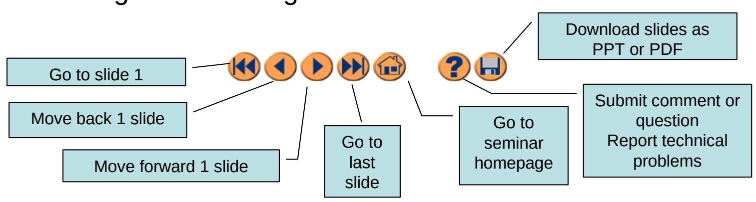

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BIOLOGICAL RESPONSE INDICATOR DEVICES FOR GAUGING ENVIRONMENTAL STRESSORS

BRIDGES

Ecological Risk: New Tools and Approaches Utilized by Superfund Research Program; RiskeLearning June 2010

Kim A. Anderson
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WHY BIOAVAILABLE ?

- **Estimating exposure concentration**
- Predicting environmental fate

- Understanding Environmental Factors on Diseases…
  - MUST develop new bio-analytical tools to measure exposure
    - L.S. Birnbaum, EHP, 2010

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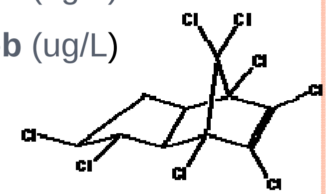
REAL WORLD-DISSOLVED CONCENTRATIONS NOT SIMPLE S_W FROM THE LABORATORY, ENVIRONMENTAL CONDITIONS EFFECT SPECIATION, BIOAVAILABILITY

Chlordane Solubility (S_w)- **56 ppb** (ug/L)

Chlordane Rainbow trout LC50 **90 ppb** (ug/L)



However when more REAL world conditions used

Chlordane Solubility* (S_w)- **28,000 ppb** (ug/L)
*Water containing 34 mg/L *dissolved* organic carbon

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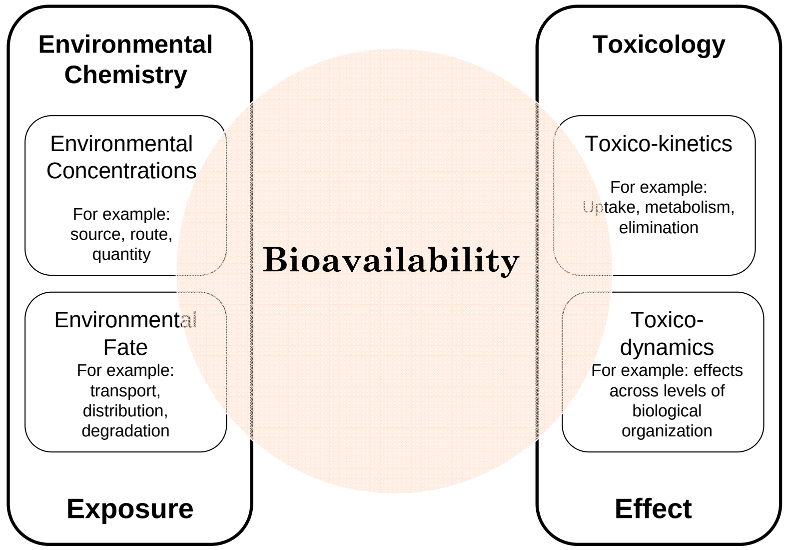
Casarett & Doull's Toxicology, 6th Ed, 2001, pg 1016-1020

BRIDGES



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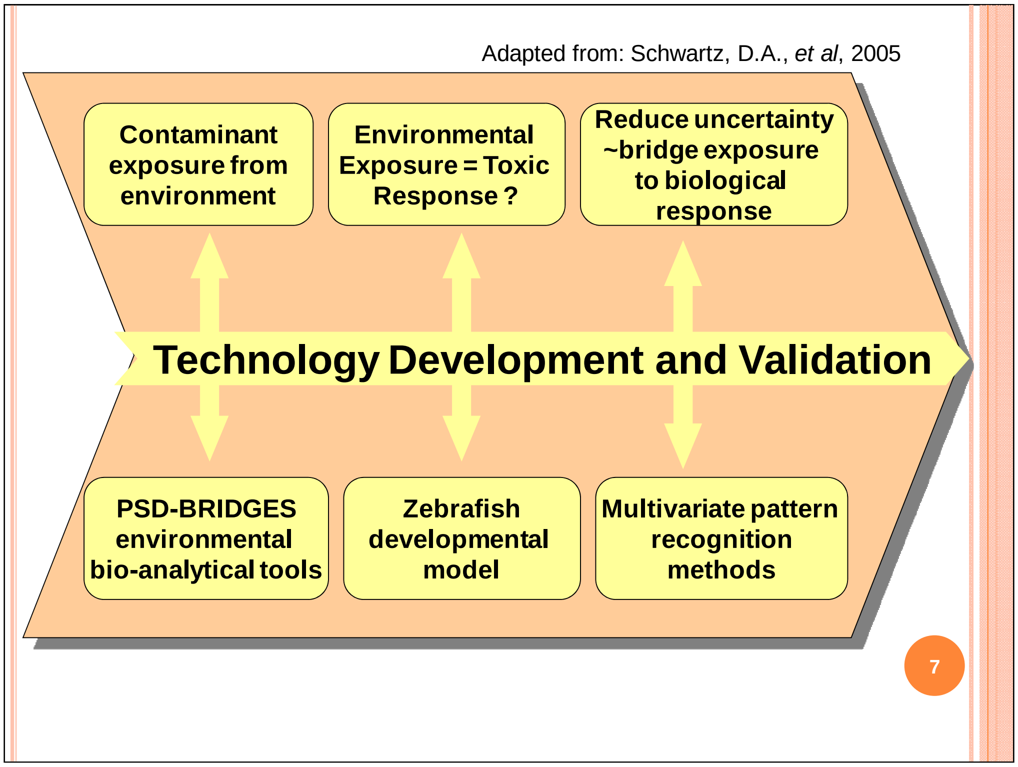
Adapted from: Anderson & Hillwalker, Ecotoxicology Bioavailability, Elsevier 2008


Adapted from: Schwartz, D.A., *et al*, 2005
Contaminant exposure from environment
Environmental Exposure = Toxic Response ?
Reduce uncertainty ~bridge exposure to biological response
Technology Development and Validation
PSD-BRIDGES environmental bio-analytical tools
Zebrafish developmental model
Multivariate pattern recognition methods
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BEYOND CHEMICAL ANALYSIS…

- Exposure dose (exposure concentration)
  - Chemical **mixtures**
  - Predicting environmental fate
- Advantage not to "lump" bioavailable processes
  - Insight will depend on *isolating* processes
  - For example FIRST step in aquatic food chain
- Integration of **space-time** into health risk framework
  - Ambient rough estimate
  - Bio-monitoring transient estimate
- Multi-media

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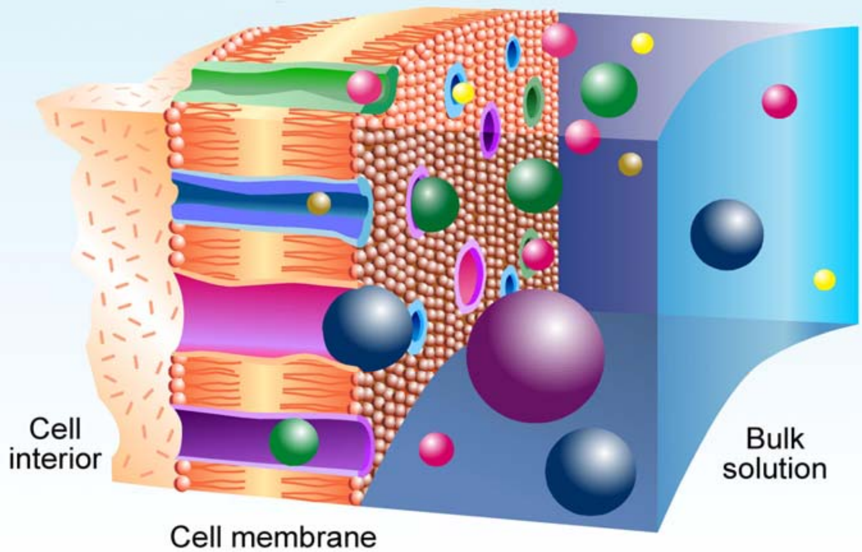
Bioavailability



PASSIVE SAMPLING DEVICES FOR EXAMPLE: LIPID FREE TUBE (LFT)

- Bioavailability processes include
  - **chemical** (cell membrane lipophilic character) and
  - **physical** (pore size ~9.5A) control of contaminant uptake.
- Passive sampling devices (such as **LFT**) mimic both chemical and physical processes
  - lipophilic membrane character, and
  - pore size ~10A

- Other types of membranes:
  - Polymer film or tubes
    - low density polyethylene
    - silicone or silastic
    - polypropylene
- Södergren, 1987 dialysis bag filled with hexane
- Huckins *et al*, 1992 developed semipermeable membrane device (SPMD) tube filled with a triolein
- **Reviewed in:** Namiesnik, J.; Zabiegalal, B.; Kot-Wasik, A.; Partyka, M.; Wasik, A., Passive sampling and/or extraction techniques in environmental analysis: a review. *Anal. Bioanal. Chem.* **2005,** 381, 279-301

