Contaminant Exposure, Food Web Transfer and Potential Effects on Ospreys (*Pandion haliaetus*) in Chesapeake Bay

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PATUXENT Wildlife Research Center

Chesapeake Bay

NASA Satellite Image of the Bay on September 13, 2011 post –Irene
http://www.nnvl.noaa.gov/images/high_resolution/836_20110912-TSM-Chesapeake.jpg

- Largest estuary in the U.S. watershed encompasses 6 states (64,299 mi^2)
- Myriad of anthropogenic threats

- 72% of Bay & tidal segments impaired by toxics
- Overharvesting

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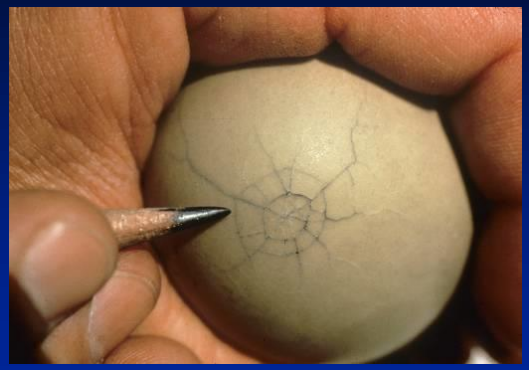
Chesapeake Bay Ospreys

- Scattered information before the 1970's
- Population decline (*pesticide DDT*)
  - Low point early 70's – 1,450 pairs
- Population recovery (*DDT ban*)
  - Mid-90's – 3,500 pairs "Osprey garden of the world"
  - Nest substrate to foster growth



D. A. Ratcliffe. 1967. *Nature* 215:208-215.

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Ospreys as a Sentinel

- High trophic level fish-eating species
- Adapts to human landscapes
- Tolerable of short-term nest disturbance
- Nests highly visible and easy to access
- Nests found across large spatial scales
- Long-lived
- Nest site fidelity
- Accumulate lipophilic contaminants
- Known sensitivity to many contaminants
- Worldwide distribution

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Contaminants and Chesapeake Bay Ospreys

- 2000-2001 large-scale ecotoxicological study:
  - Osprey productivity adequate
  - *p,p*-DDE levels ↓
  - PCBs remained high
  - PBDE flame-retardants approach LOAEL for pipping & hatching success in kestrels

U.S. EPA Regions of Concern

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Rationale and Objectives

- Decade since last large scale ecotoxicological study on ospreys in the Bay
- Limited exposure data for Bay avifauna
- May 2009 Presidential Chesapeake Bay Executive Order

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Scientific Objectives

1. "Decadal re-evaluation of contaminant exposure and productivity of ospreys nesting in Chesapeake Bay Regions of Concern"

   Examine spatial and temporal changes in contaminant exposure and osprey productivity in ROCs.

2. "Chesapeake Bay fish-osprey food web: evaluation of contaminant exposure and genetic damage"

   Reconstruct osprey dietary preferences and relate contaminant concentrations in fish to those in osprey eggs. Examine genetic damage as a biomarker of effect in osprey nestling whole blood.

3. "Exposure and food web transfer of pharmaceuticals in ospreys: predictive model and empirical data"

   Conduct in silico and in situ analyses of exposure of ospreys to pharmaceuticals.

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Scientific Objectives

1. "Decadal re-evaluation of contaminant exposure and productivity of ospreys nesting in Chesapeake Bay Regions of Concern"

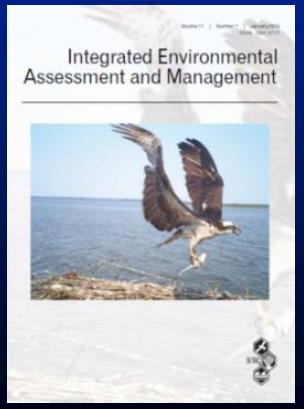
   Lazarus, R.S., Rattner, B.A., McGowan, P.C., Hale, R.C., Schultz, S.L., Karouna-Renier, N.K., Ottinger, M.A., 2015a. Decadal re-evaluation of contaminant exposure and productivity of ospreys (Pandion haliaetus) nesting in Chesapeake Bay regions of concern. Environ. Pollut. 205: 278-290.

2. "Chesapeake Bay fish-osprey food web: evaluation of contaminant exposure and genetic damage"

   Lazarus, R.S., Rattner, B.A., McGowan, P.C., Hale, R.C., Karouna-Renier, N.K., Erickson, R.A., Ottinger, M.A., 2016. Chesapeake Bay fish-osprey (Pandion haliaetus) food chain: evaluation of contaminant exposure and genetic damage. Environ. Toxicol. Chem. **Accepted with Minor Revision.**

3. "Exposure and food web transfer of pharmaceuticals in ospreys: predictive model and empirical data"

   Lazarus, R.S., Rattner, B.A., Brooks, B.W., Du, B., McGowan, P.C., Blazer, V.S., Ottinger, M.A., 2015b. Exposure and food web transfer of pharmaceuticals in ospreys (Pandion haliaetus): predictive model and empirical data. Integ. Environ. Assess. Manag. 11, 118-129.



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Research Objectives

Whole Fish

Osprey Eggs

Analytes Quantified in Eggs:
- *Organochlorine pesticides*
- *PCBs (total and congeners)*
- *PBDEs (total and congeners)*
- *New flame retardants (e.g., HBCD, TDCPP)*
- *Methoxytriclosan*

Water

Fish Plasma

Osprey Nestling Plasma

Analytes Quantified in Plasma:
Suite of 29 pharmaceuticals
- *Antidepressants*
- *Antihypertensions*
- *Antiseizures*
- *Analgesics*
- *Antilipemics*
- *Stimulants*
- *Antifungal*
- *Antihistamine*

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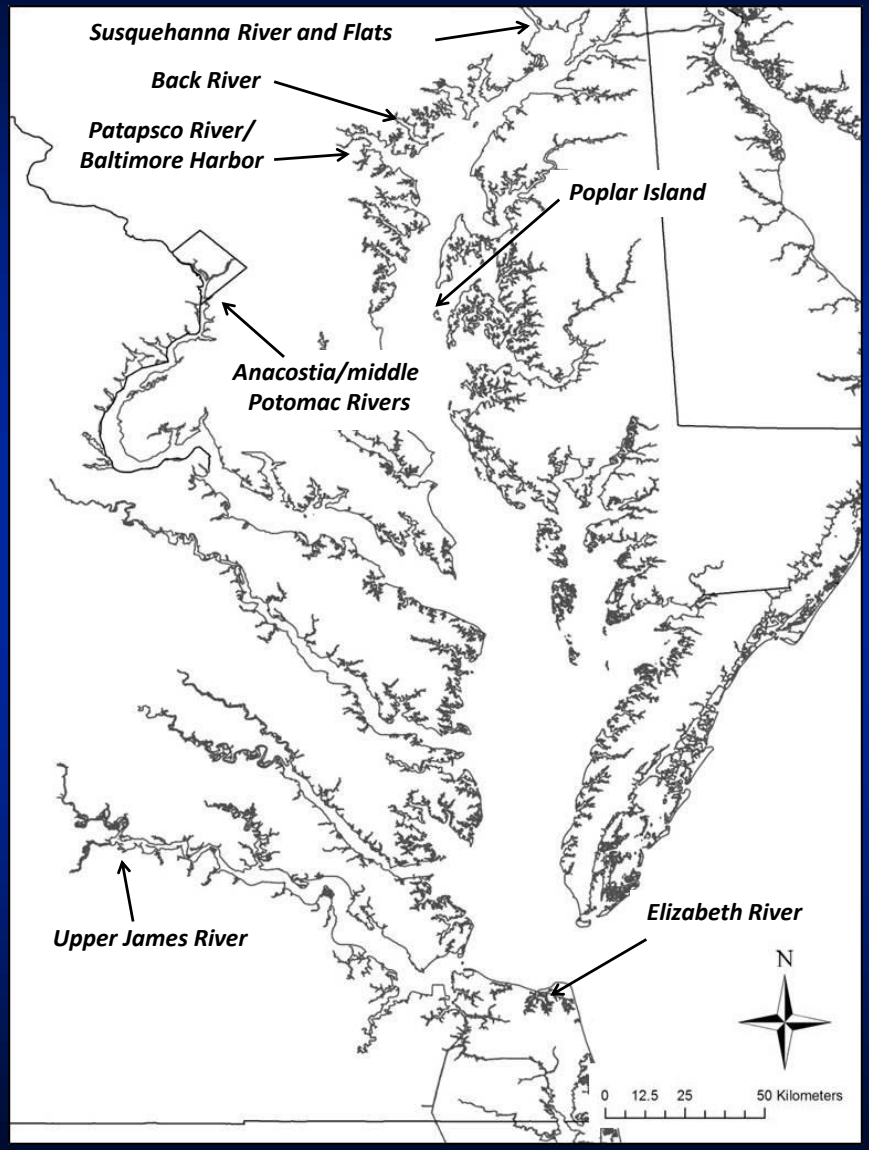
Sampling Sites (2011-2013)

