Contaminated Site Clean-Up Information

Welcome to the CLU-IN Internet Seminar

PAH and PCB Toxicity and Adaptation - Lessons Learned from Chronically Exposed Wild Populations

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

Delivered: August 19, 2010, 2:00 PM - 4:00 PM, EDT (18:00-20:00 GMT)

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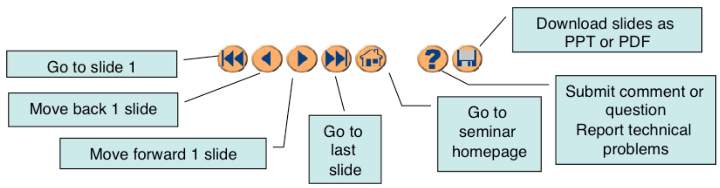
Visit the Clean Up Information Network online at www.cluin.org

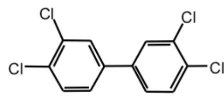
Housekeeping

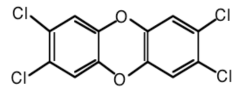
- Please mute your phone lines, Do NOT put this call on hold
- Q&A
- Turn off any pop-up blockers
- Move through slides using # links on left or buttons



- This event is being recorded
- Archives accessed for free http://cluin.org/live/archive/

Mechanisms of Evolved Resistance to Dioxin-like PCBs in Fish Inhabiting a Marine Superfund Site





Mark E. Hahn
with colleagues and collaborators

Woods Hole Oceanographic Institution, Woods Hole, MA
Boston University Superfund Basic Research Program

Superfund Research Program at Boston University

- Theme: Perturbations of reproductive and developmental processes by chlorinated and non-chlorinated organics
- Epidemiological studies/methods (2 biomedical projects)
- Mechanisms (3 biomedical and 4 non-biomedical projects)
  - *Chemicals*: PAHs, PCBs, phthalates, xenoestrogens
  - *Genes*: AHR, PXR, PPAR, ER; CYPs
  - *Models*: mammals and fish (killifish and zebrafish)

http://www.busrp.org/

Differential Chemical Sensitivity

phyla

species

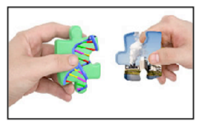
populations

individuals

• What are the mechanisms responsible for differential chemical sensitivity?

• How does chronic, multi-generational exposure to chemicals at Superfund sites influence susceptibility of resident populations?

Gene-Environment Interactions



niehs

Fundulus heteroclitus

(mummichog or Atlantic killifish)

- Species: widely distributed (estuarine)
- Individuals: limited home range
- High genetic diversity
- Large population sizes
- Short generation time (1-2 yr)

- Easily maintained and bred in laboratory
- External development; transparent embryos
- Relatively short development time (15 d to hatch)
- Sensitive to PCB/dioxin toxicity

Reviewed in Burnett, *et al.* (2007)

History: *Fundulus* and Adaptation

Adaptation (temperature): Mitton and Koehn (1975). *Genetics* **79**, 97

Biochemical genetics: Powers and Place (1978). *Biochem. Genet.* **16**, 593

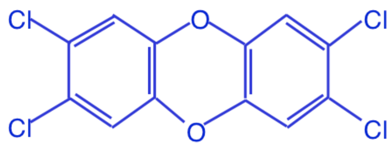
Biotransformation/P450: Burns (1976) *Comp. Biochem. Physiol.* **53B**, 443
Stegeman (1978). *J. Fish. Res. Bd. Can.* **35**, 668

Methylmercury tolerance: Weis, et al. (1981) *Mar. Biol.* **65**, 283

Dioxin resistance: Prince and Cooper (1995) *Environ. Toxicol. Chem.* **14**, 579

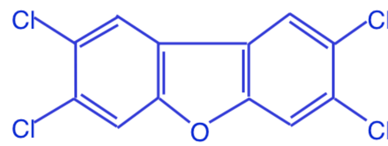
Functional genomics: Oleksiak, Churchill, and Crawford (2002) *Nat. Genet.* **32**, 261

Halogenated and Polycyclic Aromatic Hydrocarbons



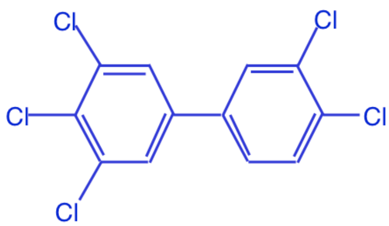
2,3,7,8-TCDD

Chlorinated Dibenzodioxins
(75 congeners)



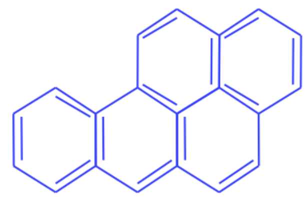
2,3,7,8-TCDF

Chlorinated Dibenzofurans
(135 congeners)



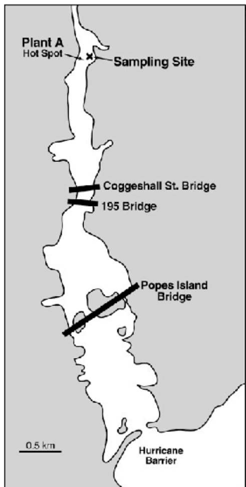
3,3',4,4',5-pentachlorobiphenyl (PCB-126)

Polychlorinated biphenyls
(209 congeners)



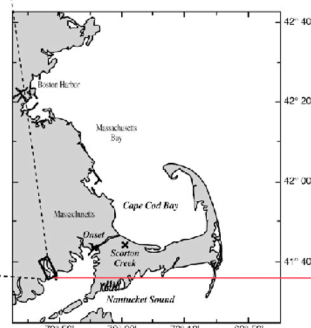
Benzo[*a*]pyrene

Polycyclic aromatic hydrocarbons



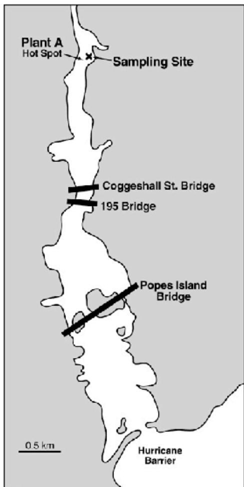
Acushnet River Estuary
(New Bedford Harbor)

Fundulus heteroclitus
(Atlantic killifish)



New Bedford Harbor

(*Fundulus* [PCB] = 272 ppm, dry wt)



Acushnet River Estuary
(New Bedford Harbor)

Fundulus heteroclitus
(Atlantic killifish)

Scorton Creek

(*Fundulus* [PCB] = 0.177 ppm, dry wt)

New Bedford Harbor

(*Fundulus* [PCB] = 272 ppm, dry wt)

Goals

- Characterize the resistance of New Bedford Harbor (NBH) killifish to PCBs (and other dioxin-like compounds)
  - Embryo-larval survival (D. Nacci, U.S. E.P.A.)
  - Altered gene expression:
    - Induction of cytochrome P450 1A (CYP1A) (molecular marker)
    - Genome-wide response

- Determine molecular mechanism of resistance.
  - Identify and characterize genes in the aryl hydrocarbon receptor (AHR) pathway in killifish
  - Test hypotheses about mechanism of resistance

- Investigate costs of resistance.

Approaches for Characterizing the PCB-Resistant Phenotype

- embryo-larval toxicity (LC20)

- *in ovo* EROD (CYP1A) activity (*in vivo*, non-destructive)
- EROD induction in cultured hepatocytes
- Immunohistochemistry (IHC) for CYP1A protein
- real-time RT-PCR for CYP1A RNA

- gene expression profiling
  - microarray
  - deep sequencing (454, Illumina)

PCB resistance in NBH killifish embryos

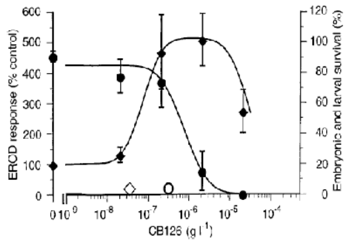


Fig. 5 *Fundulus heteroclitus*. Survival (●) and EROD (◆) responses (means ± SD, $n = 3$ bioassays) to CB126 of West Island fish embryos. Survival response model (means ± SE): $C_0 = 85.06, 10.68$; $\sigma = 0.470, 0.17$; $r^2 = 0.900$. EROD response model: $R_m = 512.68, 7.60$; $C_0 = 99.99, 0.03$; $\sigma = -0.368, 1.58$; $r^2 = 0.799$. Effective concentrations (EC) are also shown (survival: $EC_{20} = 304$ ng l^{-1}, ○; EROD: $EC_{20} = 36$ ng l^{-1}, ◇)