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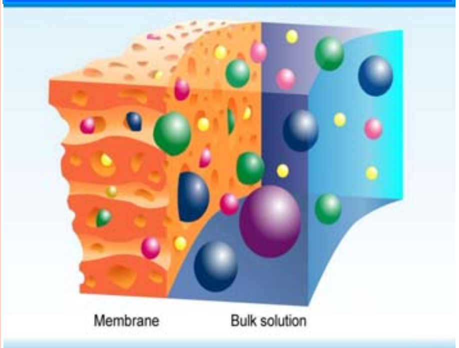
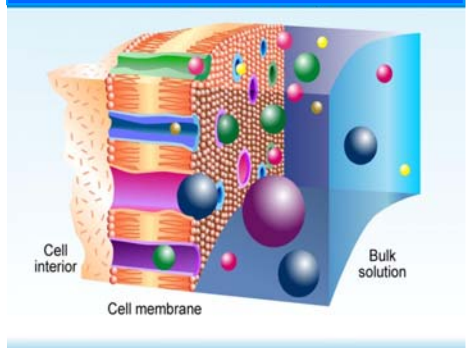
PASSIVE SAMPLING DEVICE ADEQUATE BIOLOGICAL SURROGATE

LFT Membrane



Cell Membrane



LFTs are polyethylene membranes that, similar to cell membranes, passively uptake freely dissolved (bioavailable) hydrophobic compounds.

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PASSIVE SAMPLING DEVICES DEPLOYED AT SUPERFUND SITES

- **PSD theory:** our LFTs are constructed of sealed polyethylene lay flat tubes which represent an organic lipid membrane. Like a membrane, LFTs discriminate against particulate bound material. As *in situ* time integrative passive samplers, LFTs may be deployed for extended periods of time to sequester contaminants. This **overcomes potential issues such as detection limits, bioavailable fraction collection and fluctuating contaminant concentrations**.

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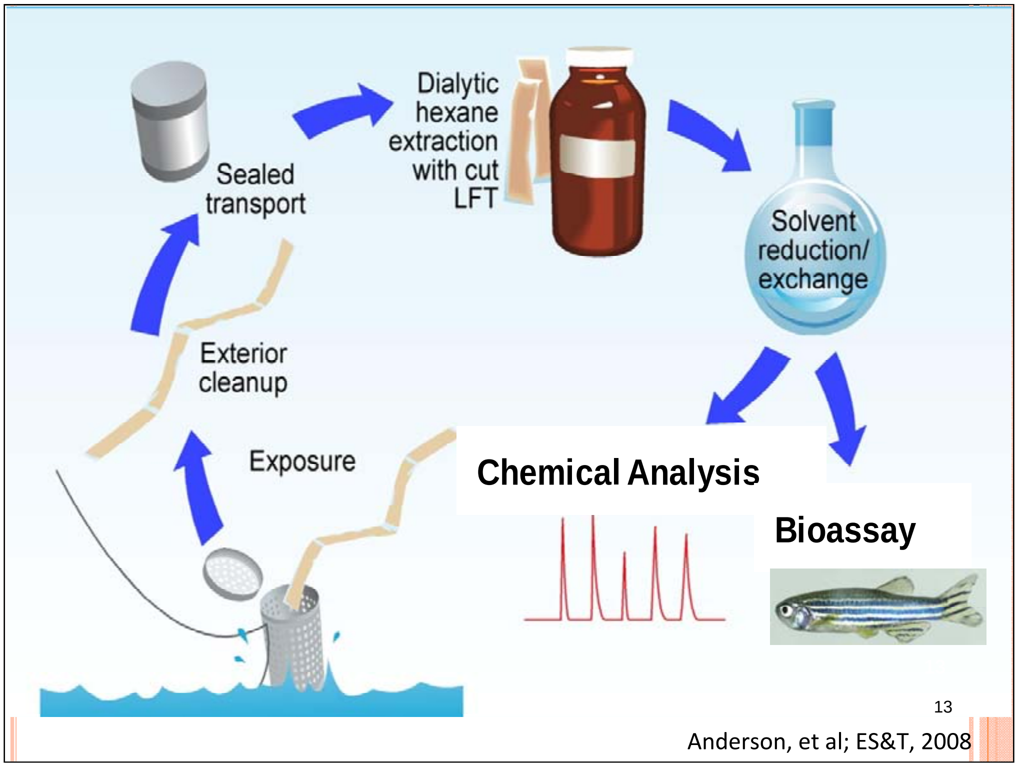
Dialytic hexane extraction with cut LFT
Sealed transport
Solvent reduction/ exchange
Exterior cleanup
Exposure
Chemical Analysis
Bioassay
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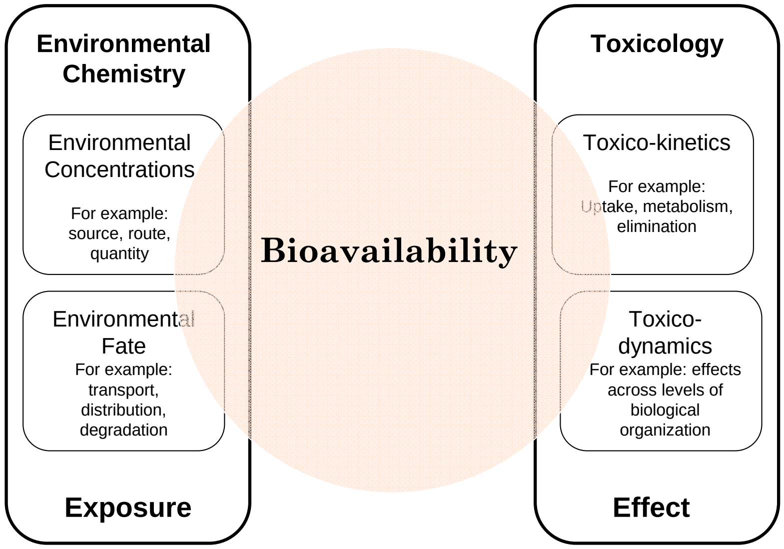
Anderson, et al; ES&T, 2008

BRIDGES: IF PROCESSES IN THE ENVIRONMENTAL SYSTEM AND IN THE BIOLOGICAL SYSTEM ARE TREATED WITH THE SAME MODELLING STRUCTURES AND TOOLS CONSISTENT EXPOSURE AND EFFECT ASSESSMENT IS POSSIBLE.



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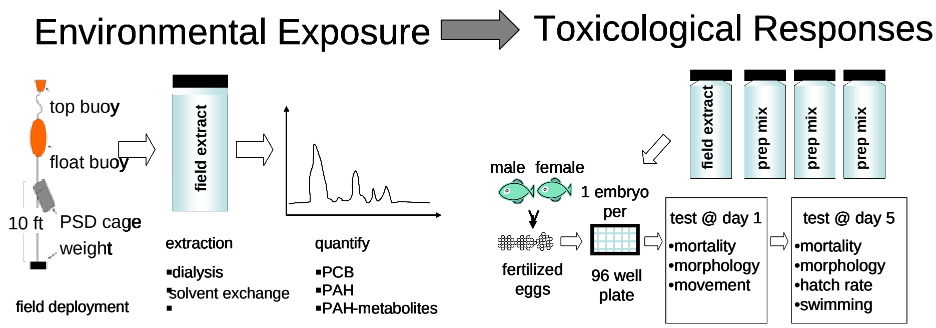
BRIDGES: **REDUCE EXPOSURE UNCERTAINTY BY ANALYZING BIOLOGICAL RESPONSES**

BRIDGES EXTRACTS WITH BIOASSAY MODELS (ZEBRAFISH, AMES, ETC) SYSTEMS



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BRIDGES BIO-ANALYTIC TOOL

Biological **R**esponse **I**ndicator **D**evices **G**auging **E**nvironmental **S**tressors

- Developed in response to the need to link environmental exposure to biological responses.

Exposure to complex mixtures of chemicals in the environment → **Biological response to toxicity**

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BRIDGES BIO-ANALYTIC TOOL

- Combines Lipid-Free Tubing (LFT) passive sampling devices with the embryonic zebrafish model.

LFTs passively concentrate the **bioavailable** fraction of chemicals from the environment:

- time integrated, biologically relevant chemical concentrations

The embryonic zebrafish developmental model is a **high throughput, whole organism, vertebrate** bioassay widely used for toxicity assessments.

- Provides a quantitative measure of the developmental toxicity of site-specific, environmentally relevant contaminant mixtures.