- U.S. EPA Regions of Concern
  - Baltimore Harbor/Patapsco
  - Anacostia/middle Potomac
  - Elizabeth River
- Susquehanna River
- James River
- Back River
- Poplar Island reference site



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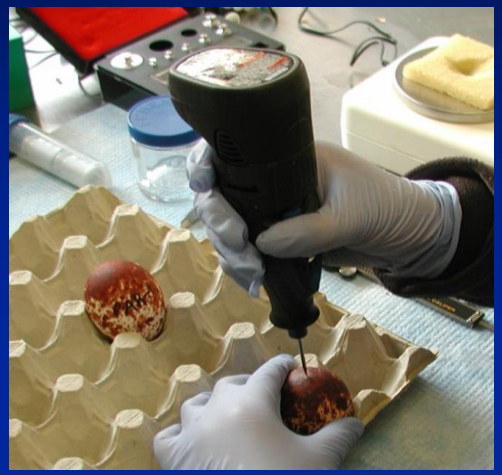
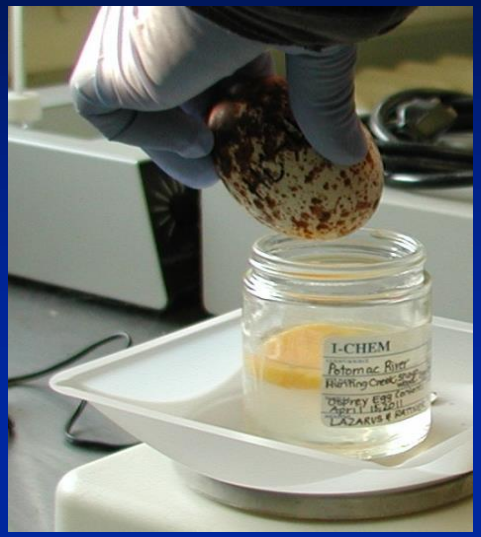
Egg Sampling & Productivity

- Collect 1 sample egg/nest, **n=64** (Organochlorine pesticides , PCBs, flame retardants, methoxytriclosan)





- Monitor fate of nest weekly (eggs, chicks, fledglings)

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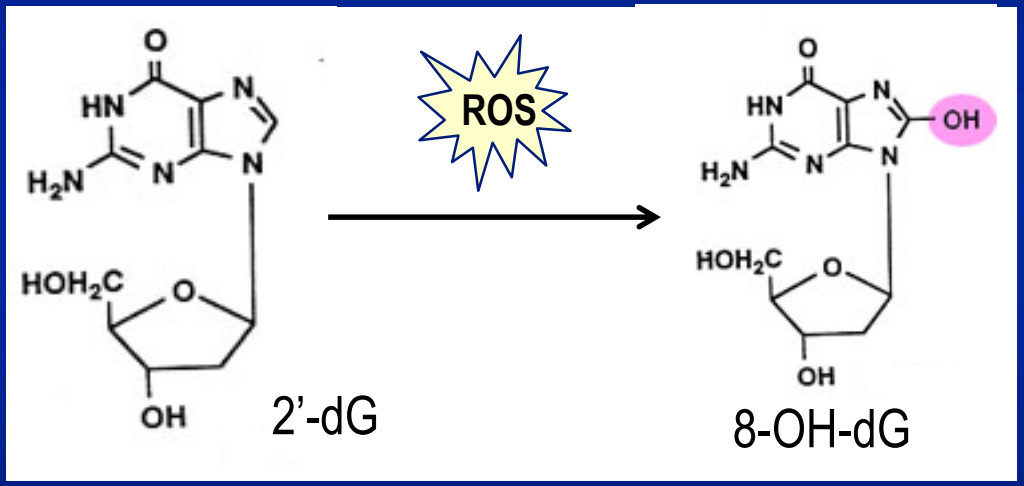
Nestling Blood Samples

- Collected nestling blood samples from 40-45 day old chicks to measure oxidative DNA damage (8-hydroxy-2'-deoxyguanosine) in nucleated red blood cells



- Whole blood analyzed at PWRC (DNA/RNA oxidative damage EIA)

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Food Web Sampling Methods

Water near
WWTP outfall
2-3 sites/tributary

Reconstruction of
osprey diet

Plasma/Whole Fish
2-3 dominant species in
osprey diet
(Size range: 25-35 cm)

Osprey nestling eggs/blood
40-45 day old nestlings for
organics, pharmaceuticals,
and genetic damage

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Analytical Methods

- Eggs and whole fish analyzed at VIMS (GC-MS and UPLC-APPI/MS) for organochlorine pesticides, PCBs, PBDEs, alt-BFRs and methoxytriclosan

- Water, fish plasma and osprey nestling plasma analyzed at Baylor University (isotopic dilution LC-MS/MS)

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Results

1. Osprey productivity
2. Eggshell thickness
3. Contaminants in Eggs
4. Genetic damage
5. Biomagnification factors
6. Pharmaceuticals

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Osprey Productivity

Site	Fledglings/active nest	
	2000-2001 *(Rattner et al. 2004)*	2011-2013 (Lazarus et al.)
Reference Site (South R. 2000-01 & Poplar I. 2011-13)	1.07	1.33
Baltimore Harbor/Patapsco (2011)	1.07	1.43
Anacostia/middle Potomac (2011)	0.88	1.23
Elizabeth River (2012)	1.53	1.00 (1.28)
Susquehanna River (2013)	-	1.80
James River (2012)	-	1.17
Back River (2013)	-	1.00






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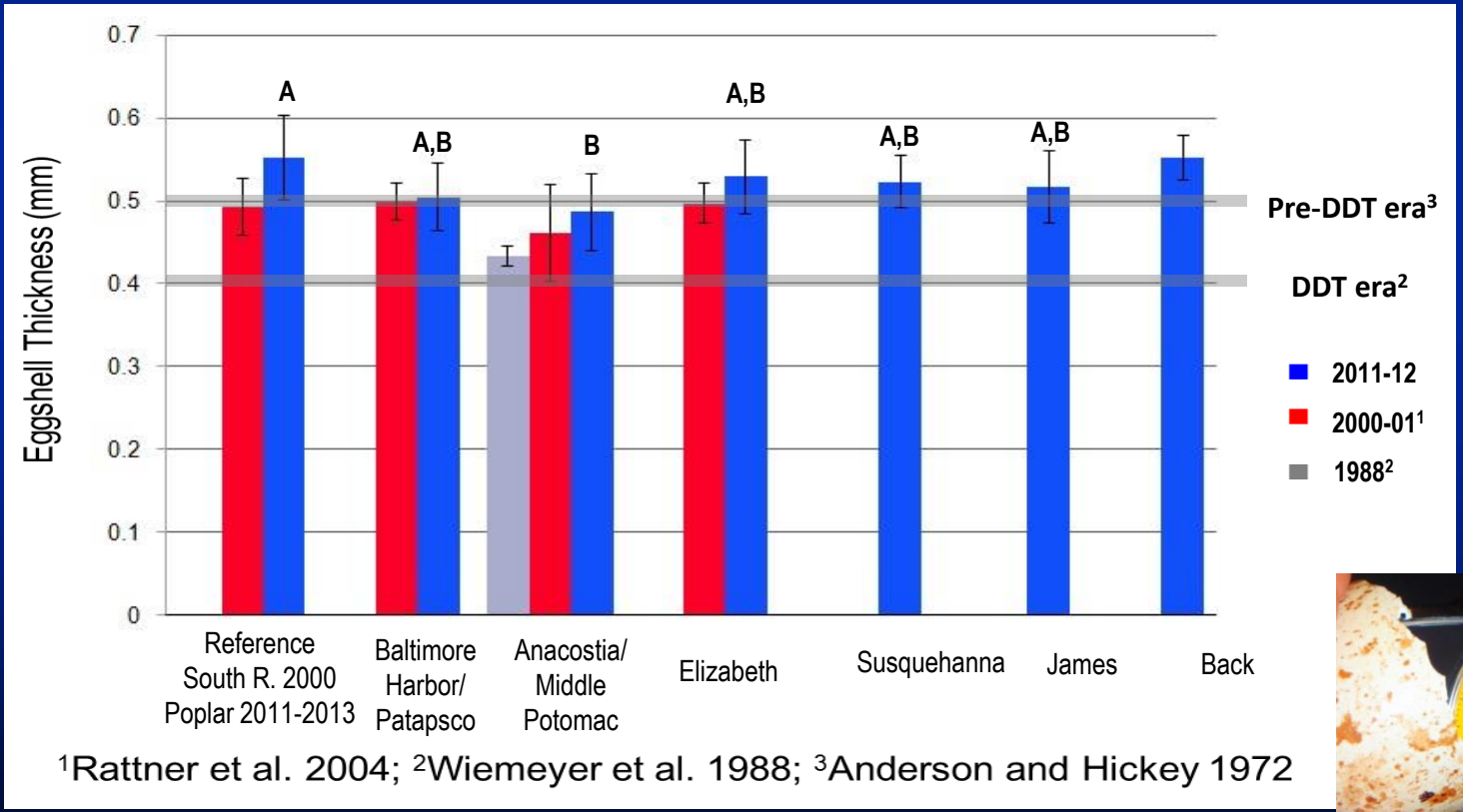
Eggshell Thickness