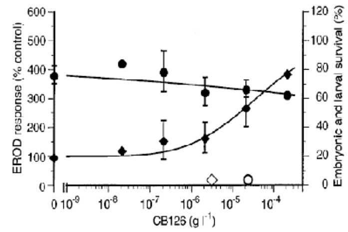


Fig. 6 *Fundulus heteroclitus*. Survival (●) and EROD (◆) responses (means ± SD, $n = 3$ bioassays) to CB126 of New Bedford Harbor fish embryos. Survival response model (means ± SE): $C_0 = 82.36, 4.85$; $\sigma = 4.85, 7.60$; $r^2 = 0.988$. EROD response model: $R_m = 97.83, 480.60$; $C_0 = 99.93, 0.93$; $\sigma = -1.275, 1.22$; $r^2 = 0.945$. Effective concentrations (EC) are also shown (survival: $EC_{20} = 23770$ ng l^{-1}, ○; EROD: $EC_{20} = 3092$ ng l^{-1}, ◇)

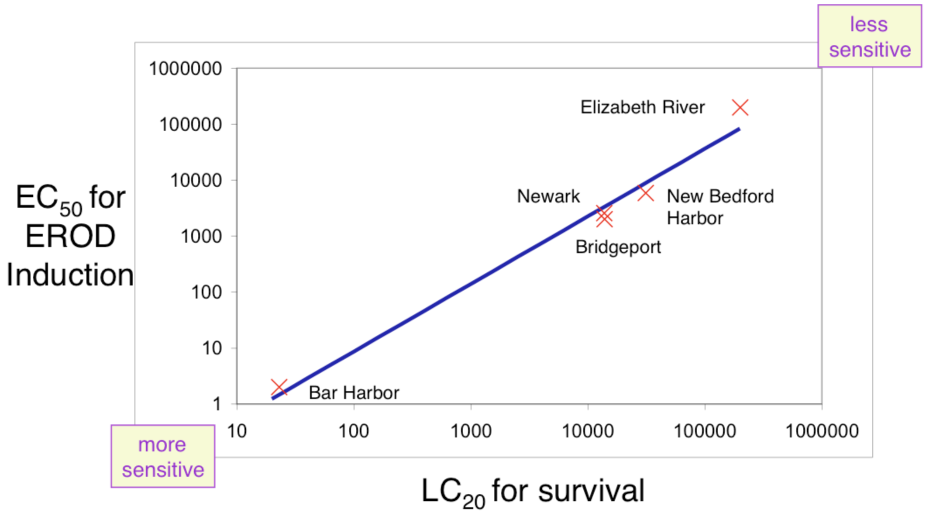
- Embryos resistant to toxicity of PCB-126
  (~78x less sensitive)
- Embryos resistant to EROD (CYP1A) induction
  (~85x less sensitive)

West Island: LC_{20} = 304 ng/L

NBH: LC_{20} = 23,770 ng/L

Nacci *et al.* (1999) *Mar Biol* **134:** 9

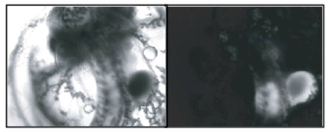
Sensitivity to EROD (CYP1A) induction by PCB-126 predicts sensitivity to lethal and sublethal toxicity of PCB-126



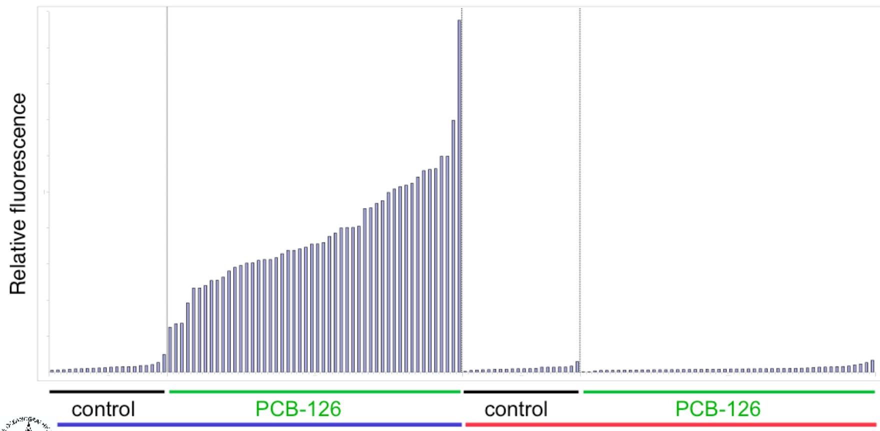
from data in Nacci *et al.* (2010) *Estuaries and Coasts* **33:** 853

In ovo EROD activity (CYP1A) in killifish embryos exposed to PCB-126

Franks, Timme-Laragy, *et al.* (unpublished)
5 dpf, 48 hr exposure



Method from
Nacci et al 1998

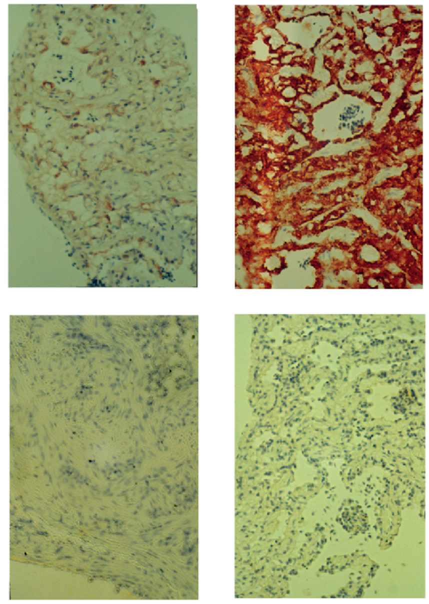


Immunohistochemical staining of CYP1A in killifish heart:

Scorton Creek

New Bedford Harbor

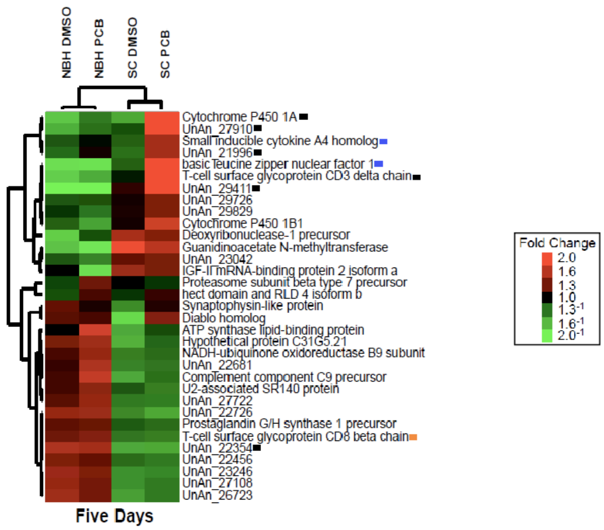
control | TCDF



Bello *et al.* (2001) *Toxicol. Sci.*

Gene expression profiles in PCB-126-exposed killifish embryos:

Genome-wide loss of responsiveness to AHR-dependent signaling

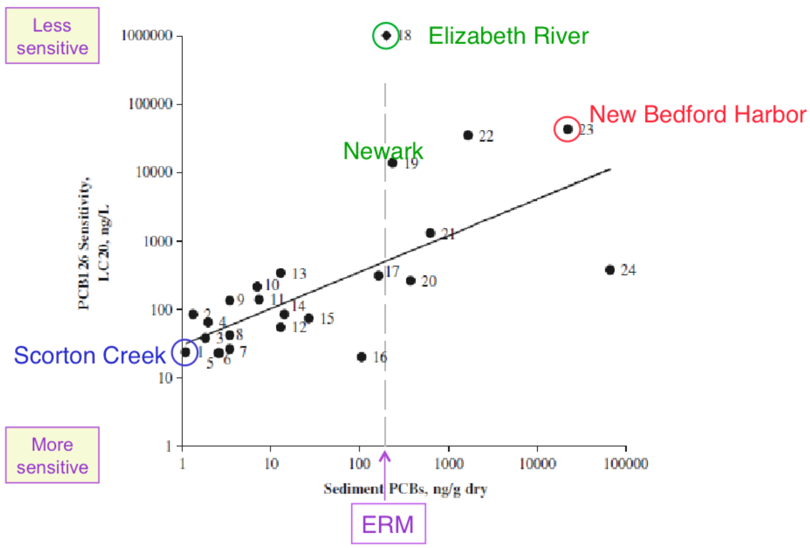


Oleksiak, Jenny *et al.*, manuscript in preparation. See also Whitehead *et al.*, *Mol. Ecol.* (in press)

New Bedford Harbor killifish: Summary of phenotype

- NBH killifish resistant to embryo-larval toxicity of PCBs
- NBH killifish resistant to CYP1A induction by dioxins / PCBs / PAHs
    - transcriptional (mRNA)
    - all tissues & life stages
    - cross-resistance but some chemical specificity (TCDD vs. BNF)
- Genome-wide loss of responsiveness to PCB-126
- Resistance is heritable (genetic)