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Beyond Chemical Analyses: Bio-Analytical Tools

Passive Sampling Devices

- Sources, Fate
- Space and Time-integrated flux
- Ambient solute/ vapor concentration
- Bioassays
- Organism Exposure & BCF
- Sample Enrichment

DEPLOYMENT SITES

SUPERFUND SITES

Provides a **quantitative** measure of the developmental **toxicity** of **site-specific**, **environmentally relevant contaminant mixtures**

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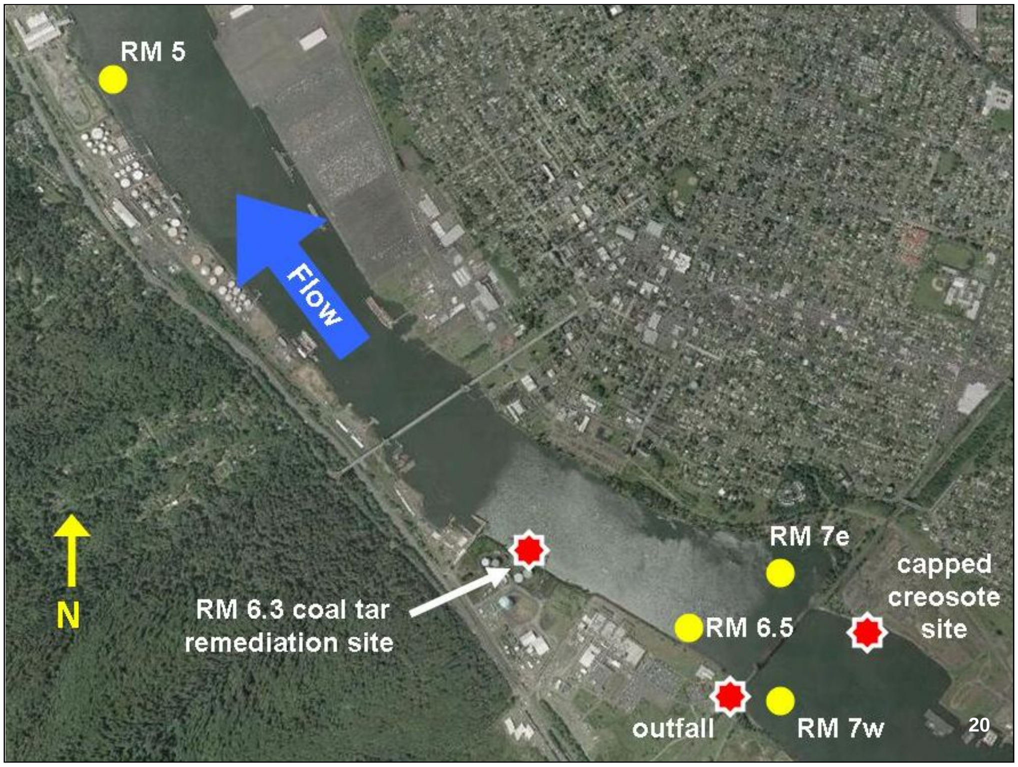
RM 5
Flow
RM 7e
capped creosote site
N
RM 6.3 coal tar remediation site
RM 6.5
outfall
RM 7w
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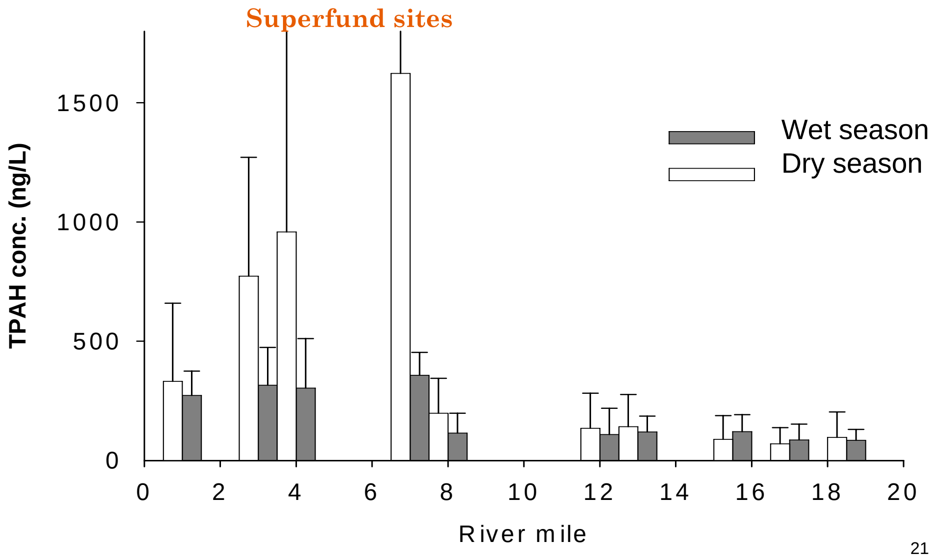
SPATIAL AND TEMPORAL PAHS IN A MODEL HARBOR

Estimated bioavailable ΣPAH



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SOURCES OF PAHS IN THE ENVIRONMENT

- Biogenic (minor)
- Petrogenic
  - Generated by geological processes
    - NATURAL- seeps, coal outcrops
    - ANTHROPOGENIC –fossil fuel release (0.2-7% PAHs)
- Pyrogenic
  - Generated by high temperature combustion of organic matter
    - NATURAL –forest fires, piare fires
    - ANTHROPOGENIC- wood stoves, car exhaust, coal tar

ANDERSON'S SAMPLING SITES, deployed May 11-13, June 8-10, June10- x, 2010

EXAMPLES:
PASSIVE SAMPLING DEVICES IN GULF OF MEXICO
PRE-, POST-IMPACT DEEPWATER HORIZON BLOWOUT

- 4 sites FL, AL, MS, LA
- Early, May, Early June
- *Paired* Air and Water
- *Paired* Chem- and Bio-
- Grande Isle hit by oil

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BEYOND, NEXT CHEMISTRY

- Expanded PAHs
  - 302 isomers
  - alkylated
- **Oxygenated PAHs**
  - facilitated by dispersant
  - more bioavailable
  - as toxic as PAHs
- Screening method imperative for mixture assessment
  - **1,200+** analytes

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PAHS FROM DEEPWATER HORIZON SPILL
OVER 40 PAHS QUANTIFIED, OVER 1200 CONTAMINANTS SCREENED

Over 40 PAHs quantified, as but a few examples of those detected include:(x=present, nd=none detected):

PAHS	Fl air	AL air	MS air	LA air	FL water	AL water	MS water	LA water
Naphthalene	x	x	x	x	x	x	x	x
Naphthalene-2methyl		x	x	x	x	x	x	x
Naphthalene-1methyl	x	x	x	x	x	x	x	x
Fluoranthene	x	x	x	x	x	x	x	x
Benzo[a]anthracene	x	x	nd	nd	x	x	x	x
Benzo[a]pyrene	nd	nd	nd	nd	nd	nd	x	nd

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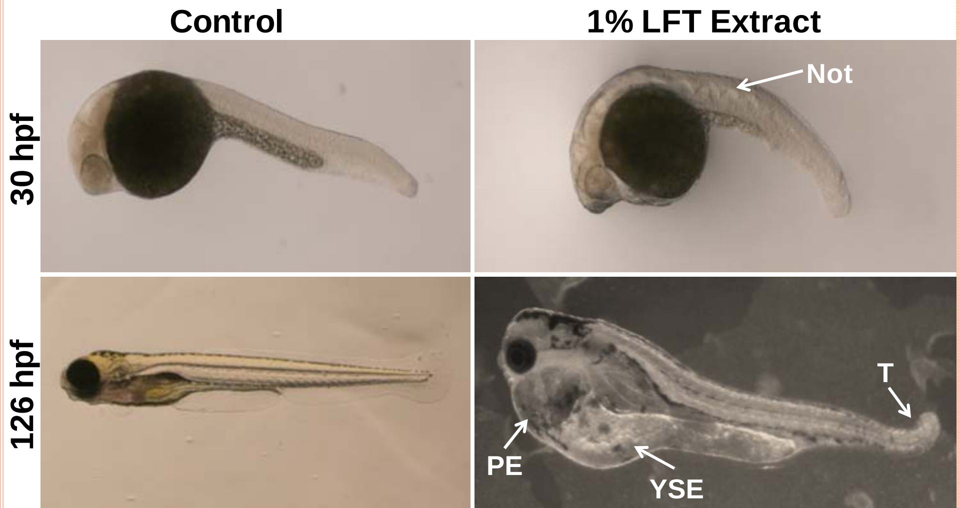
SITE-SPECIFIC BIOLOGICAL RESPONSES



Not= notochord waviness; PE= pericardial edema; YSE= yolk sac edema; T= bent tail

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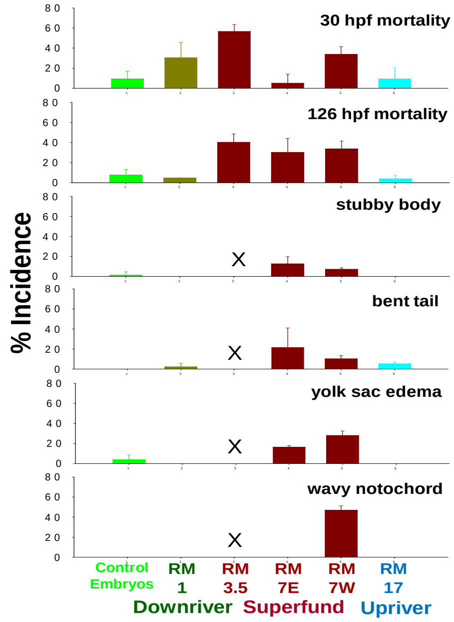
SITE-SPECIFIC BIOLOGICAL RESPONSES