- No relationship between *p,p'*-DDE and eggshell thinning in this study
- Shells on Anacostia smaller compared to Poplar (p=0.0058)
- Of 30 eggs only 1 (Baltimore Harbor) had *p,p*-DDE residues in 95% CI for 10% shell thinning (1.2-3.0 µg/g ww)



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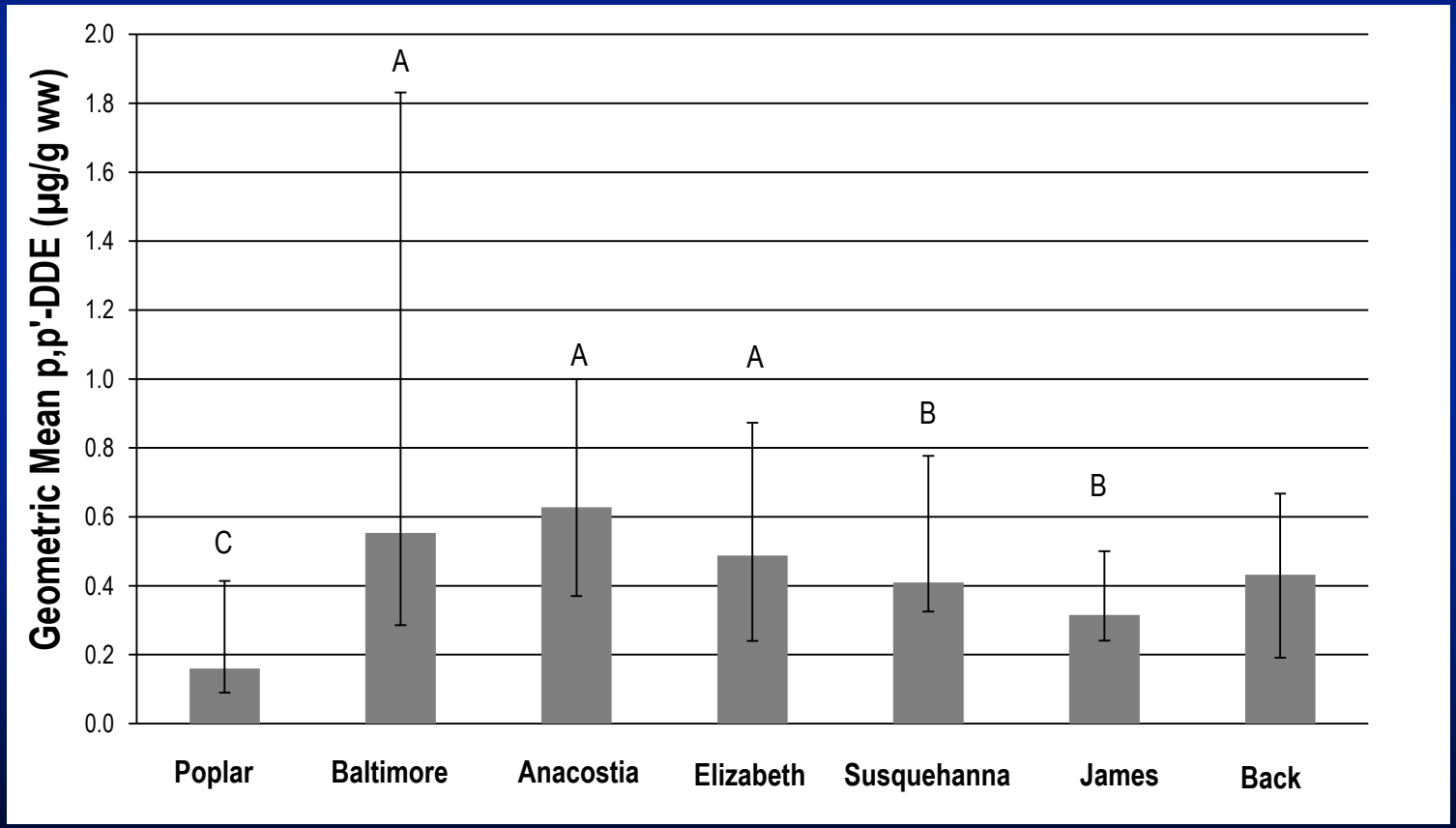
Organochlorine Pesticides in Osprey Eggs

- 8 of 10 of the most abundant pesticides declined by 10-75%
- *p,p'*-DDE greatest on Baltimore Harbor, Anacostia and Elizabeth Rivers. Greatest detect in Baltimore Harbor (1.83 µg/g ww)



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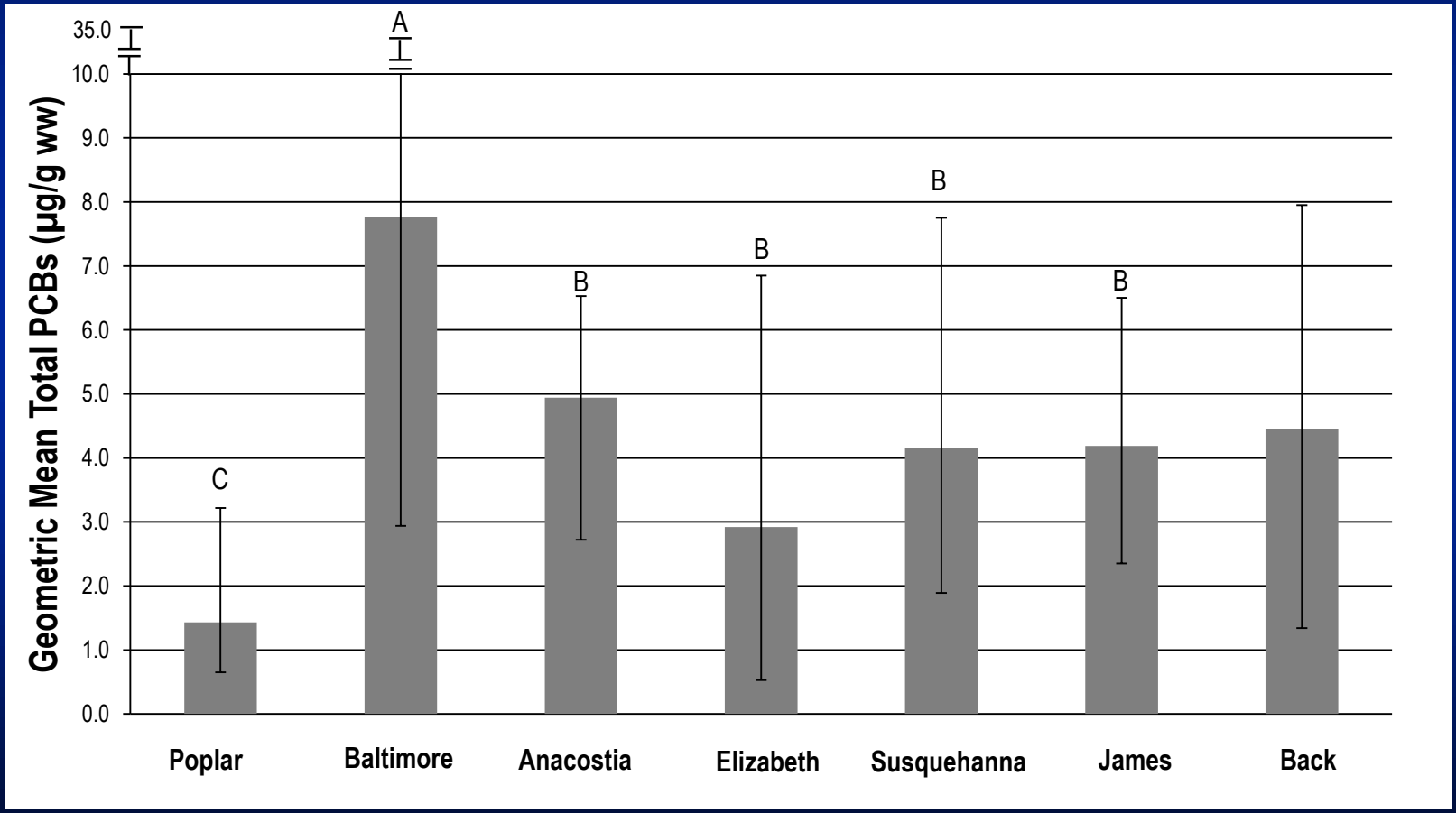
Bars indicate minimum and maximum values on geometric means, letters $p<0.05$

PCBs in Osprey Eggs

- Concentrations at all sites were 3-4 times greater than at Poplar Island. Total PCBs were highest in Baltimore Harbor/Patapsco River (up to 35 µg/g ww in Curtis Creek).