Sensitivity to PCB-126 is related to sediment [PCB]



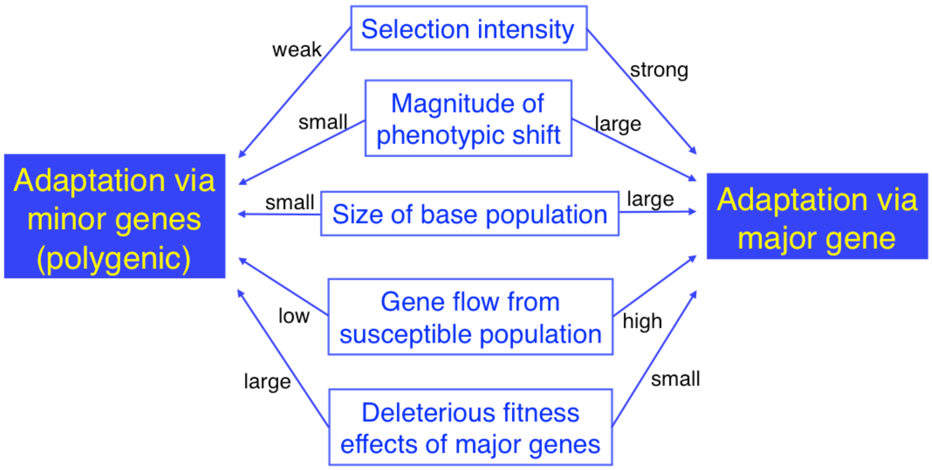
Nacci *et al.* (2010) *Estuaries and Coasts* **33:** 853

What is the mechanism of resistance?

Some approaches used to investigate

- **Comparative gene expression profiling**
  - basal (Fisher & Oleksiak, 2007; Oleksiak 2008; Bozinovic & Oleksiak, 2010)
  - induced (Whitehead *et al.* 2010; Oleksiak *et al.*, in prep.)
- **Population genomics** (Williams *et al.* 2009, 2010)
- **Population genetics**
  - Quantitative trait loci (QTL) (Nacci and colleagues)
  - candidate gene approach (Hahn and colleagues)
- **Experimental manipulations**
  - gene knock-down (Clark *et al.* 2010)
  - model systems (zebrafish) (Billiard *et al.* 2006)

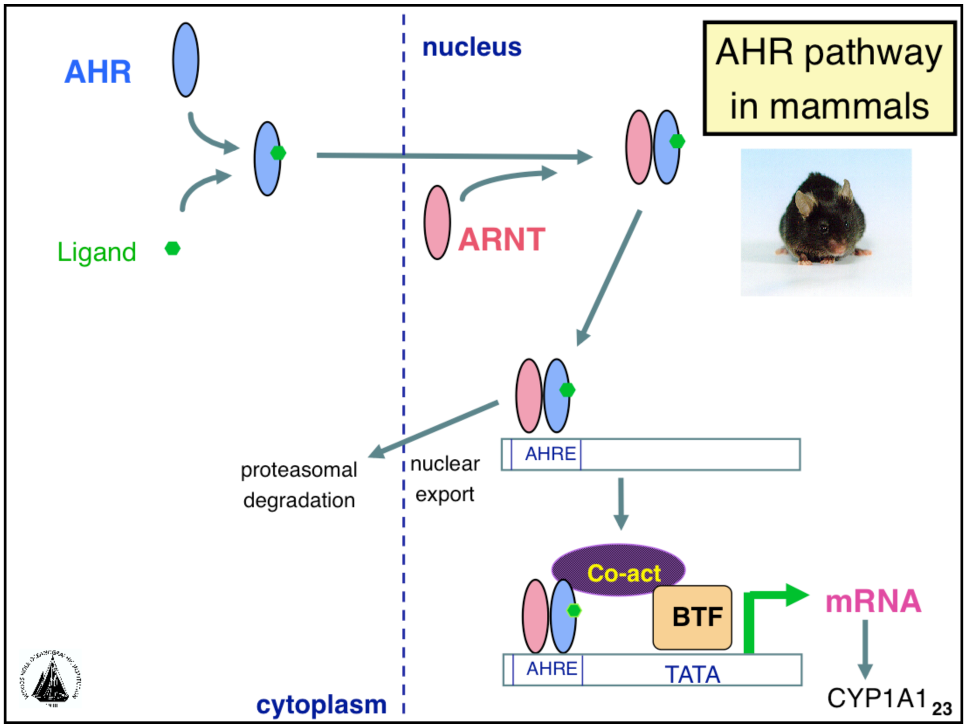
Major gene or polygenic?



Modified from Woods & Hoffman (2000), ch. 9 in *Demography in Ecotoxicology* (Kammenga & Laskowski, Eds.)

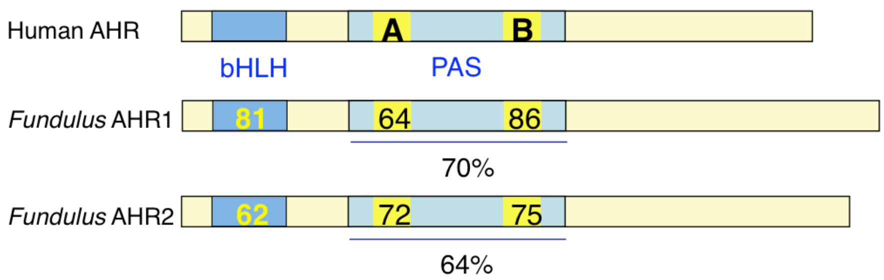
Aryl Hydrocarbon Receptor
(Ah Receptor, AHR, Dioxin receptor)

- Transcription factor / bHLH-PAS gene family
- High-affinity binding (nM) of dioxins, planar PCBs, PAHs
- Ligand-dependent regulation of gene expression
  - Phase I: Cytochrome P450 1A (CYP1A)
  - Phase II: UGT, GST, NQO
  - <u>many</u> others not involved in biotransformation
- Required for toxicity of dioxins and dioxin-like PCBs in mice and fish
- Controls sensitivity to dioxin-like compounds in birds, mouse strains, rat strains


AHR pathway
in mammals
AHR
Ligand
nucleus
ARNT
proteasomal
degradation
nuclear
export
AHRE
Co-act
BTF
mRNA
AHRE
TATA
CYP1A1
cytoplasm
23

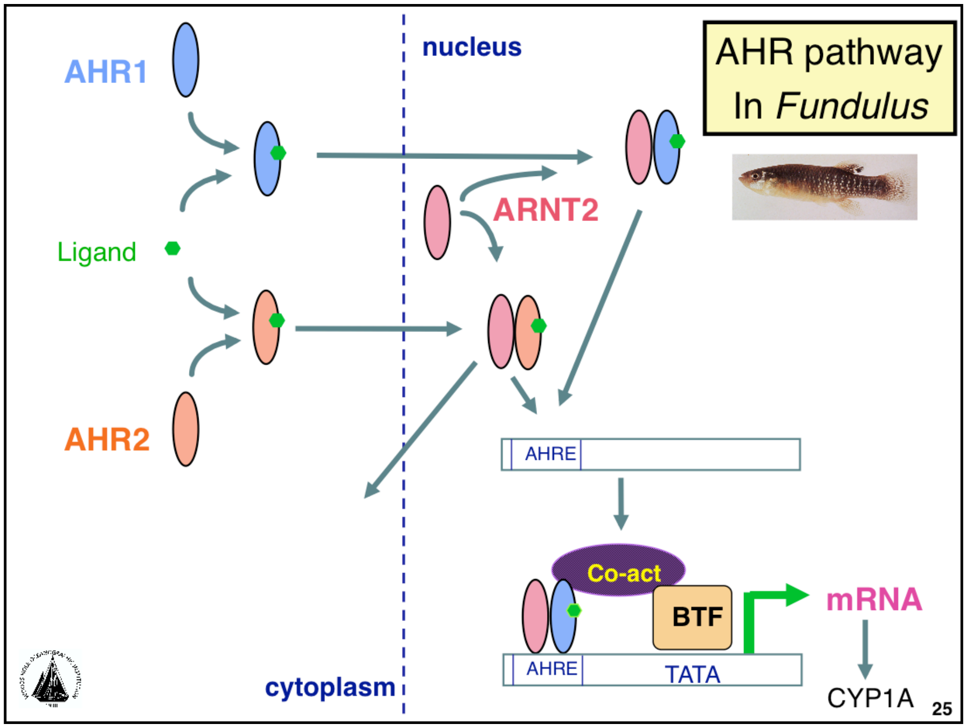
Two *AHR* genes in *Fundulus heteroclitus*