- 6 of 18 biological responses were significantly different in exposed embryos compared to controls (MLR, likelihood ratio, p<0.05; n=941)
- Significant differences between sites were observed for biological responses.
  - Hillwalker *et al*, 2010

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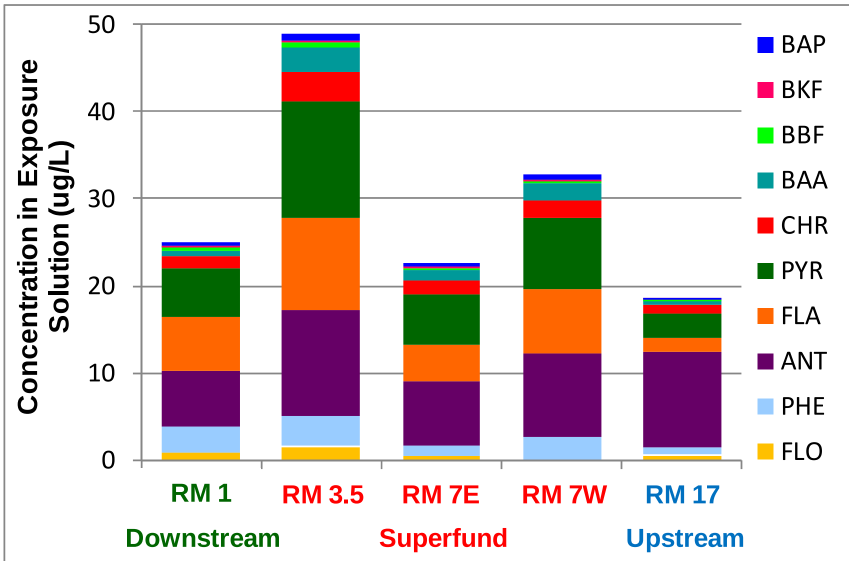
SITE-SPECIFIC CHEMICAL PROFILES



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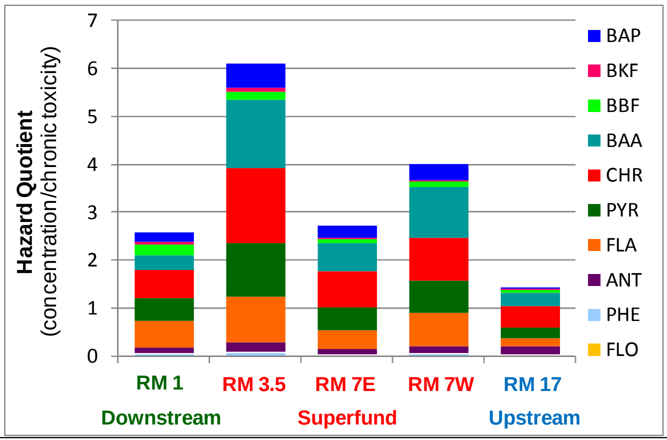
BAP= Benzo(a)pyrene; BKF= Benzo(k)fluoranthene; BBF= Benzo(b)fluoranthene; BAA= Benz(a)anthracene; CHR= Chrysene; PYR= Pyrene; FLA= Fluoranthene; ANT=Anthracene; PHE=Phenanthrene; FLO=Fluorene

SITE-SPECIFIC HAZARD QUOTIENT PROFILES

- Divide the concentration by the chronic toxicity value
  - (Neff *et al*, Integr. Environ. Assess. Manage., 2005)
- Normalizes the concentrations by relative toxicity
- Provides a more realistic estimate of the relative contribution of each chemical to the observed biological responses



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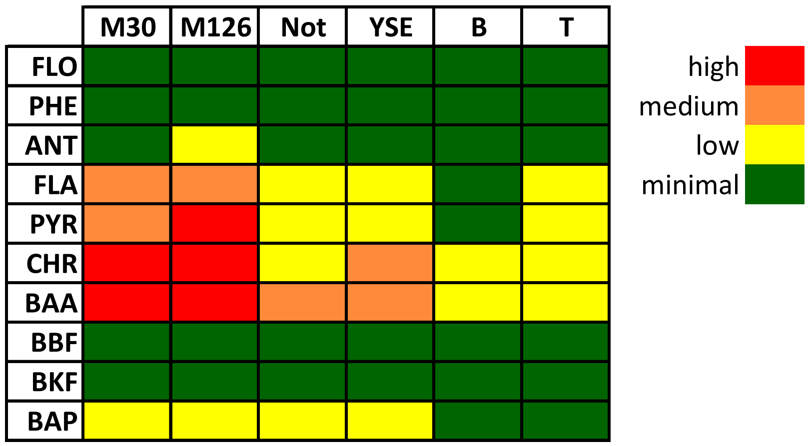
CHEMICAL/BIOLOGICAL ASSOCIATION

- Simple Model: Multiply biological response (% incidence) matrix by PAH hazard quotient matrix to determine **relative contribution of individual PAHs to observed biological responses**:



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M30= 30 hpf mortality; M126= 126 hpf mortality; Not= Wavy Notochord; YSE= Yolk Sac Edema; B= Stubby Body; T= Bent Tail

INTEGRATED ENVIRONMENT AND HEALTH

- embryonic zebrafish exposed to environmental contaminant mixtures obtained from passive samplers deployed in a model river system show site-specific biological responses that can be associated with differences in the chemical profiles of the sites

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BRIDGING: AN INNOVATIVE APPROACH TO QUANTIFYING RISK AT A MGP REMEDIATION PROJECT USING PASSIVE SAMPLING DEVICES

$$Exposure = \frac{C \times CF \times IR \times EF \times ED}{BW \times AT}$$

PSD mass-to-mass concentrations replace the shellfish/fish tissue concentrations normally used here

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CHEMISTRY + BIOLOGY
INTEGRATED ENVIRONMENTAL AND HUMAN HEALTH

- Adding passive sampler concentrations to health risk models increases spatial and temporal precision, improves risk estimates, reduces animal collection, and reduces costs.

- Passive samplers can supplement fish/shellfish data in health assessments to provide specific spatial and temporal contaminant information and thereby help public health and remediation professionals more precisely evaluate and relate exposure and risk

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INTEREST IN PASSIVE SAMPLING DEVICES

PSD PROVIDED TO SBR RESEARCHERS UPON REQUEST

- **Passive Sampling Devices Attributes:**
  - sequesters specific fraction (dissolved, bioavailable)
  - comparative data rapidly developing
  - iterative (captures episodic events)
  - composite without mechanical equipment or on-site power requirements
  - no seasonal issues with PSD compared w/ organisms
  - very low detection limits possible with relative analytical ease
  - minimizes decomposition or chemical change transport/storage
  - does not lump biological processes
  - Less expensive
  - Easier to replicate spatially
  - Greener technology
  - adequately mimics biological
  - not as many negatives as organisms with more positives than grabs…
  - *in-situ* assessment of environmental contaminants that are most biologically relevant
  - have ability to use in bio-assays
  - easily able to do exposure concentration dependent bio-assays

- **Using PSDs for Risk Assessment**
- Advantages
  - Inexpensive
  - Easy to analyze
  - Site specific
  - Bio-available fraction
  - No metabolic activity
  - Nondestructive

$\cong$

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Bioavailable Air and Water Passive Sampling Gear for Gulf of Mexico and Deepwater Horizon Oil Spill
BRIDGES: Biological Response Indicator Devices for Gauging Environmental Stressors pictured: K.Hobbie, K.Anderson, S.Allan, L.Tidwell
Funded in part by NIEHS Superfund Center "PAHs: New technologies and emerging human health risks" P42ES016465 and Environmental Health Science Center NIEHS P30ES000210

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Food Safety & Environmental Stewardship

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Chemical Reaction Kinetics Model
uptake and release of contaminant

Air/Water   Passive Sampling Device   Air/water

Rate to change of the concentration:

$$dC_m/dt = k_u C_w - k_e C_s$$

Conc at any *t* is determined by competing rates of uptake and release

Performance Reference Compounds Uptake and Release of Contaminant

$$N = N_0 \exp(-k_e t)$$

Where N_0 is the amount present at $t = 0$, N is the amount present after

$$k_e = -\ln(N/N_0)/t$$

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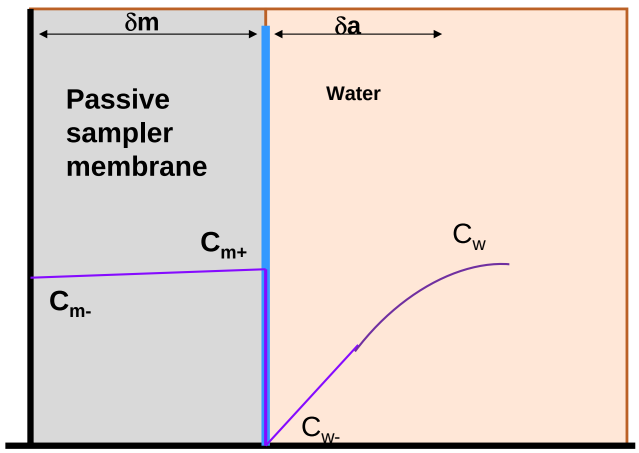
MASS TRANSFER COEFFICIENT MODEL