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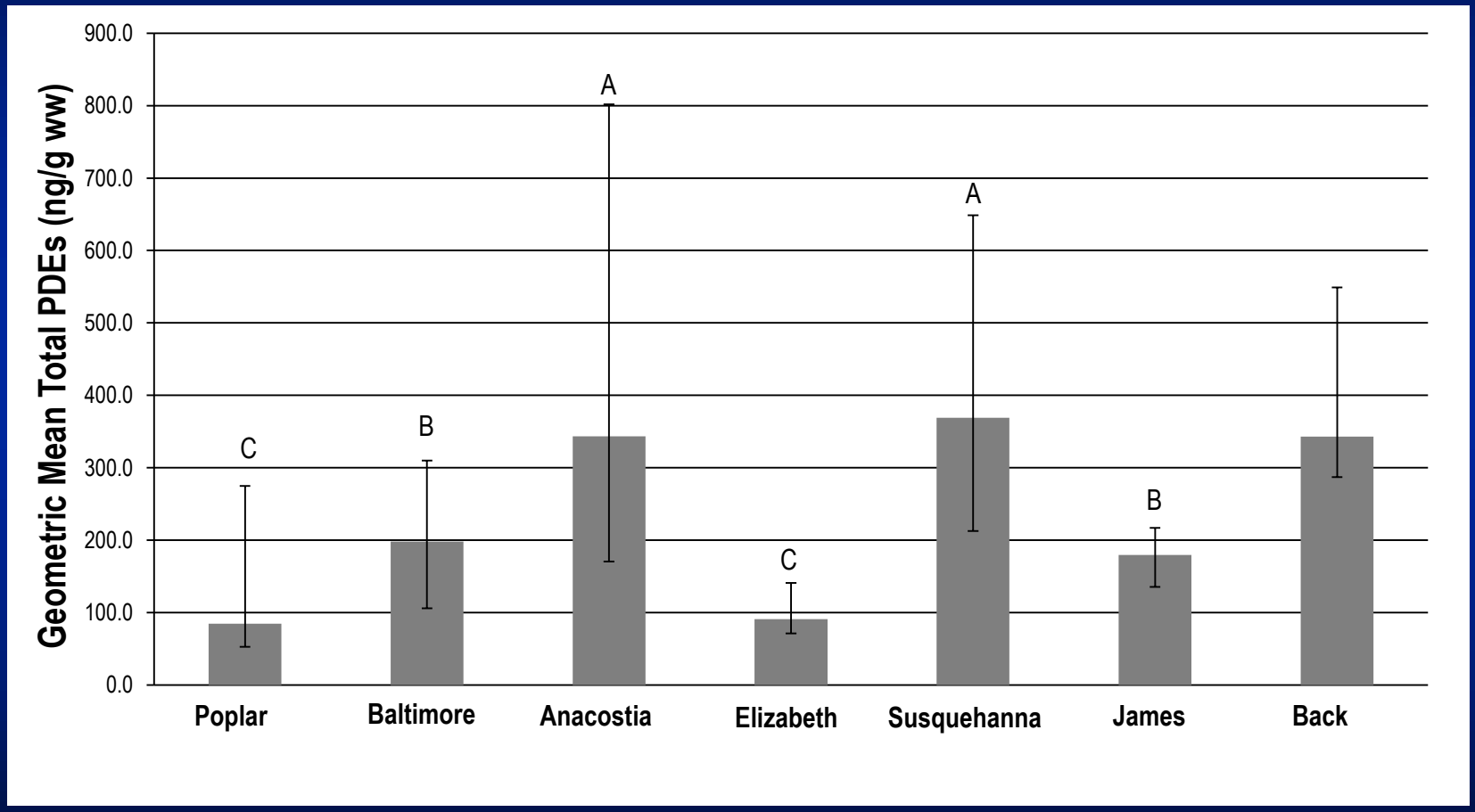
Bars indicate minimum and maximum values on geometric means, letters $p<0.05$

PBDEs in Osprey Eggs

- Total PBDEs highest on the Anacostia/middle Potomac Rivers (up to 802 ng/g ww) and the Susquehanna River (up to 649 ng/g ww).



Bars indicate minimum and maximum values on geometric means, letters $p<0.05$

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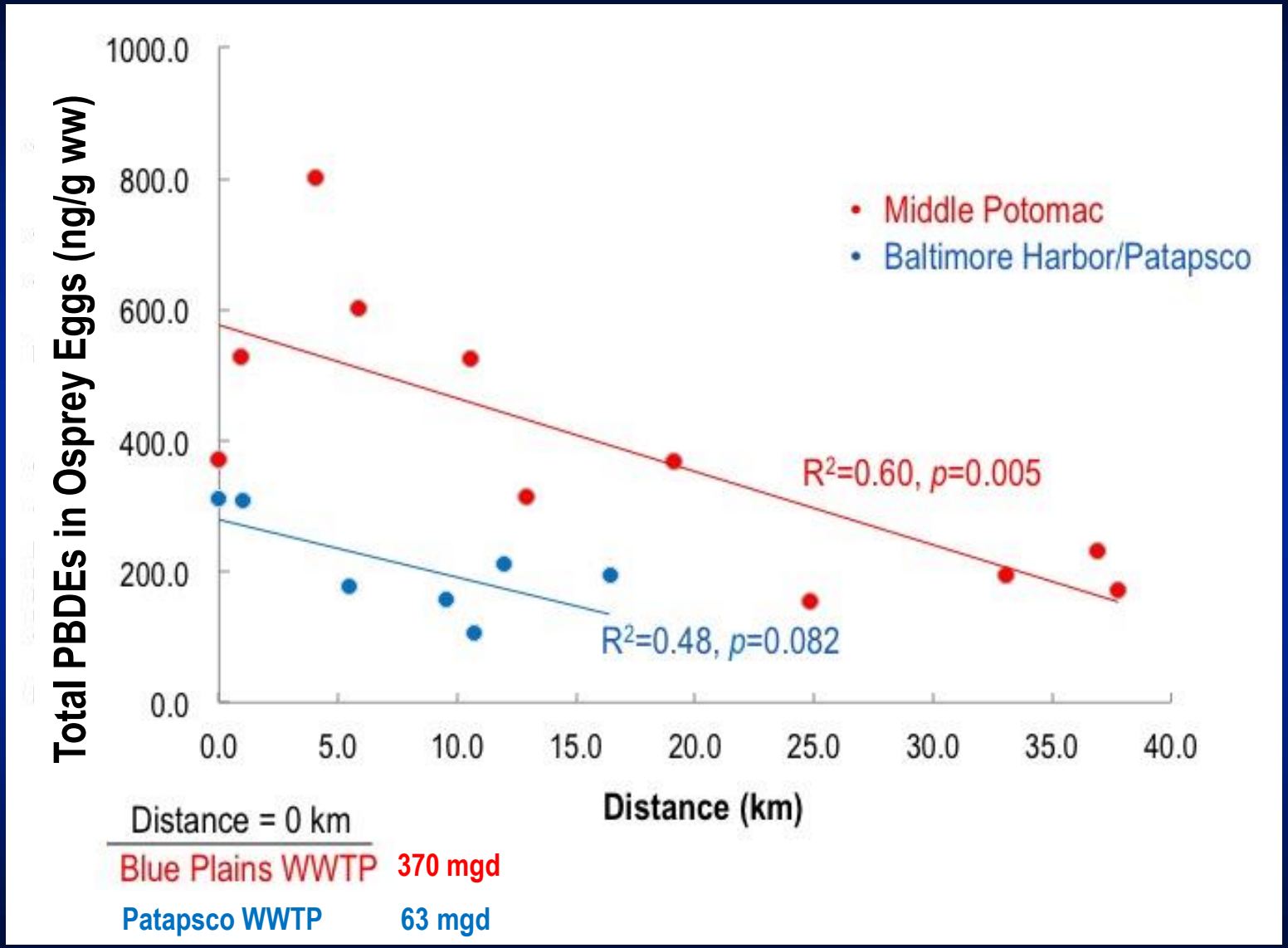
PBDEs in Osprey Eggs: Spatial Patterns