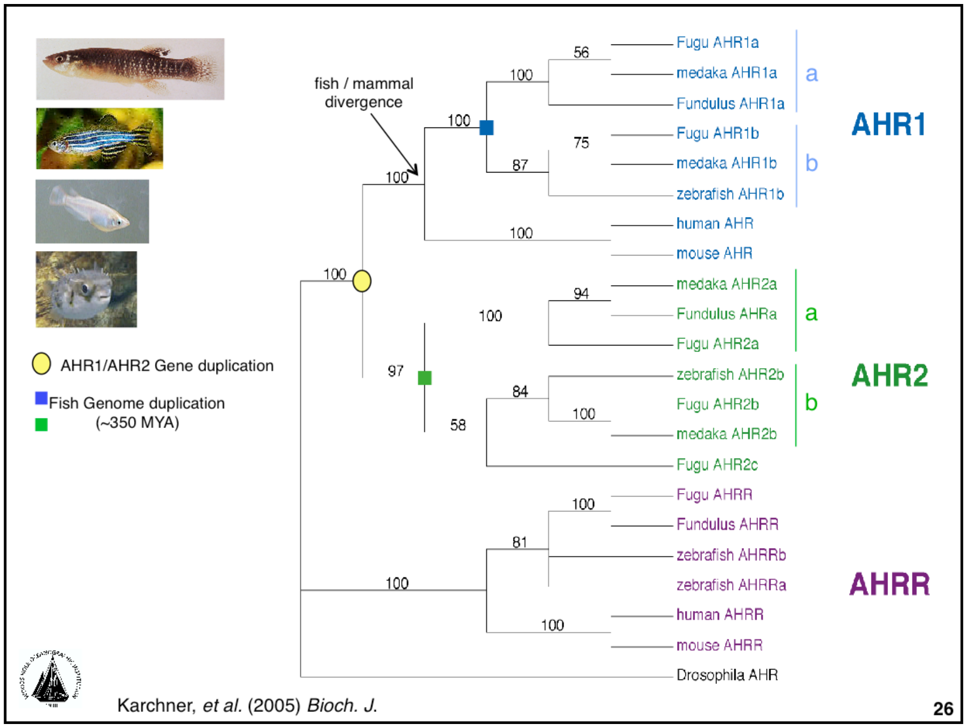
(% amino acid identity compared to human AHR)



- Both exhibit specific binding of [^{3}H]TCDD
- Both exhibit ligand- and ARNT-dependent binding to AHRE sequences
- Both transcriptionally active
- Different tissue-specific patterns of expression

Hahn, *et al.* (1997) *PNAS*; Karchner, *et al.* (1999) *J. Biol. Chem.*


AHR pathway
In *Fundulus*
nucleus
AHR1
ARNT2
Ligand
AHR2
AHRE
Co-act
BTF
mRNA
AHRE
TATA
cytoplasm
CYP1A
25



Discovery of 2 AHRs in Fundulus led to more extensive exploration of AHR diversity and evolution in fishes.

Found that AHR1/AHR2 gene duplication preceded divergence of lineages leading to fish and tetrapods, but that AHR2 lost from mammals.

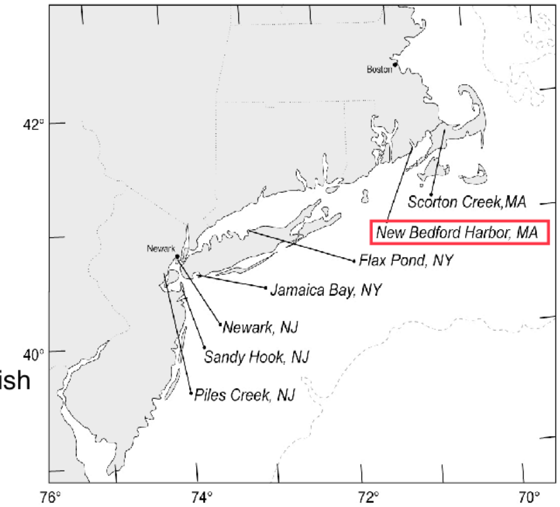
AHRs in fish underwent additional diversification associated with fish-specific genome duplication.

Fundulus AHR signaling pathway, targets, and related genes identified in *Fundulus*

Gene	Reference
CYP1A & promoter	Morrison et al 1995; Powell et al 2004
AHR1, AHR2	Hahn et al 1997; Karchner et al 1999
ARNT2	Powell et al. 1999
AIP (Ara9)	Tanguay et al (unpublished)
AHRR	Karchner et al 2002
HIF-2α	Powell et al 2002
HIF-1α, HIF-3α	Rees et al (in prep)
CYP1C1	Wang et al 2006
CYP1B1, CYP1C2, CYP1D1	Zanette et al 2009
CYP19A1, CYP19A2	Greytak et al 2005
ERα, ERβa, ERβb	Greytak et al 2007
ESTs (various)	Oleksiak et al 2001

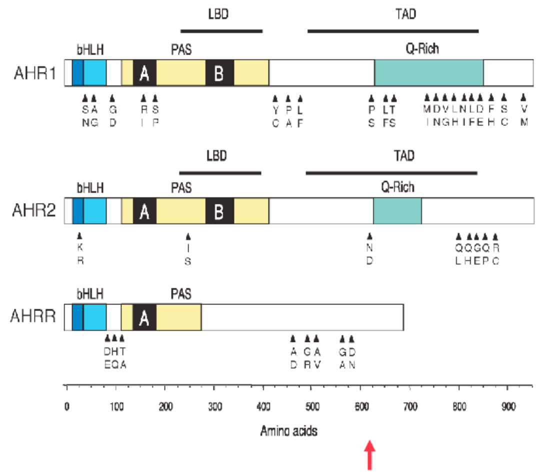
AHR allelic diversity in *Fundulus heteroclitus*

- Collect fish (≥15 per site)
- Isolate RNA
- Sequence AHR1, AHR2, & AHRR cDNAs from each fish
- Identify SNPs
- Infer haplotypes
- Population genetic analysis
- Functional characterization



AHR allelic diversity in *Fundulus heteroclitus*

Reitzel, Karchner, Franks et al. Manuscript in preparation



	#nt coding	# sites	# fish	cSNPs	ns-cSNPs	haplotypes (ns-cSNPs)
AHR1	2832	7	101	45	21	46
AHR2	2853	7	74	30	8	26
AHRR	2040	5	54	38	8	13

AHR Population Genetics

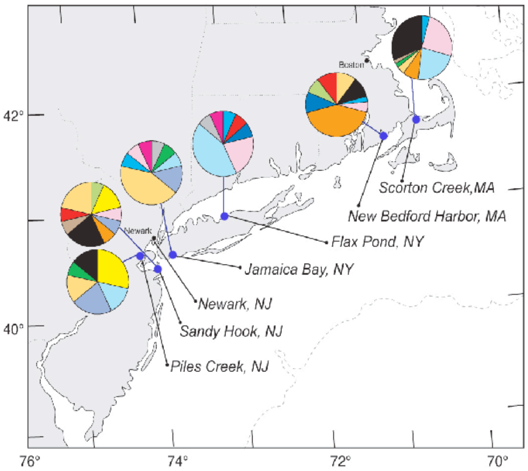
	AHR1	AHR2	AHRR
Site-specific differences in overall genetic diversit y	No	No	No
Population structure?	Yes	Yes	Yes
Isolation by distance?	Yes	No	--

AHR2 pairwise F_{ST} values

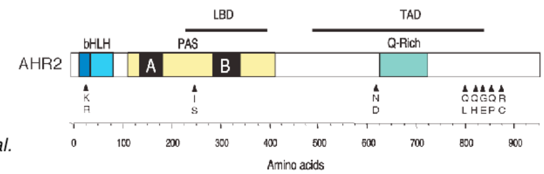
	NBH	SC	JB	FP	PC	SH
NBH	0					
SC	**0.14197**	0				
JB	**0.15459**	**0.17283**	0			
FP	**0.21634**	0.04684	**0.27903**	0		
PC	**0.12756**	**0.12998**	**0.1143**	**0.14203**	0	
SH	**0.16712**	**0.12239**	*0.06531*	**0.11681**	0.01223	0
Newark	0.1604	**0.36783**	*0.65272*	*0.57407*	0.34064	*0.49863*

Reitzel, Karchner,Franks et al.
Manuscript in preparation

AHR allelic diversity
in *Fundulus heteroclitus*



AHR2
haplotypes
(alleles)
(non-synonymous SNPs only)



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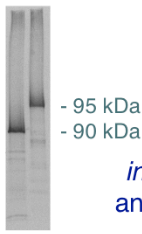
Manuscript in preparation