SCHEMATIC OF CONC DISTRIBUTION INSIDE AND OUTSIDE THE PSD



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WATER (OR AIR) CONCENTRATION

$$R_s = V_m K_{mw} k_e$$

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Rs = sampling rate

$$C_w = N/V_m K_{mw}(1 - \exp((-R_s t/V_m K_{mw}))$$

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Ecological Factors Controlling Mercury Bioaccumulation in Aquatic Food Webs

June 30, 2010

Celia Chen
Department of Biological Sciences
Dartmouth College
Hanover, NH

Where, What, and How

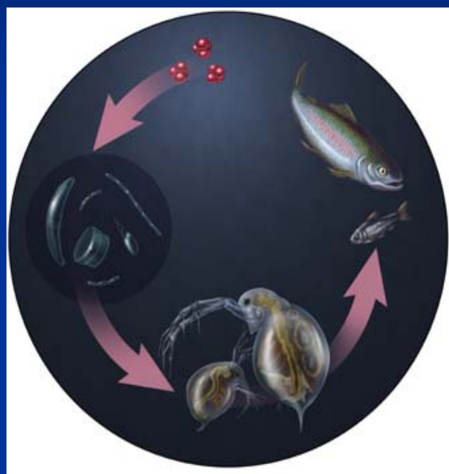

Illustration by W. Scavone

- Aquatic Ecosystems
  - Lakes
  - Estuaries
- Metals
  - Hg
- Approaches
  - Field studies
  - Mesocosm studies

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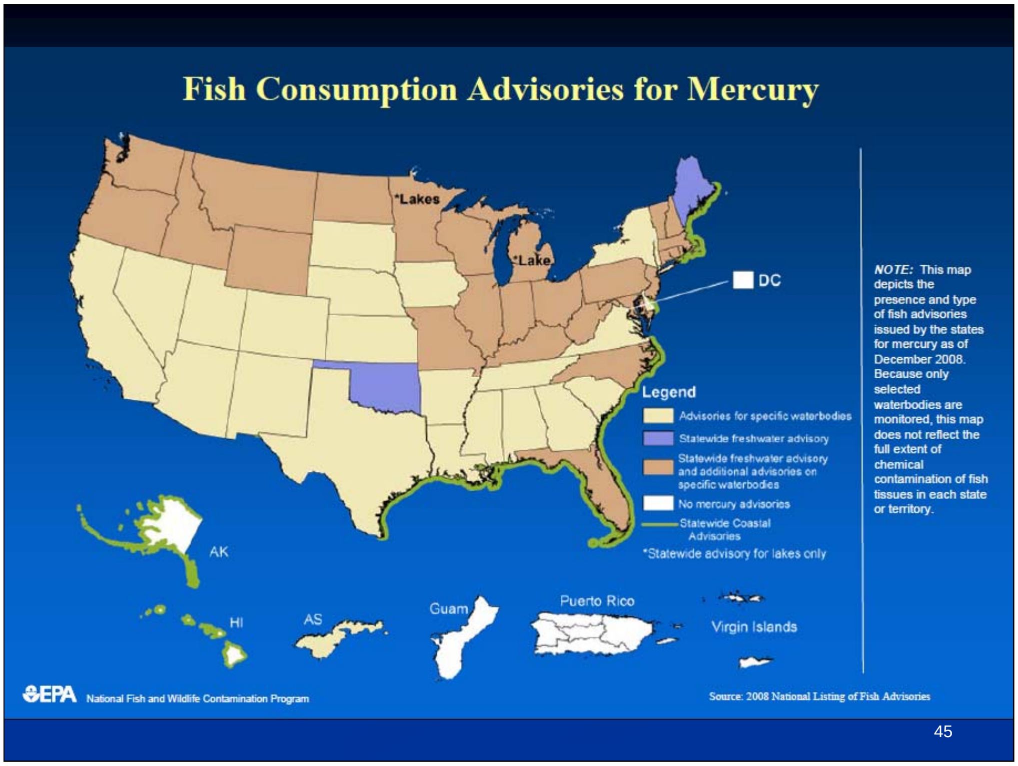
Ecological Processes

Trophic Transfer occurs when concentrations in predators are related to concentrations in their prey.

Biomagnification occurs when metal concentration increases with each level of a food chain.

Biomass dilution occurs when metal concentration decreases with increasing plankton biomass

Feeding Pathway distinguishes the route through which metals are transferred in food webs (benthic vs. pelagic)



Fish Consumption Advisories for Mercury
*Lakes
*Lake
DC
Legend
Advisories for specific waterbodies
Statewide freshwater advisory
Statewide freshwater advisory and additional advisories on specific waterbodies
No mercury advisories
Statewide Coastal Advisories
*Statewide advisory for lakes only
AK
HI
AS
Guam
Puerto Rico
Virgin Islands
NOTE: This map depicts the presence and type of fish advisories issued by the states for mercury as of December 2008. Because only selected waterbodies are monitored, this map does not reflect the full extent of chemical contamination of fish tissues in each state or territory.
EPA National Fish and Wildlife Contamination Program
Source: 2008 National Listing of Fish Advisories
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Toxicity of MeHg

In Humans:
>95% retained from diet

Main organs affected:
- •Brain
- •Kidney
- •Fetal CNS

Effects: Impairs hearing, speech, vision, gait

Most damage thought to be irreversible.

drfrenchfry.wordpress.com

Mad Hatter's Syndrome 1800's

Minimata Disease 1956

Chemical Forms of Hg

- Elemental Hg^0
  - Mostly atmospheric
  - Long residence time 0.5-2 yrs
- Oxidized Hg^{2+}
  - Dominant form in atmosphere and water
  - Reactive gaseous Hg (RGM)
  - Particulate Hg (PHg)
- CH_3Hg^+ monomethylmercury (MeHg)
  - Toxic form
  - Bioavailable form

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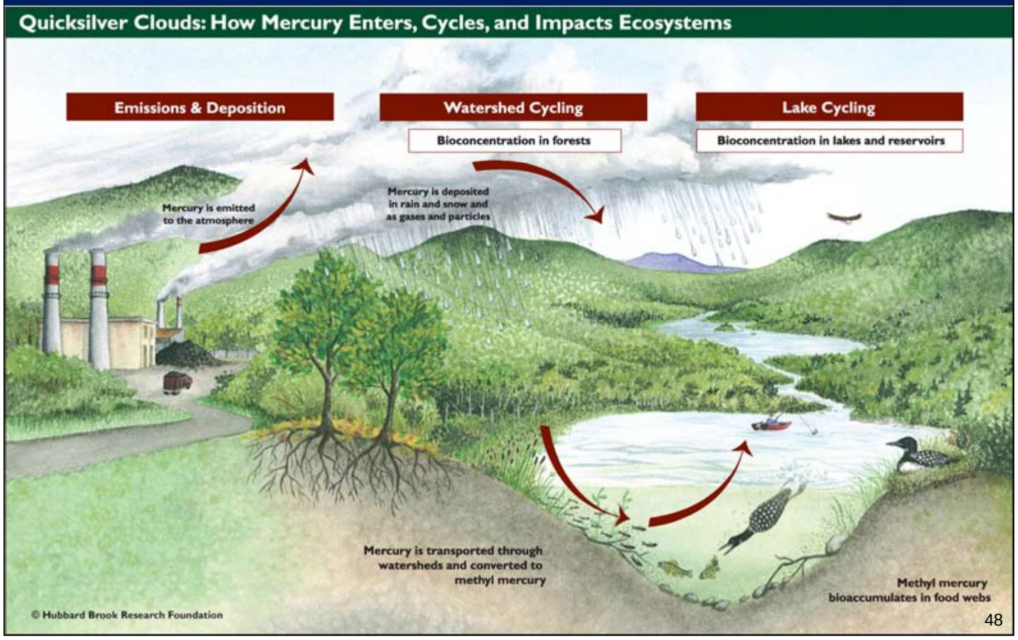
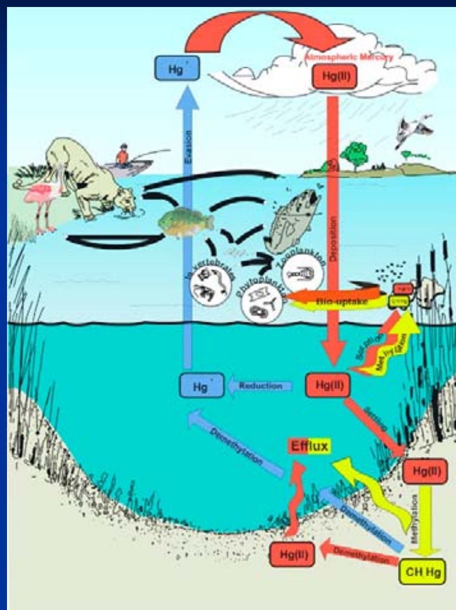
Mercury Sources, Transport, and Fate