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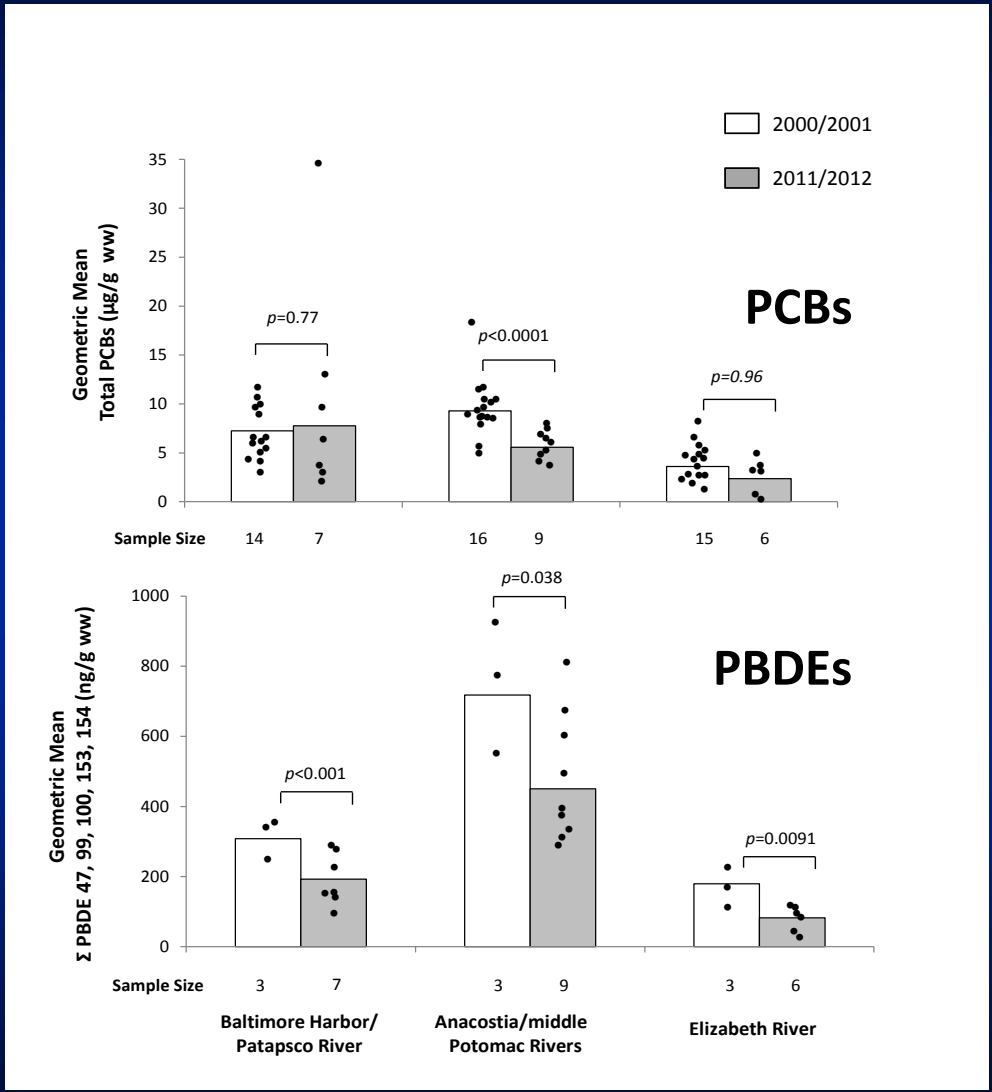
A Decadal Perspective

- *p,p*-DDE residues low (<1.83 µg/g ww) declined ~60% on the Anacostia/middle Potomac since 2000-2001
- PCBs declined significantly on Anacostia/middle Potomac…but not elsewhere
- PBDEs declined significantly across all sites, greatest values reported in vicinity of WWTP



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Alt-BFRs in Osprey Eggs

- Detected 5/5 alt-BRFs (α-HBCD, BTBPE, DBDPE, TBB and TBPH) in eggs
- Most frequently detected in Baltimore Harbor/Patapsco River
- Highest residues of α-HBCD, BTBPE TBB and TBPH on the Anacostia/middle Potomac in the vicinity of Blue Plains WWTP
    - α-HBCD (max: 3.03 ng/g ww)
    - BTBPE (28.7 ng/g ww)
    - TBB (30.3 ng/g ww)
    - TBPH (7.37 ng/g ww)
- All alt-BFRs ~ ¼ of the PBDE flame retardants concentrations

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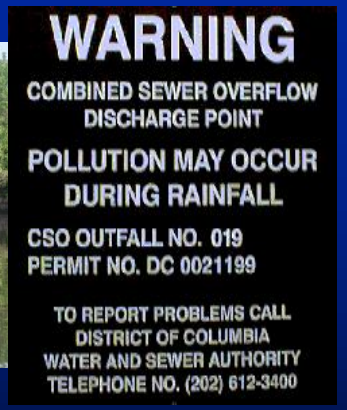
Methoxytricolosan in Osprey Eggs

- Methoxytriclosan detected in all 9 samples from the Anacostia/middle Potomac (1.29-7.40 ng/g ww) & one sample from Baltimore Harbor (5.55 ng/g ww)
  - Highest in vicinity of Blue Plains
  - Second highest in Curtis Creek in vicinity of Patapsco WWTP

- Potential marker of domestic wastewater

Blue Plains WWTP:
Capacity: 370 MGD



Combined Sewer Overflow
Anacostia River

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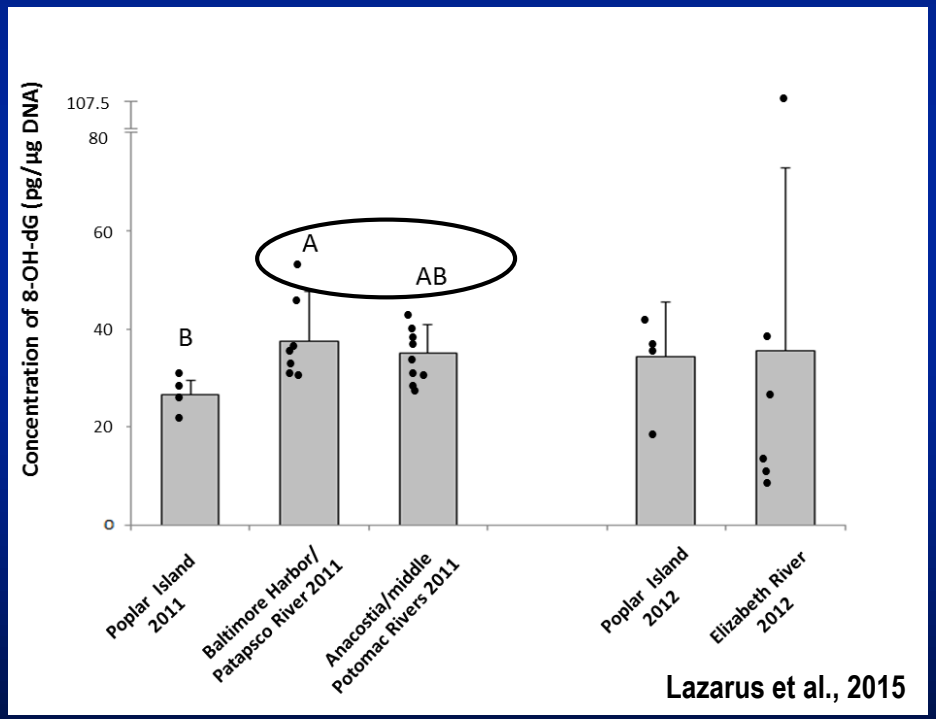
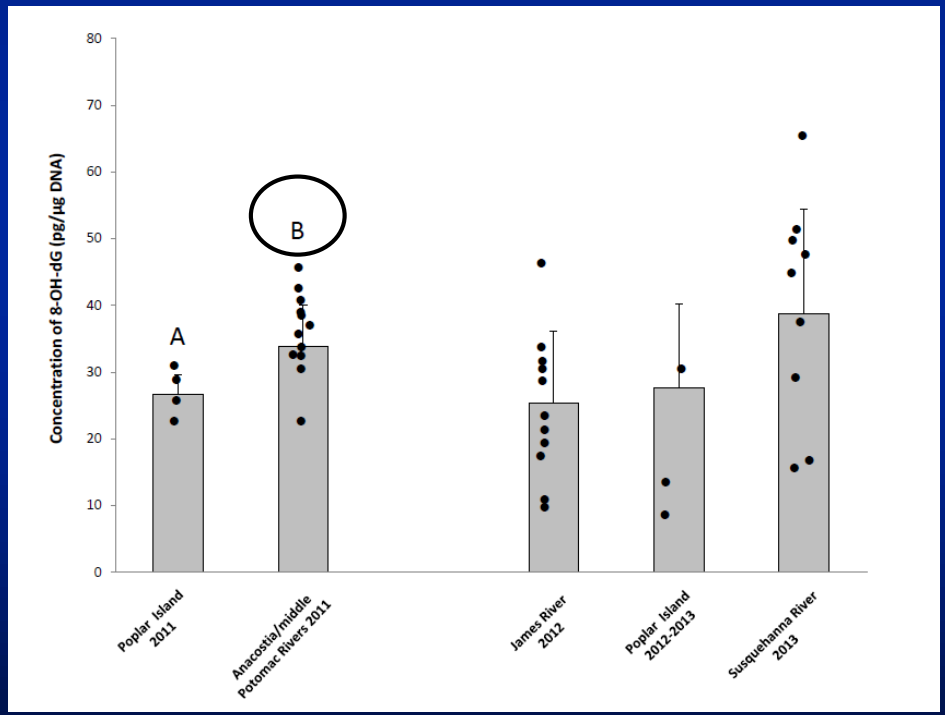
DNA Damage (8'-OH-dG)

- Conducted assays in 2011 and in 2014 (2012 and 2013 data)
- Incidence of DNA damage higher in Baltimore Harbor/Patapsco River and on the Anacostia/middle Potomac River; outlier on Elizabeth R. in 2012, no rationale for its exclusion