In vitro AHR2 expression and functional analysis

Isolate and sequence cDNA
for AHR variants

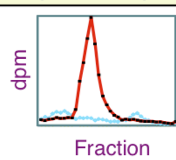
ATGTATGCTGGA
CGCAAGAGGAG
AAAACCCGTCC
AGAGAGCGCTG
AA………

subclone into expression vector

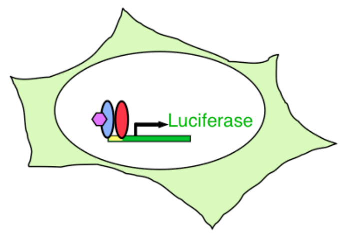


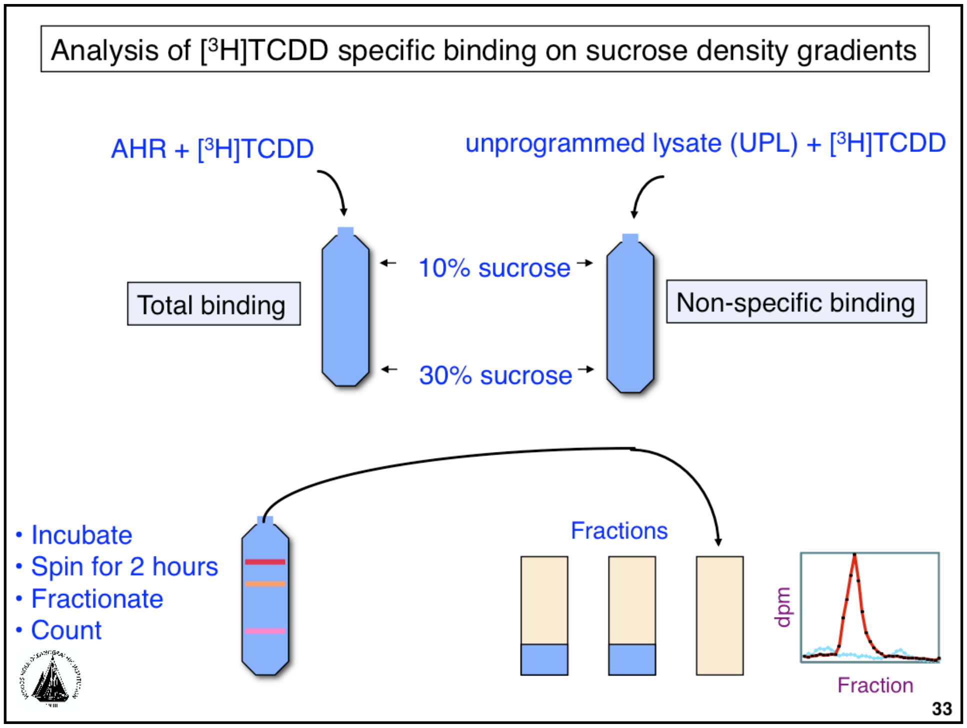
in vitro transcription
and translation (TNT)

Ligand-binding assays
on expressed protein



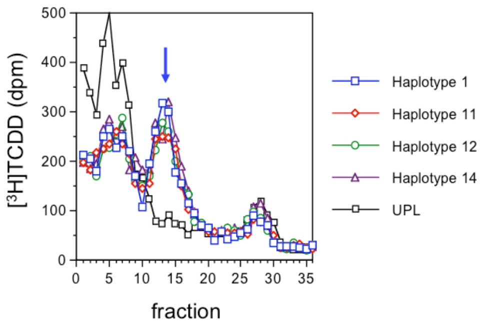
Transient transfection assays
to measure
transactivation of reporter gene

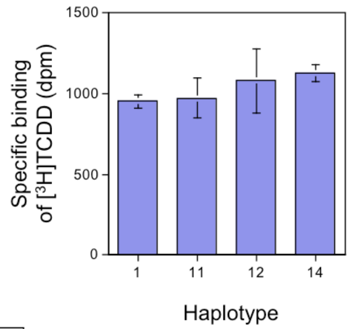


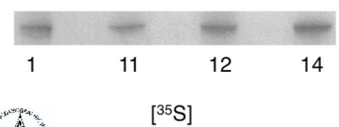


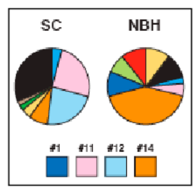
Analysis of [3H]TCDD specific binding on sucrose density gradients
AHR + [3H]TCDD
unprogrammed lysate (UPL) + [3H]TCDD
Total binding
10% sucrose
30% sucrose
Non-specific binding
• Incubate
• Spin for 2 hours
• Fractionate
• Count
Fractions
dpm
Fraction
33

Functional analysis of AHR2 variants:
[^{3}H]TCDD binding



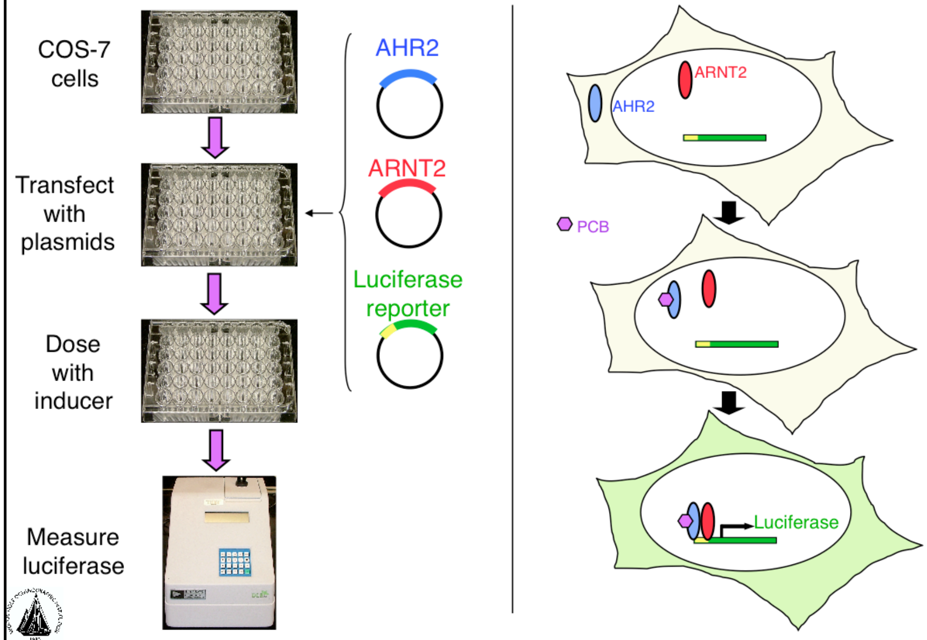






Karchner, Franks et al.
Manuscript in preparation

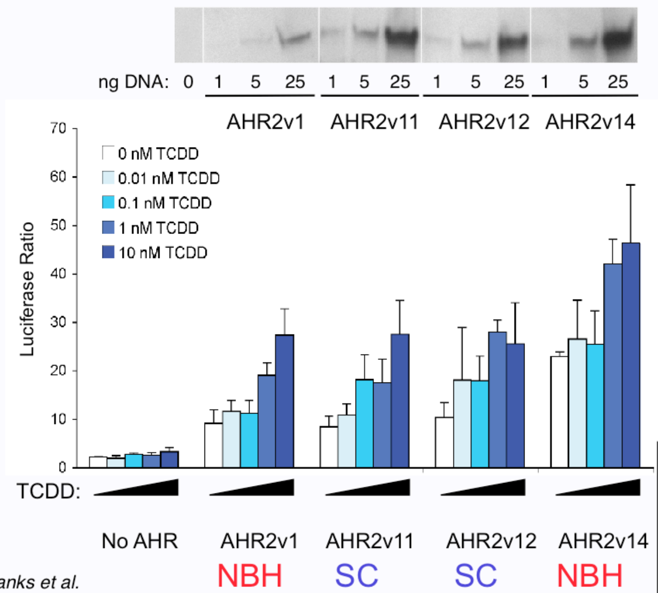
Cell Transfection Assay

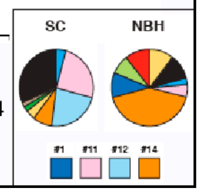


Functional analysis of AHR2 variants:
Transactivation (TCDD)

A.

B.

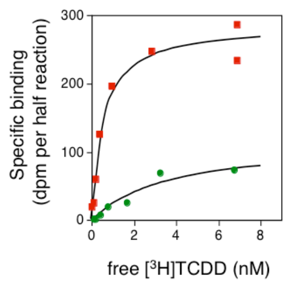




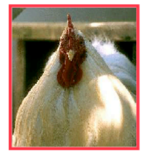
Karchner, Franks et al.
Manuscript in preparation

Are the methods sensitive enough to detect biologically relevant differences in AHR function?