In-Lake Mercury Processes



USGS SOFIA 2000

- Hg enters lake as Hg(II)
- Hg methylation occurs in sediment producing MeHg
- MeHg and Hg(II) are fluxed from sediments to water
- MeHg and Hg(II) are taken up by particulates and phytoplankton
- MeHg is preferentially assimilated and transferred up the food web

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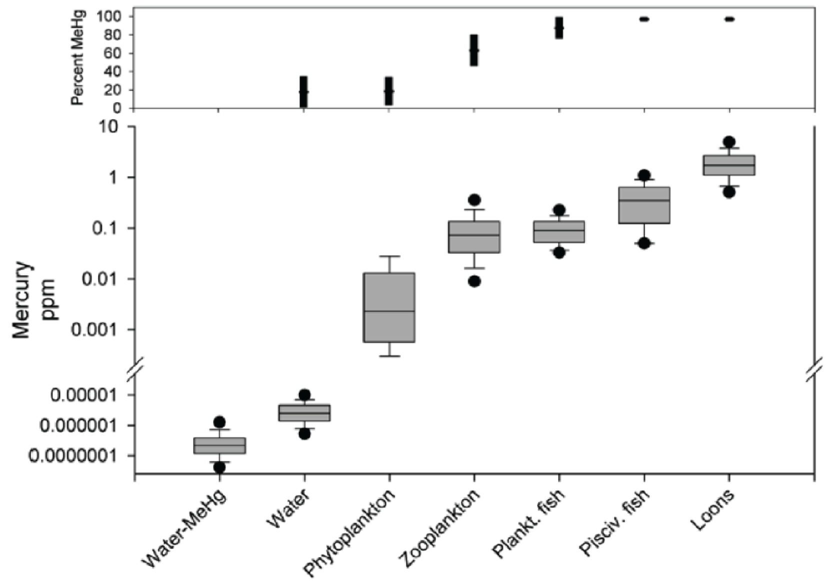
Biomagnification of MeHg



Driscoll et al. 2007

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1. Field Approaches in Lakes to Investigate Metal Bioaccumulation and Trophic Transfer

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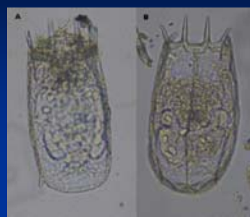
Pelagic Food Webs in Lakes



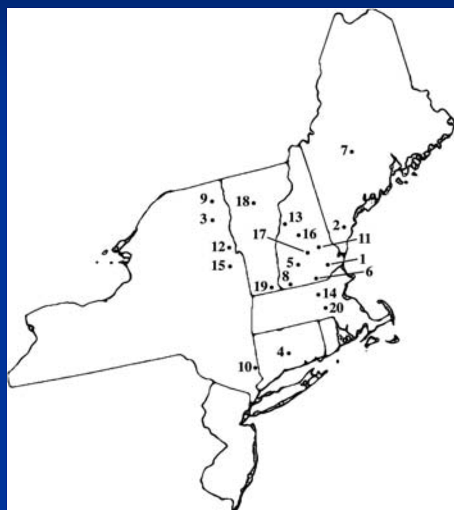
Rotifers,
Herbivores

Copepods,Omnivores

Cladocerans, Herbivores

Planktivores, Piscivores

Field Survey of 20 Lakes in the Northeast US



- **Environmental variables**
- **Phytoplankton and zooplankton metal concentrations**
- **Phytoplankton and zooplankton taxonomy**
- **Concentrations of metals in fish tissue**

Lake Characteristics

Variable	Range
Lake area	5.5 – 902 hectares
Watershed Area	0.9 – 799 hectares
Maximum Depth	2.0 – 20.9 meters
pH	6.0 – 9.3

Field Sampling and Sample Analysis

- Trace metal clean technique
- Plankton metal and taxonomy samples (45-202 and >202 μm)
- pH, TN, TP, DO, Cond., DOC, Chlorophyll
- Calculated variables (BMF, Food web structure)

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Metal Analysis: Dartmouth Trace Element Analysis Core

- Acid digestions
- High resolution ICP-MS (Trace Metal Lab Facility)
- Hg analysis via cold/vapor ICP-MS

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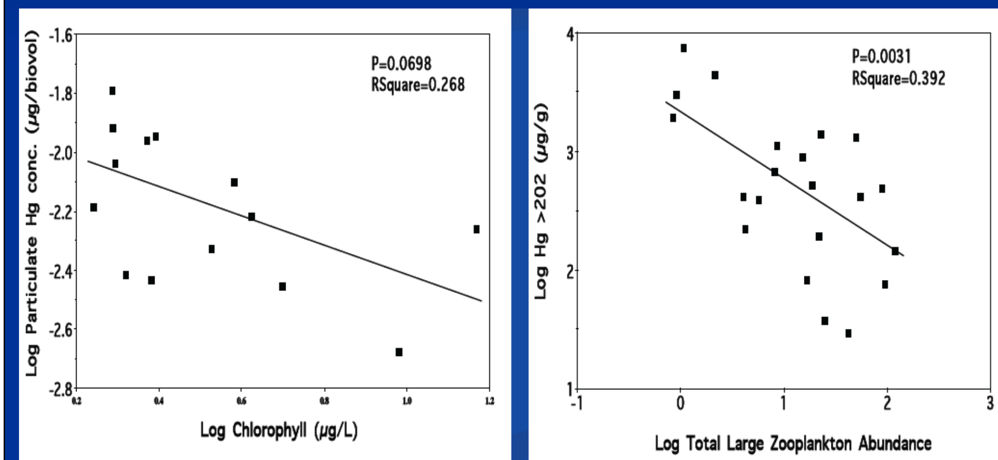
Biomass Dilution: Phytoplankton and Zooplankton Densities Decrease Hg Bioaccumulation



Chen and Folt 2005

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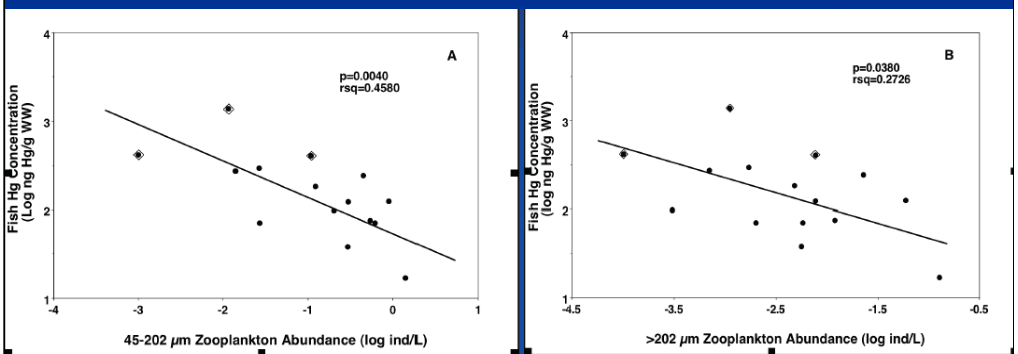
Biomass Dilution:
Higher Zooplankton Density Relates to Lower Hg in Fish



Chen and Folt 2005

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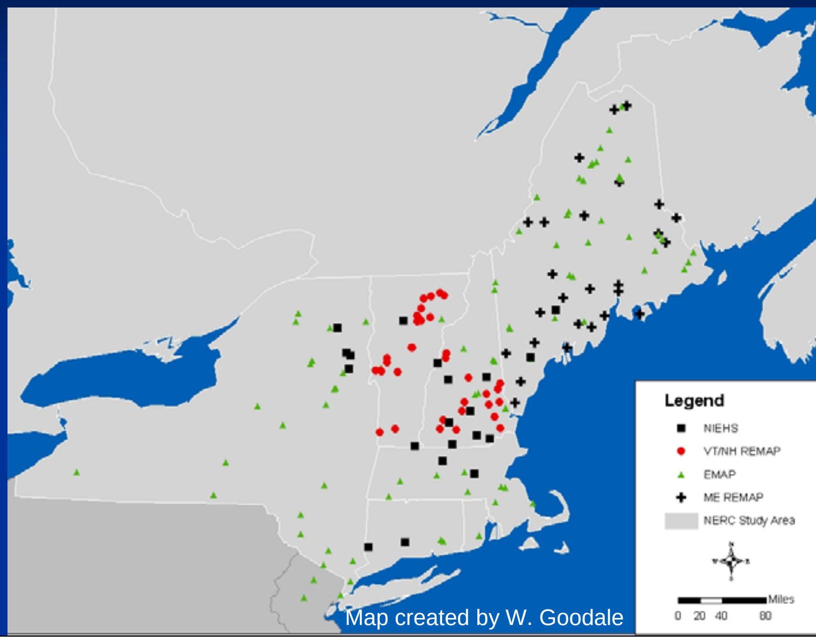
Comparison of Multi-Lake Datasets from the NE US



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Predictors of Hg Bioaccumulation