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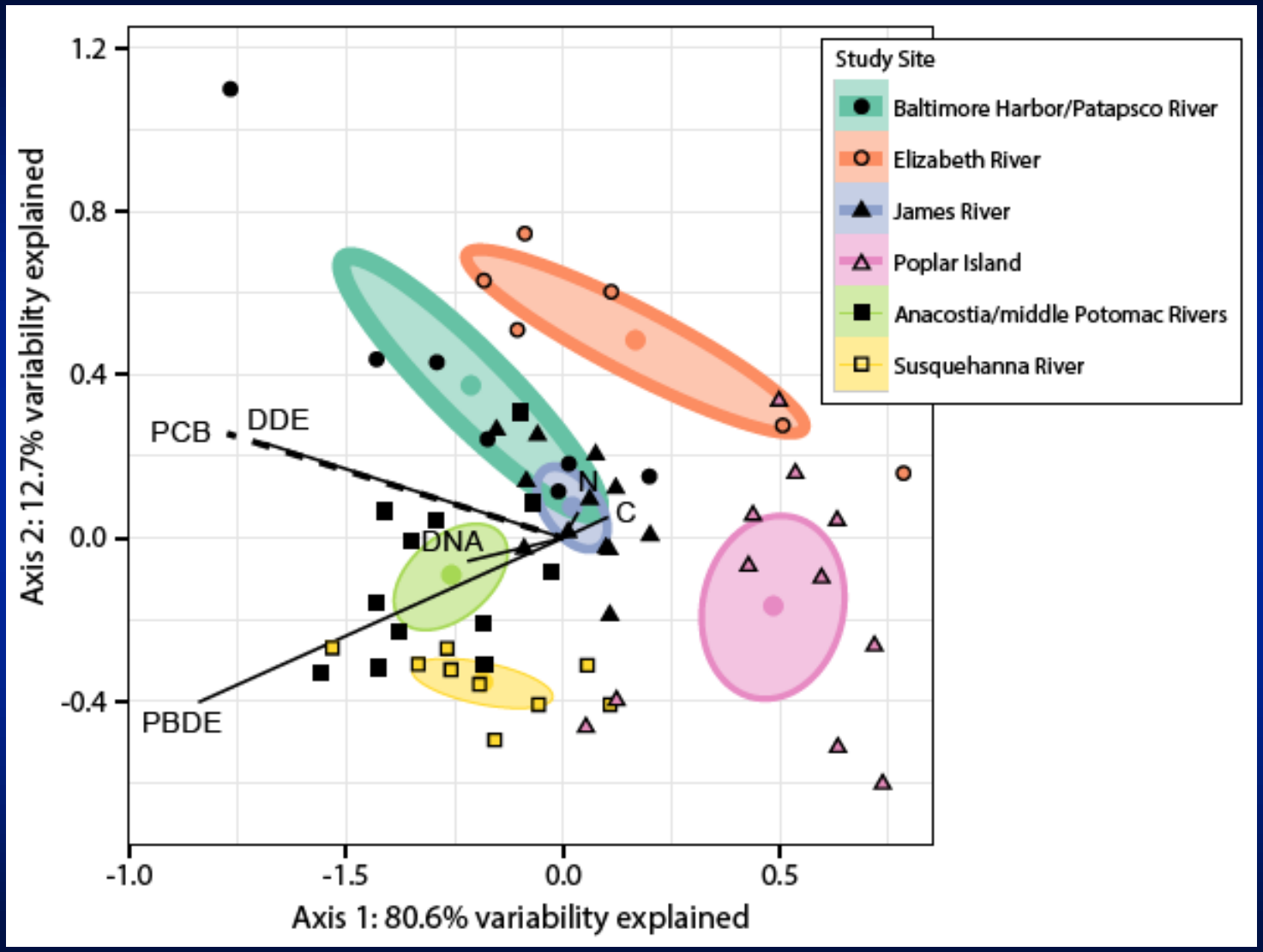
Redundancy Analysis (RDA)



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Reconstruction of Osprey Diet

- > 2000 images from game cameras, direct observations and identification of prey remains

Poplar Island	Potomac River	James River	Susquehanna River
47.8% Rockfish	27.5% Gizzard Shad	19.8% Gizzard Shad	33.2% Gizzard Shad
44.3% Menhaden	23.6 % Catfish	79.3% Carp	30.4% Catfish
4.7% Perch	19.6% Carp		12.4% Carp





<5% Composed of other sp. crappie, needlefish, herring, bass, perch, eel, hogchocker

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Contaminant Transfer in Food Web

Biomagnification Factor (BMF)

total PCBs x 23.4 ww

total PBDEs x 17.9 ww
BDE 47 x 18.9 ww
BDE 100 x 20.5

p,p'-DDE x 16.5 ww

BDE 99 x 14.2 ww

p,p'-DDD, cis-chlordane, trans-chlordane, cis-nonachlor, trans-nonachlor alt-BFRs and methoxytriclosan had BMFs < 5

Similar on both a wet and lipid weight basis

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Pharmaceuticals in the Environment

- ~ 4.02 billion R_x in U.S. per year
- Top prescribed: Zoloft®, Celexa®, Xanax®
- Top 4 grossing: Lipitor®, Plavix ®, Nexium® and Abilify®

Photo credit: www.healthyconsumer.com

- Enter environment from many sources
- Detected in many matrices

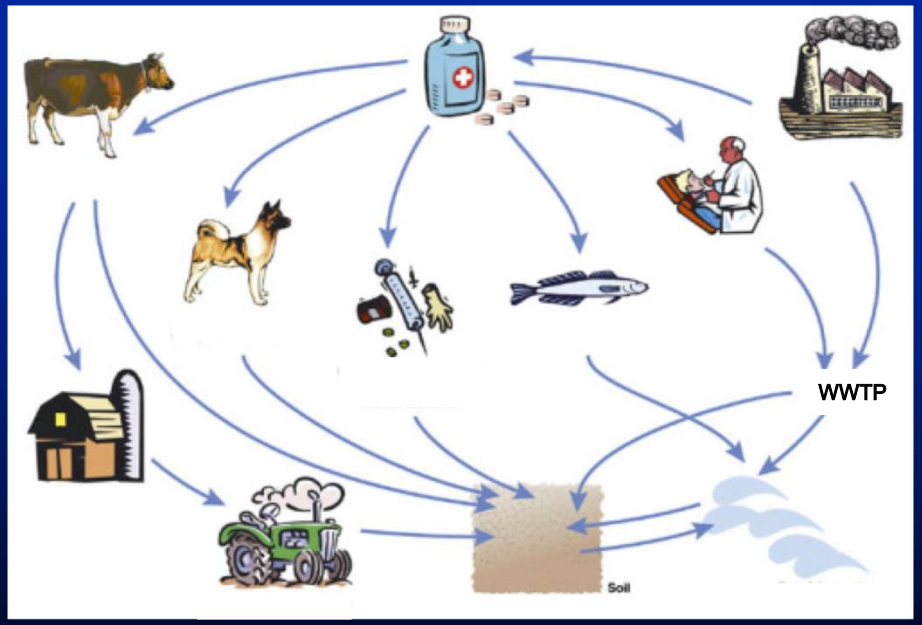

Photo credit: http://www.york.ac.uk/environment

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Pharmaceuticals in Wildlife

Photo credit: www.conservationindia.org

- Secondary poisoning by diclofenac
- Population-level effects
- Species endangerment

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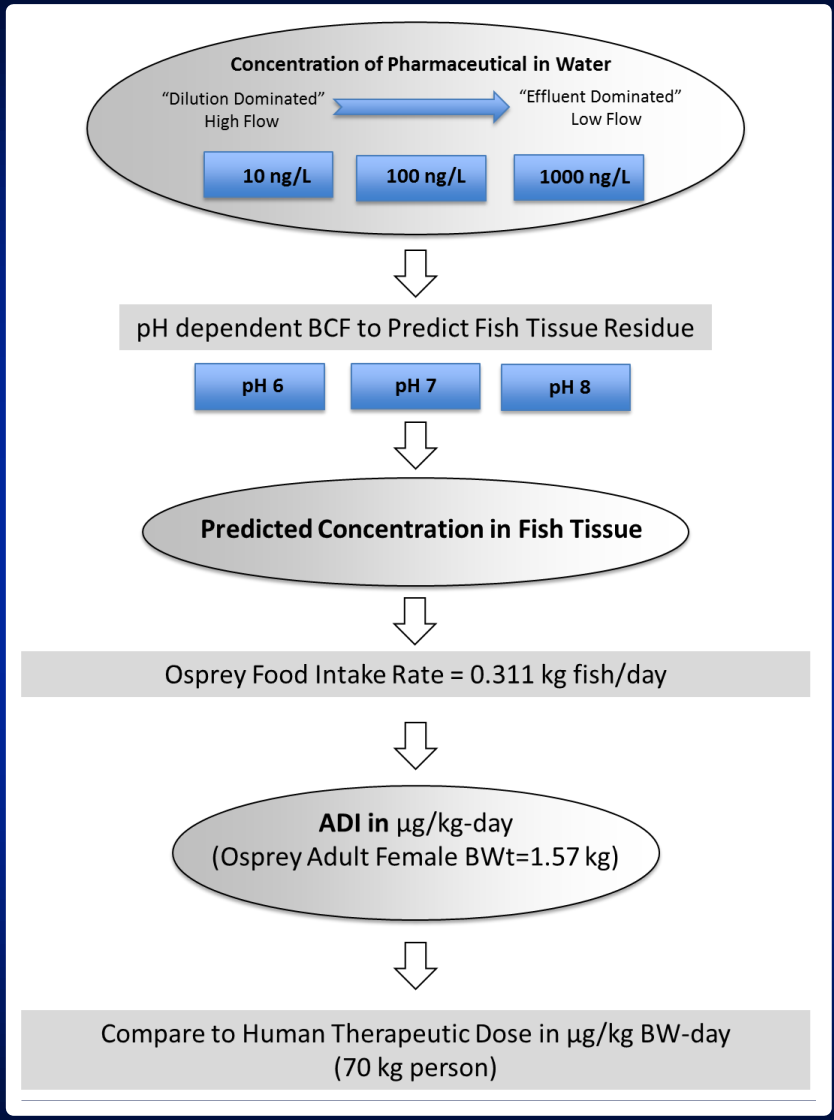
In Silico Assessment