Tern (resistant species) AHR has lower affinity for dioxin

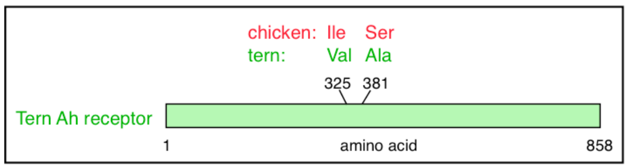


Chicken K_D = 0.52 nM



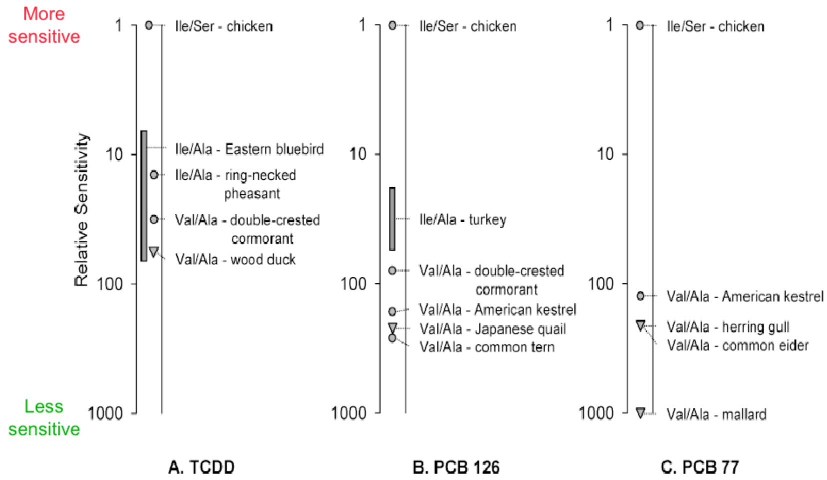
Tern K_D = 3.73 nM

63 amino acid differences; two are critical



Karchner *et al.* (2006) PNAS **103:** 6252

AHR aa325 and aa381 predict sensitivity in birds



Head, Hahn, & Kennedy (2008) ES&T

What are the costs of chemical resistance?

- Enhanced PCB/dioxin accumulation and biomagnification
- Enhanced sensitivity to certain PAH or other chemicals that require induced CYP1 for detoxication
- Altered sensitivity to xenoestrogens (ER cross-talk)?
- Altered sensitivity to oxidative stress (NRF2 cross-talk)?
- Enhanced sensitivity to environmental stressors (e.g. hypoxia; HIF cross-talk)?
- Altered immune function?

Effect of exposure to hypoxia (2 days) and PCB-126 on survival of larval killifish from SC and NBH

Larvae exposed @ 50 dpf

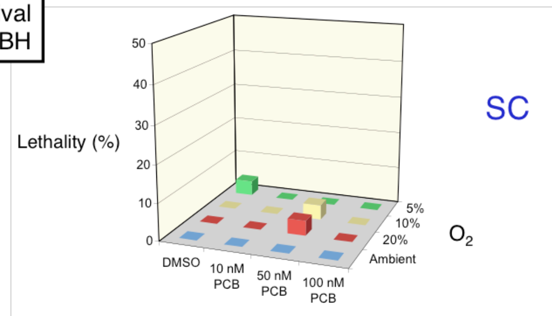
[O_2] (mg/L) (SC, NBH)

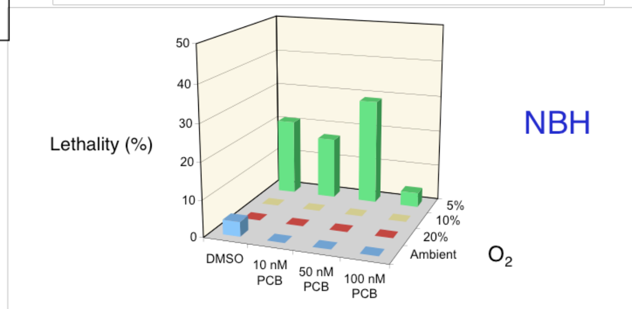
Ambient: 8.13, 7.81

20%: 7.45, 6.90

10%: 4.38, 4.14

5%: 3.26, 3.03



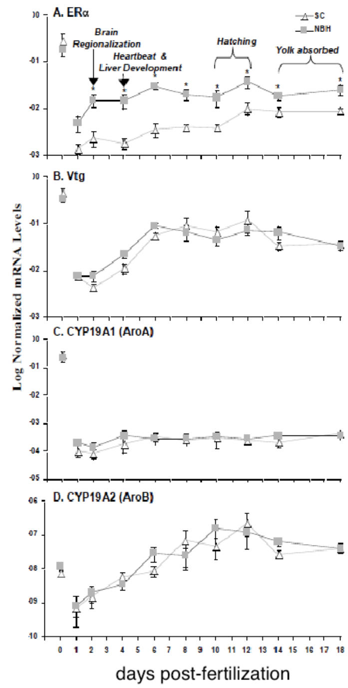


Gene expression analysis:
In progress

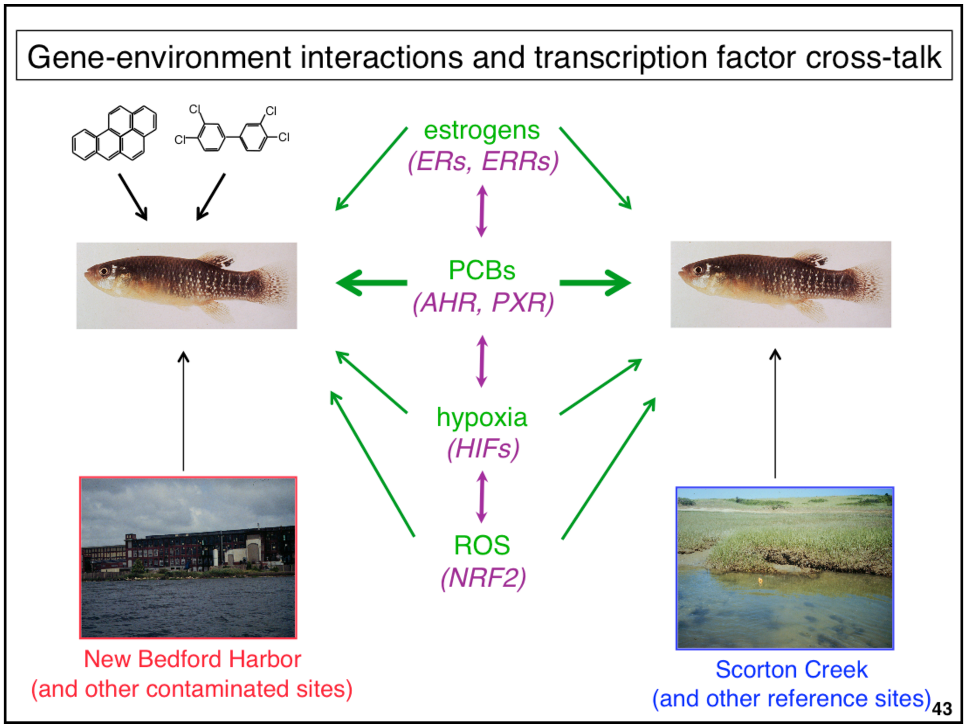
Jenny *et al*., in prep.

NBH fish also show evidence for exposure to xenoestrogens and altered estrogen signaling.

- Plasma vitellogenin
- Hepatic vitellogenin mRNA
- CYP19A2 (aromatase)
- ERα



Greytak et al. (2005) *Aquat Toxicol* **71:** 371
Greytak & Callard (2007) *Gen Comp Endocrinol* **150:** 174
Greytak, Tarrant, Hahn, & Callard (2010) *Aquat Toxicol* **99:** 291



Gene-environment interactions and transcription factor cross-talk
Cl
Cl
Cl
Cl
estrogens
(ERs, ERRs)
PCBs
(AHR, PXR)
hypoxia
(HIFs)
ROS
(NRF2)
New Bedford Harbor
(and other contaminated sites)
Scorton Creek
(and other reference sites)
43

Take-home points

- Long-term exposure can lead to evolved resistance to dioxin-like compounds.
- There is cross-resistance to compounds that act via same mechanism, but…
- The degree of resistance is compound- and life-stage-specific.
- In resistant populations, EROD/CYP1A expression is NOT a good biomarker of exposure.
- Sensitivity to induction of EROD/CYP1A and other genes is a good marker for sensitivity to lethal and sublethal toxicity.
- Mechanistic studies can promote understanding and predictive capability, but mechanisms are not always easily determined.
- Fish often have duplicated genes that complicate extrapolation of molecular mechanisms from results in laboratory rodents.
- The beneficial effects of resistance may be offset by costs involving altered sensitivity to other stressors.

Woods Hole Oceanographic Institution

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Sue Bello
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John Stegeman

Univ. of Miami

Margie Oleksiak

U.S. E.P.A.