Variable Category	Variables	NIEHS	REMAP VT-NH	EMAP	ME REMAP
Physical	Lake area	X	X	X	X
	Watershed area	X	X	X	
	Depth (max.)	X	X	(WLR)	X
	Elevation			X	X
				X	
Chemical	pH	X	X	X	X
	Alkalinity	X	X (Epi ANC)	X (ANC)	X
	DOC	X	X (Epi DOC)	X	X
	SO4		X (Epi SO4)	X	
	Conductivity	X	X	X	X
	Total P	X	X	X	
	Total N	X		X	
	N:P	X			
Landuse	Population		X (911 count)		X
	Disturbed	X		X	
	Residential				
	Hay/Perm. Past,		X	X (AG)	
	Comm/Ind/Transp.		X	X (URB)	
	Forest		X	X	
	Wetland	X	(Forest/Wetl.)	X	
	Road density	X		X	
Ecological	Linkages	X	X		
	Max. chain length	X	X		
	Chorophyll	X		X	X
	Algal biomass	X			
	Zoop. abundance	X	X	X	X
	Zoop. Biomass	X			
	Richness	X	X		X
Hg conc.	Aqueous	X	X (Epi THg)		
	Zooplankton	X	X		
	Fish	X (MX)	X (YP)	X (MX)	X (MX)

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Common Variables

Spearman Rank Correlation, P<0.05

Variable Category	Common Variables	Relationship to Hg in Fish
Physical	Lake area (3/4) Watershed area (2/3)	(+)
Chemical	pH (3/4) ANC (alkalinity) (3/4) Conductivity (2/4) SO4 (2/2) Nutrients (2/3)	(-)
Land Use	Residential, Agricultural, Commercial Industrial, Roads, Disturbed (3/3)	(-)
Ecological	Zooplankton abundance (3/4)	(-)

Bonferroni Adjustment

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2. Experimental Approach in Mesocosms to Investigate Biomass Dilution and Trophic Transfer

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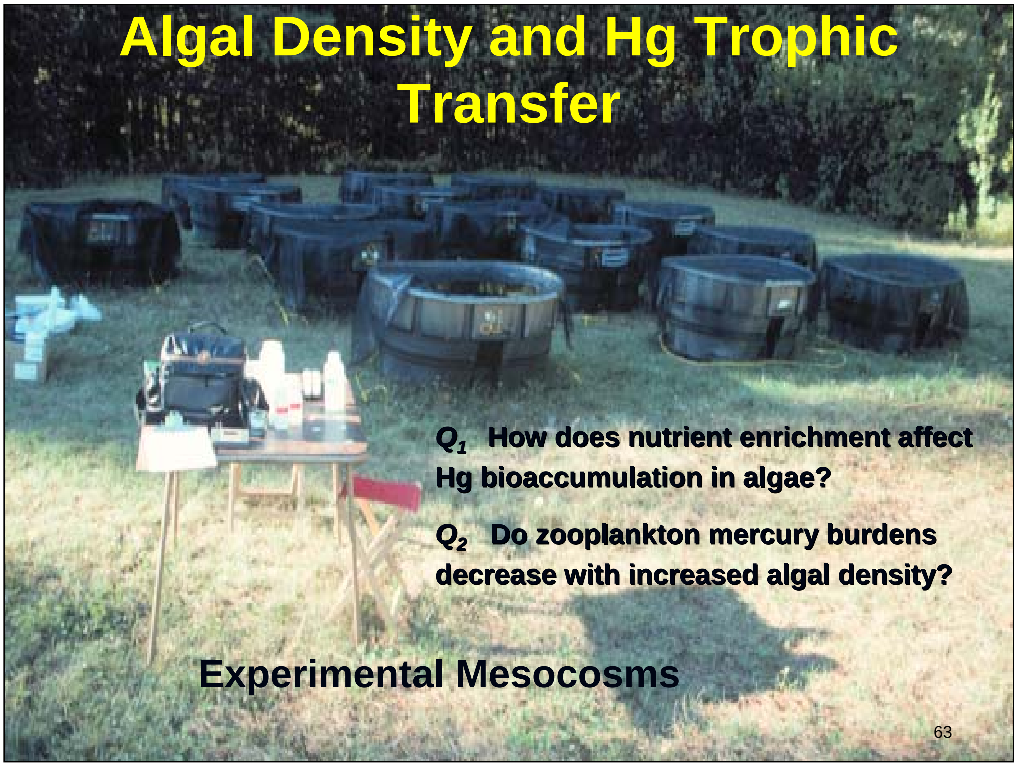
Algal Density and Hg Trophic Transfer
Q_1 How does nutrient enrichment affect Hg bioaccumulation in algae?
Q_2 Do zooplankton mercury burdens decrease with increased algal density?
Experimental Mesocosms
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Experimental Design

- Two tanks at each nutrient level
- N and P ($\mu g \bullet L^{-1}$) all at 30:1 atomic ratio
- Stable isotopes of Hg (**$CH_3{}^{200}Hg^+$, $^{201}Hg^{2+}$**)
- Hg bioaccumulation in algae and zooplankton (daphnia)

100.4 : 7.4 oligotrophic	201 : 14.8 mesotrophic	302.6 : 22.3 mesotrophic
1x	**2x**	**3x**
403.5 : 29.7 mesotrophic	504.4 : 37.2 eutrophic	605.3 : 44.6 eutrophic
4x	**5x**	**6x**

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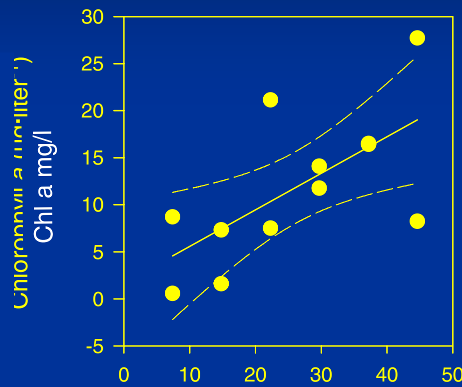
Pickhardt et al. 2002

Added N:P Increased Algal Biomass



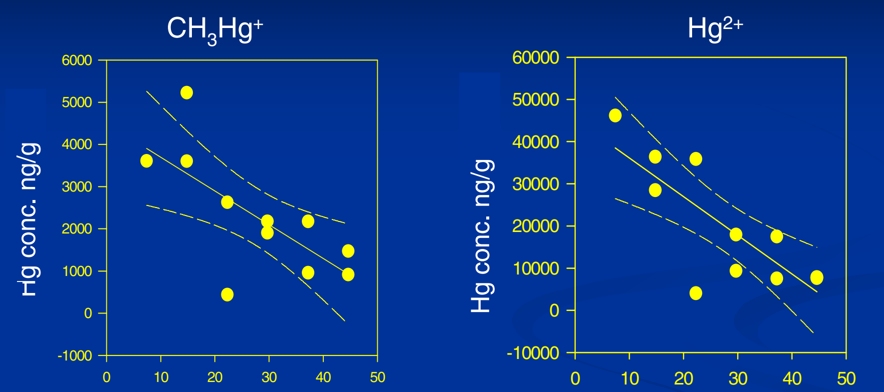
Chl. *a* = 0.39(µg P) + 1.69

n =12, R^2 = 0.431, P < 0.021

Inorganic Phosphorus Added to Tanks (µg·liter^{-1})

Pickhardt et al. 2002

Strong Algal 'Dilution' of Hg at 24 hours



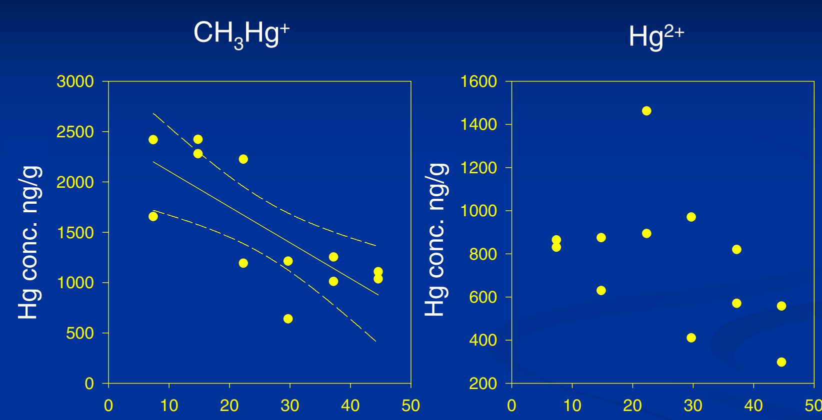
Inorganic Phosphorus Added to Tanks (µg·liter^{-1})

CH_3Hg^+ = -80.14(µg P) + 4502
n = 11, R^2 = 0.499, P < 0.016

Hg^{2+} = -917(µg P) + 45290,
n =11, R^2 = 0.623, P < 0.004

Pickhardt et al. 2002 66

Hg in *Daphnia* at 3 Weeks



Inorganic Phosphorus Added to Tanks (μg·liter^{-1})