- Environmentally relevant screening-level exposure model in food web framework to identify those that warrant further examination

- Key characteristics for bioaccumulation:
  - Low dilution scenario
  - High bioconcentration factor (BCF) at environmental pH
  - Limited metabolism



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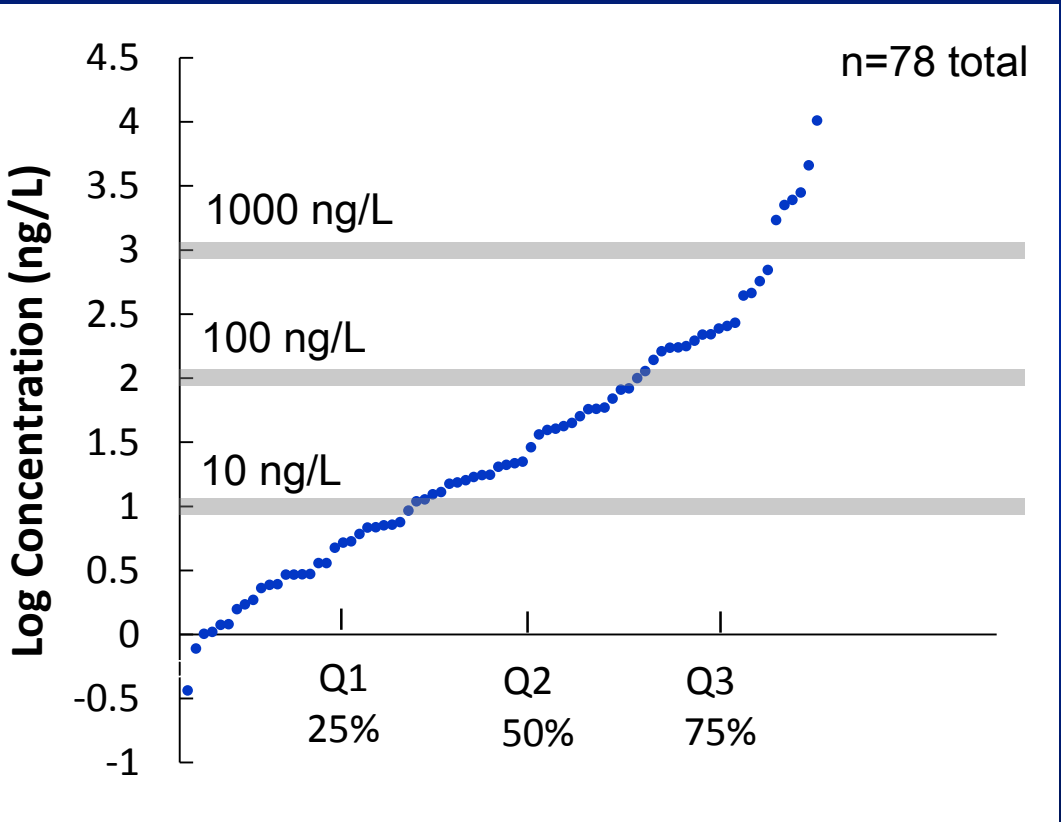
Exposure Assessment Environmentally Realistic

Concentrations of 18/24 analytes detected in water across 4 study sites



Average pH

Poplar Island (1 site)	6.81
Anacostia/Potomac (3 sites)	7.56
James River (3 sites)	7.75
Back River (1 site)	7.88

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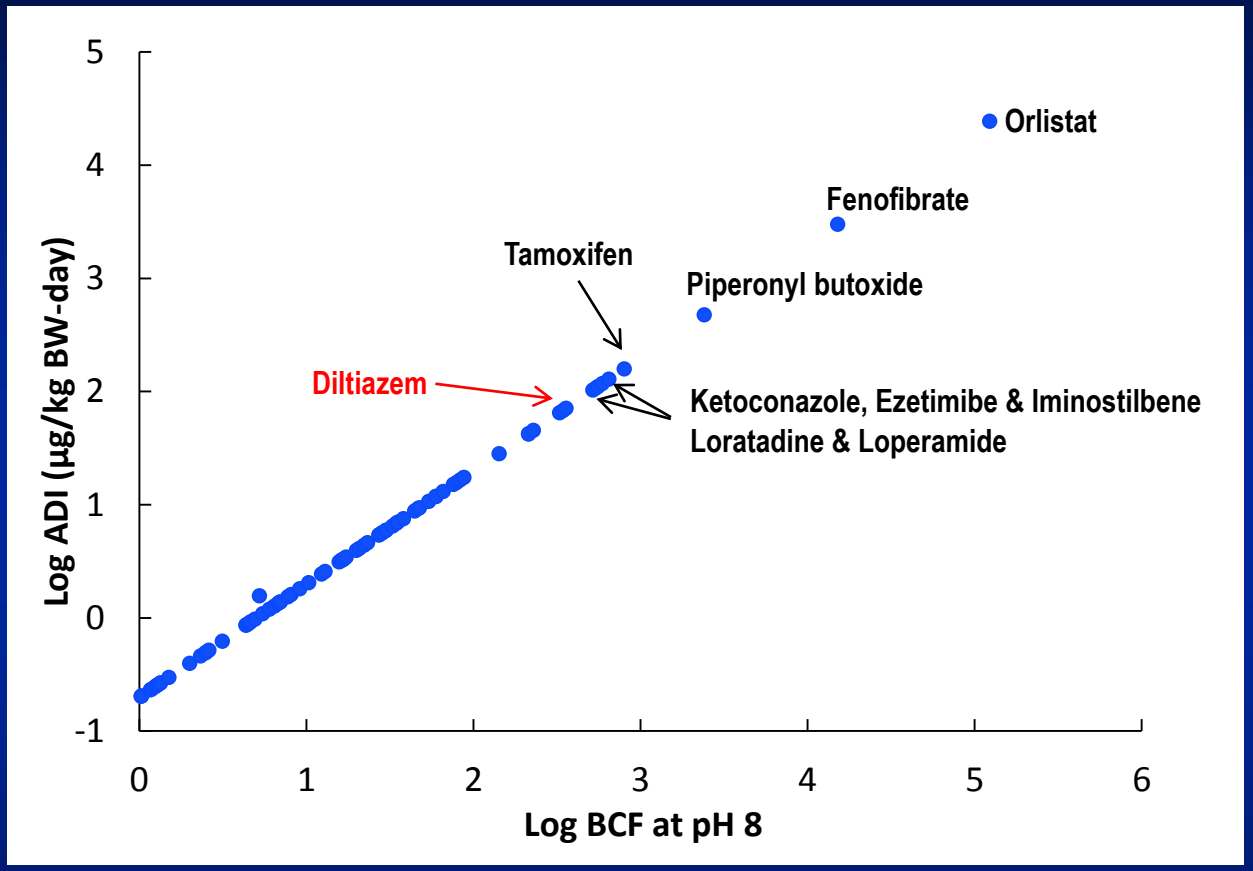
In Silico Assessment

- BCF vs. ADI for 83 analytes BCF > 1.00 at 1,000 ng/L pH 8



TOP 9 ADI > 100 µg/kg BCF > 500	
1	**Orlistat**
2	**Fenofibrate**
3	Piperonyl butoxide
4	**Tamoxifen**
5	Ketoconazole
6	Ezetimibe
7	Iminostilbene
8	Loratadine
9	**Loperamide**

- 4/9 easily available over the counter and 2/9 rank high in sales

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In situ Empirical Findings

Class	Analytes	Water	Fish Plasma						Osprey Nestling Plasma
			Catfish sp.	Gizzard Shad	Carp	White Perch	Rockfish	Atlantic Menhaden	
Analgesic	Acetaminophen	✓							
	Codeine	✓		✓					
Antibiotics	Sulfamethoxazole	✓							
	Trimethoprim	✓							
	Erythromycin	✓							
Anticoagulant	Warfarin	✓							
Antihistamine	Diphenhydramine	✓	✓	✓	✓	✓	✓		
Antihypertensives	Propanolol	✓							
	Diltiazem	✓	✓	✓	✓	✓	✓		✓
	Atenolol	✓		✓					
Anti-inflammatories	Diclofenac	✓							
	Celecoxib	✓					✓		
Antilipemic	Gemfibrozil	✓							
Antiseizure	Carbamazepine	✓	✓	✓	✓		✓		
Artificial Sweetener	Sucralose	✓		✓					
Psychostimulant	Methylphenidate	✓							
	Diazepam	✓							
Parasiticide	Ivermectin								
Stimulant	Caffeine	✓	✓						
Antidepressants	Paroxetine								
	Fluoxetine								
	Norflyoxetine								
	Sertraline								
	Desmethylsertraline								