Diane Nacci
Denise Champlin

Duke University

Rich Di Giulio
Joel Meyer

Environment Canada

Sean Kennedy
Jessica Head
Reza Farmahin

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NOAA Sea Grant (Woods Hole)

Mechanisms of PAH Developmental Toxicity and Evolved Resistance: The Elizabeth River Story

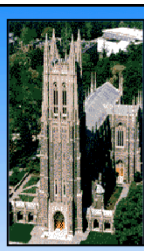
Richard Di Giulio

August 19, 2010

NICHOLAS SCHOOL OF THE ENVIRONMENT
DUKE UNIVERSITY

integrated toxicology environmental health program @ DUKE UNIVERSITY

sbrp Superfund Basic Research Program



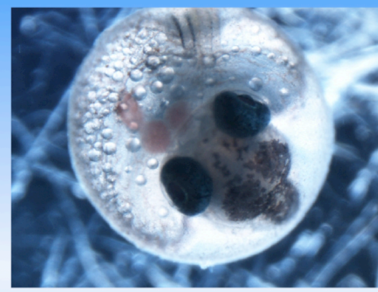
The Duke University Superfund Research Center
(established in 2000)

Theme: Developmental Effects and Later Life Consequences of Superfund Chemicals

3 biomedical and 2 non-biomedical projects

Chemicals: organophosphates, PAHs, PBDEs and nanomaterials

Case study: the Elizabeth River population of the Atlantic killifish, *Fundulus heteroclitus*



The *Fundulus* model:

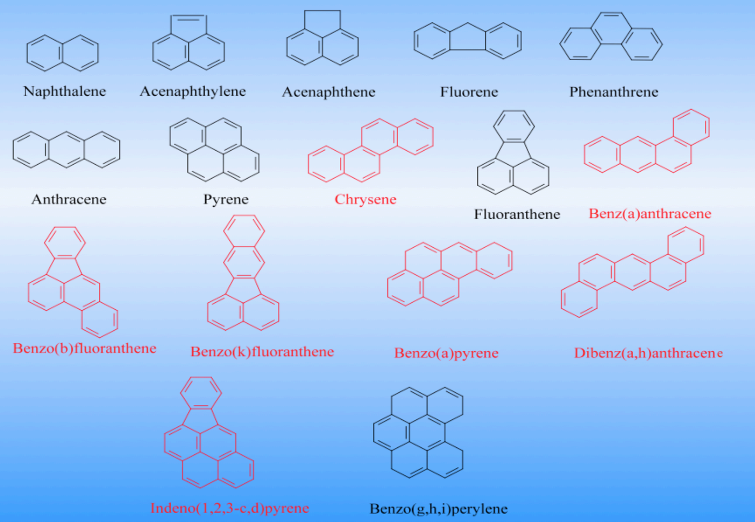
- **plentiful ecological, physiological and genetic information**
- **important in estuarine food webs**
- **widely distributed, but limited home range**
- **readily cultured, rapid development (~ 14 days to hatch)**
- **adults small enough (4-8 g) but large enough for biochemistry**
- **large (2 mm), transparent eggs**
- **gene sequencing well underway**

49
York River
Chesapeake Bay
King's Creek (ref)
James River
Atlantic Ocean
Elizabeth River
Microsoft

Atlantic Wood Industries, Elizabeth River

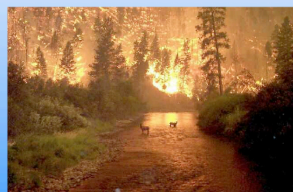
- Heavily contaminated with hydrocarbons derived from creosote (mainly PAHs)
- Site of wood treatment facilities since 1926
- Classified as Superfund site in 1990
- Sediment extracts highly toxic to *Fundulus* embryos & larvae from clean sites

Polycyclic Aromatic Hydrocarbons



EPA Class B2 Carcinogens

Sources of PAHs: Ubiquitous



And oil spills!

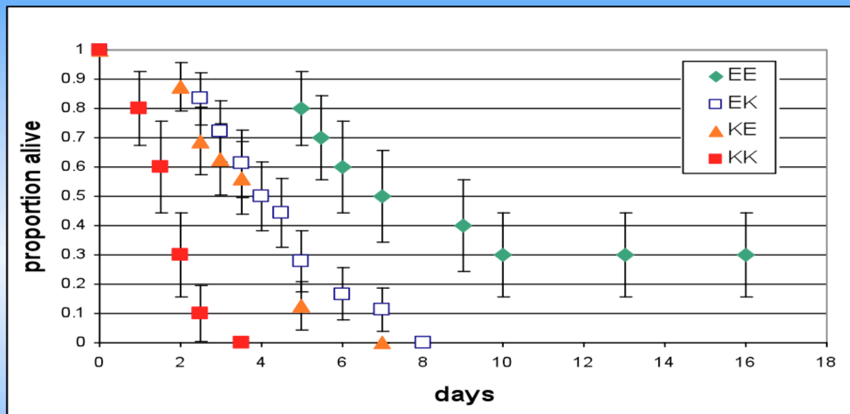
The Elizabeth River *Fundulus* population:

- Displays elevated rates of liver cancer
- But ecologically thriving
- And embryos resistant to acute effects of sediments

Questions

- Are adaptations displayed by Elizabeth River *Fundulus* genetically-based?
- What biological effect is driving the adaptation?
- What are the evolutionary consequences of the adaptation?
- What mechanisms underlie toxicities and adaptations?
- What are the ramifications for environmental management and policy?

Larval survival in ER sediment pore water

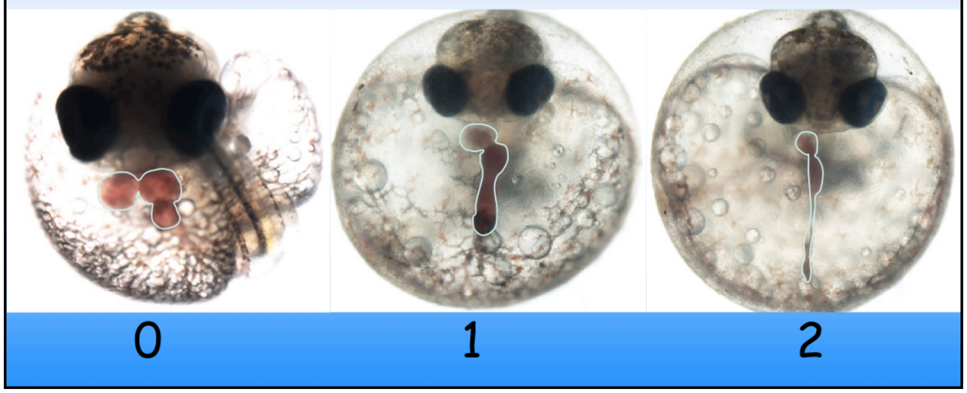


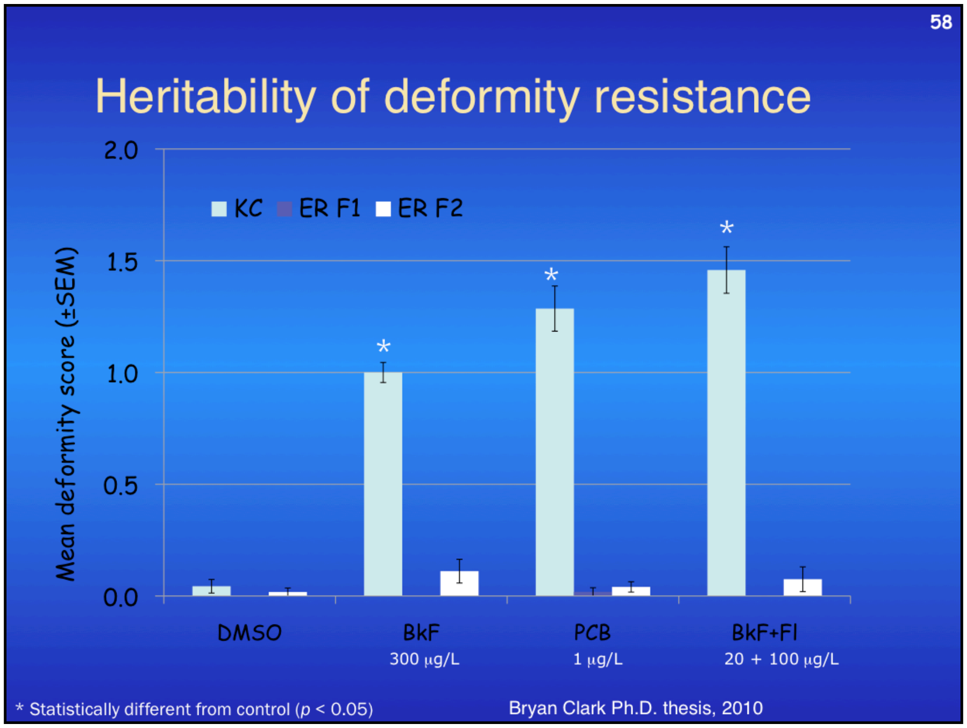
Survival analysis indicates that EK and KE hybrids are indistinguishable from each other (but distinct from EE and KK)

Meyer et al., 2002, *Toxicol Sci* 68:69-81

PAH-induced cardiac teratogenesis

- Similar in appearance to blue-sac caused by DLCs
- Caused by some individual PAHs, but certain mixtures much more potent




58
Heritability of deformity resistance
Mean deformity score (±SEM)
2.0
1.5
1.0
0.5
0.0
KC
ER F1
ER F2
*
*
*
DMSO
BkF
300 µg/L
PCB
1 µg/L
BkF+Fl
20 + 100 µg/L
* Statistically different from control (p < 0.05)
Bryan Clark Ph.D. thesis, 2010

Earliest report of a biochemical alteration associated with resistance in Elizabeth River *Fundulus*:

Van Veld and Westbrook. 1995. Evidence for depression of cytochrome P4501A in a population of chemically resistant mummichog (*Fundulus heteroclitus*). Environ. Sci. 3:221-234.