CH_3Hg^+ = -265(μg P) + 2465
n = 12, R^2 = 0.554, P < 0.0056

Hg^{2+} = -78.7(μg P) + 1041
n = 12, R^2 = 0.213, P > 0.130

Pickhardt et al. 2002 67

3. A Field Approach in Coastal Marsh Ecosystems to Investigate Feeding Pathways of Trophic Transfer

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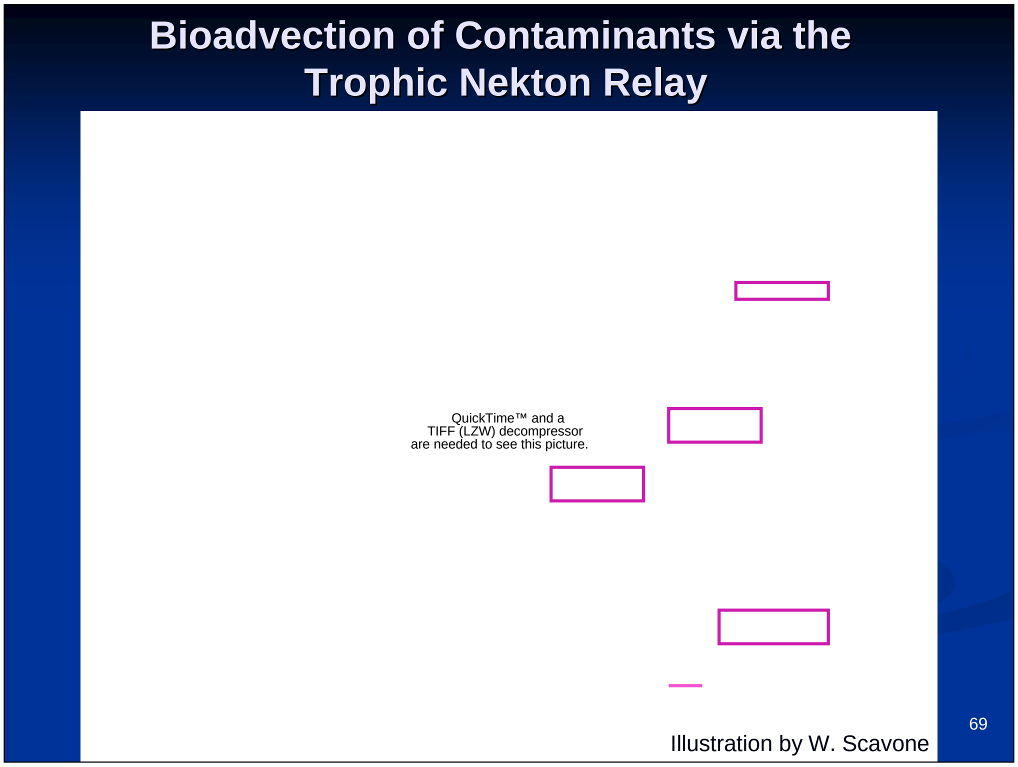
Bioadvection of Contaminants via the
Trophic Nekton Relay
QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.
Illustration by W. Scavone
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Estuarine Studies in New England

Sampling in the Field

Biotic Samples:

Primary consumers: Snails, Mussels

Secondary consumers: Killifish, Green Crabs

Metal Analysis

-whole organisms freeze dried & homogenized

-Hg speciation by isotope dilution purge and trap GC-ICPMS at Dartmouth

Isotope Analysis

-separate set of samples collected in field simultaneously

-whole organisms freeze dried and homogenized

$\delta^{13}C$, $\delta^{15}N$

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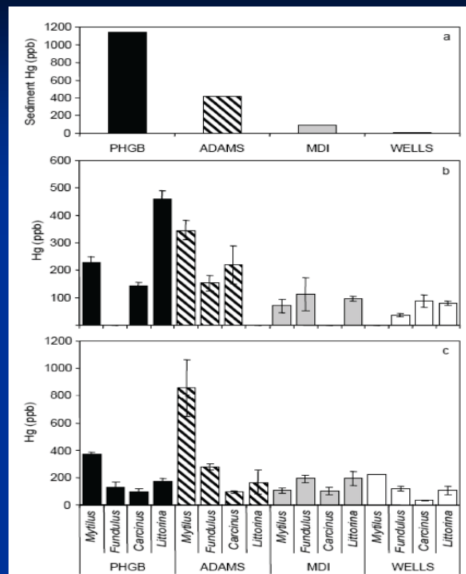
Variation in Sediments vs. Biota (2003-4)



Sediment conc. varies by 200X

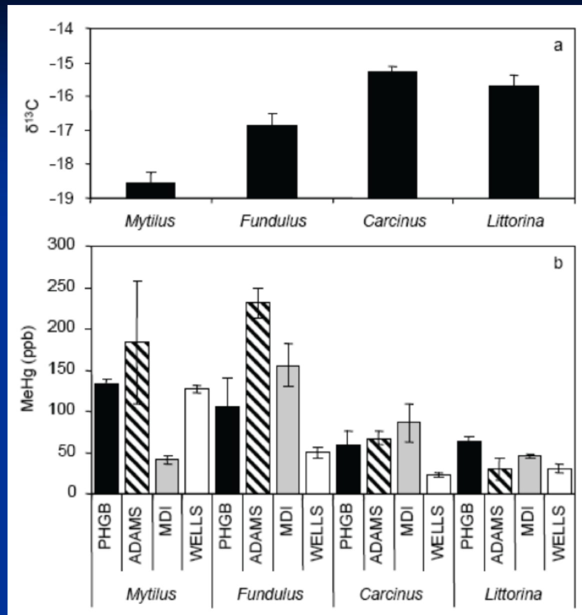
Four Species:
Mussels
Killifish
Green crab
Snail

Biotic conc. vary by 2-4X

Concentrations (ug/g DW) of THg in: a) sediments and b) four focal species collected in 2003 (not all four species collected at all sites) and c) 2004. 72

Chen et al. 2009

Relationship of Feeding Pathway and MeHg



Benthic

Pelagic

MeHg and Food source: a) Delta ^{13}C signatures and b) MeHg concentrations (ug/g DW) in four focal taxa across four GOM sites.

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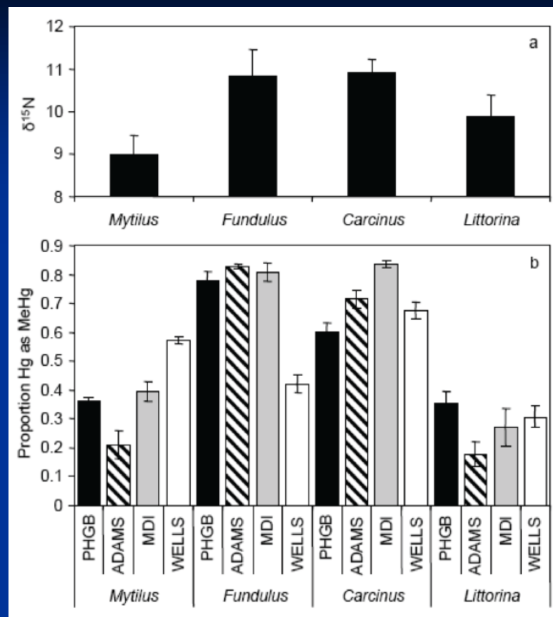
Relationship of Trophic Level to % of THg as MeHg



Higher TL

Lower TL

MeHg and trophic level: a) Delta ^{15}N signatures and b) % of THg as MeHg in four focal taxa across four GOM sites.

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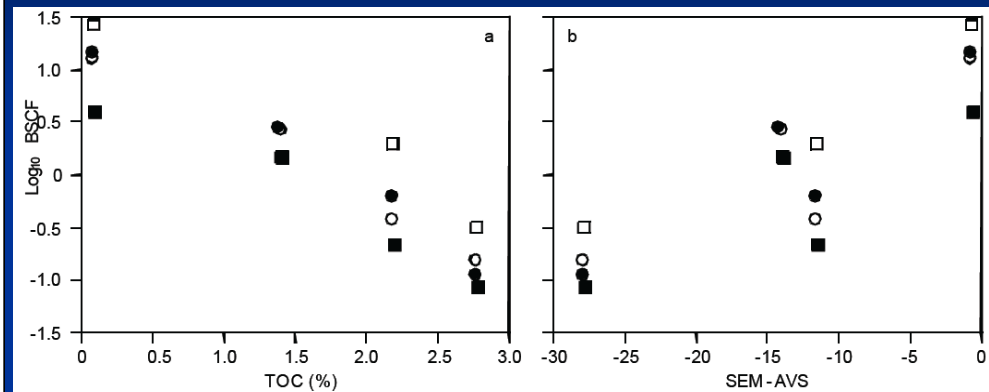
Relationship of Sediment Carbon to Bioaccumulation of Hg

BSCF = Benthic-sediment concentration factor = Hg in organism/Hg in sediment



Relationship of biotic-sediment concentration factors (BSCF) across sites and four focal taxa to: a) TOC and b) SEM-AVS. Symbols: (open square) *Mytilus*, (solid square) *Carcinus,* (open circle) *Littorina*, (solid circle) *Fundulus.*

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Overall Conclusions

- Metal fate in aquatic food webs is determined by a number of ecological processes:

  Trophic Transfer

  Biomagnification.

  Biomass dilution

  Feeding Pathway

- These processes are present across lake and estuarine systems

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Acknowledgments


Illustration by W. Scavone

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