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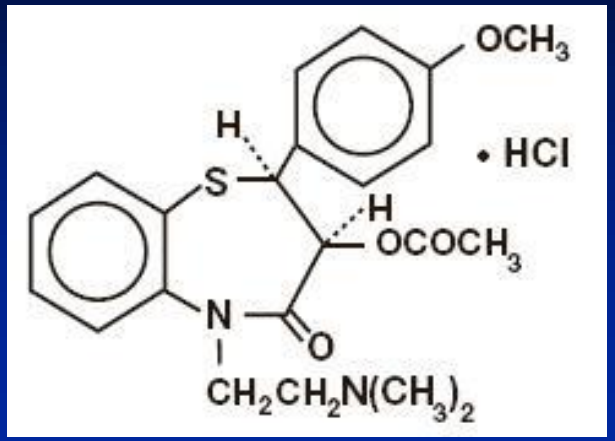
Diltiazem/Cardiazem

- Anti-hypertensive drug
- Ca^{2+} channel blocker



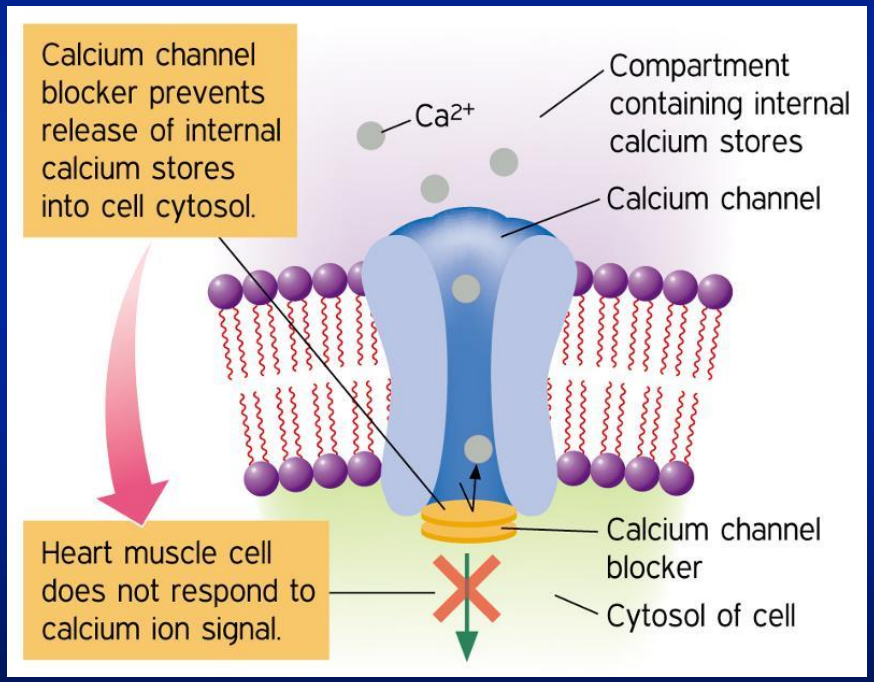


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Photo Credit: http://www.m2c3.com/chemistry/VLI/M2_Topic4/M2_Topic4_print.html

Diltiazem in Nestling Plasma

- Diltiazem detected in plasma from all osprey nestlings

		2011		2012		2013	
	Poplar (2011-2013)	**Baltimore/ Patapsco**	**Anacostia/ Potomac**	**Elizabeth**	**James**	**Back**	**Susquehanna**
Geo Mean (ng/mL)	2.19^{C}	$3.79^{A,B}$	4.52^{A}	0.97^{D}	0.91^{D}	$2.35^{B,C}$	$1.43^{C,D}$
Range	0.605-4.46	2.89-5.11	3.50-**8.63**	0.56-1.32	0.54-1.36	1.05-4.28	1.05-2.09
detects/n	13/13	8/8	13/13	6/6	12/12	7/7	10/10

C_{max} (Human): 30 ng/mL

Superscripts indicate differences in [diltiazem], $p < 0.04$

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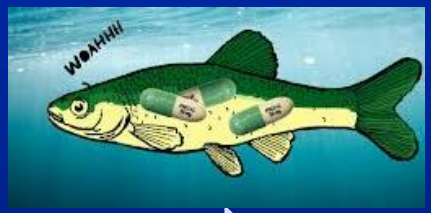
Lazarus et al. 2015

Diltiazem in Wildlife

- Although below HTD, findings indicate it can bioaccumulate water→ fish (26.1x) →osprey (4x)

- Cases of fatalities in humans due to overdose (2,500-8,000 µg/L > HTD of 30 µg/L)

- Paucity of effect threshold data for birds and lower vertebrates make interpretation challenging



- Application of our knowledge of mammalian pharmacology, but many uncertainties

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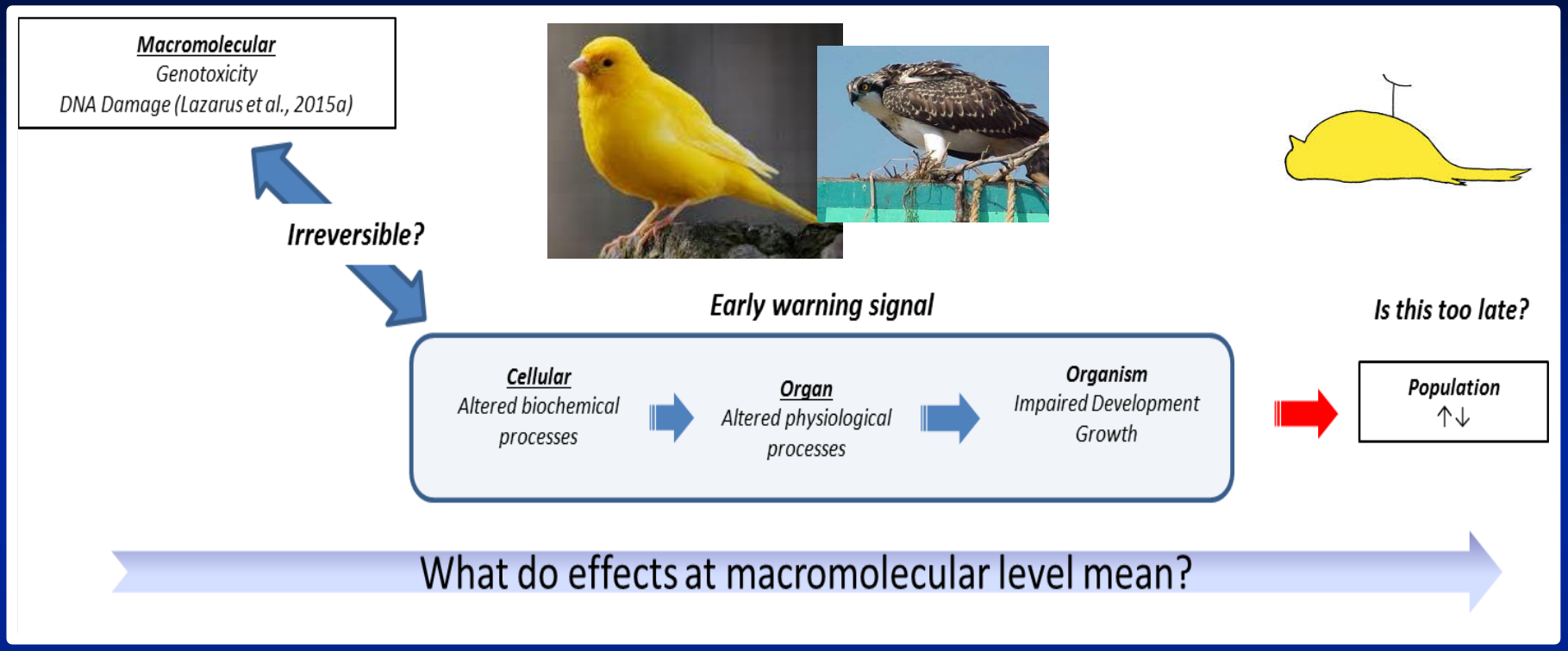
Hierarchy of Effects



Can our "canary in the coal mine" be at the cellular or macromolecular level?

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Final Conclusions

- Reproduction adequate to sustain population
- DDE levels ↓ in eggs & no evidence of shell thinning
- No relationship between contaminants and osprey productivity in the Bay
- PCB concentrations remained unchanged or slightly lower BUT there are a few high values in industrialized areas (i.e., Baltimore Harbor)
- PBDEs ↓ ~40% across all sites; remain elevated near WWTP; <adverse effect levels
- Diltiazem detected in osprey plasma but limited knowledge of effects
  - *In Silco* tools help identify drugs that may warrant further attention
- Marginal evidence of DNA damage in ROCs… could have subtle effects on fitness
- Ospreys have demonstrated their resilience in the face of anthropogenic threats

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