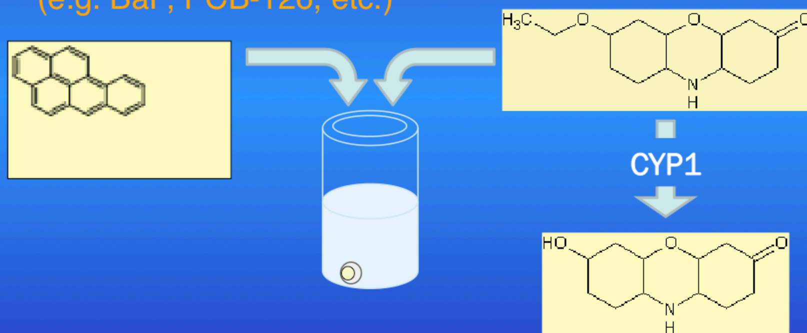
Demonstrated lack of induction in wild-caught ER males vs KC fish, with either 3-MC or ER sediment exposures

Dr. Peter Van Veld
Virginia Institute of Marine Sciences

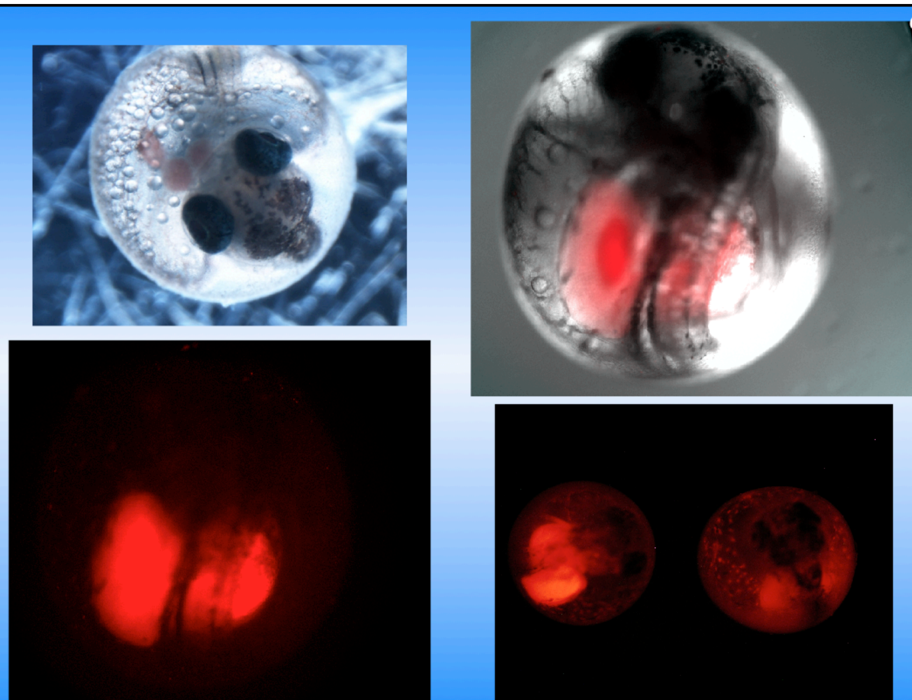
In ovo ethoxyresorufin-*o*-deethylase (EROD) assay

AHR agonist
(e.g. BaP, PCB-126, etc.)

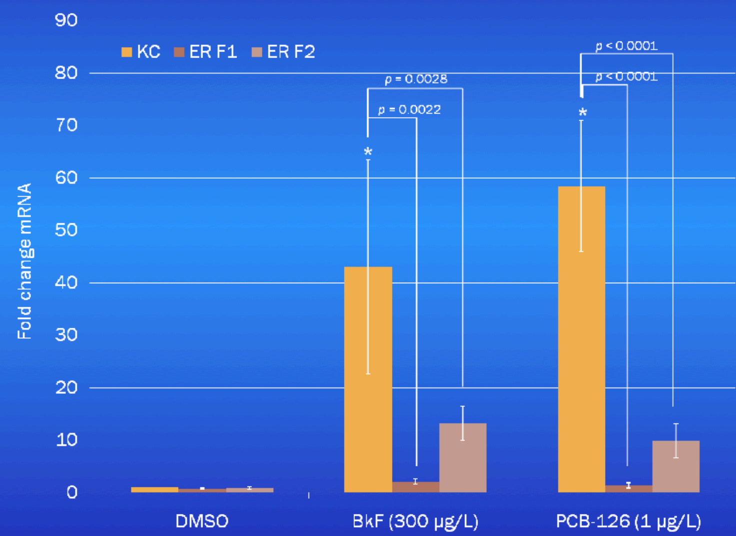
Ethoxyresorufin



Nacci et al, 2005, Techniques in Aquatic Toxicology, CRC



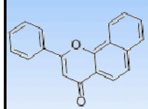
Heritability of resistance to CYP1A mRNA induction



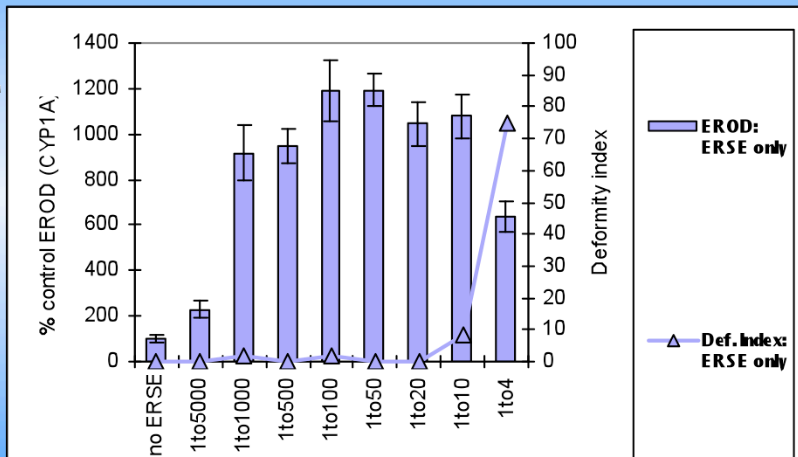
Bryan Clark Ph.D. thesis, 2010

Mechanisms of PAH Toxicity and Adaptation

Effects of 100 μg/L α-naphthoflavone (ANF) on ERSE induced EROD and deformities

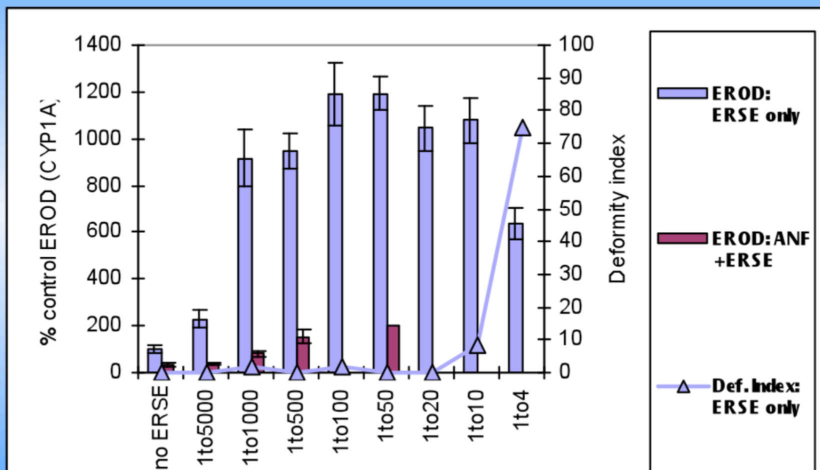


ANF

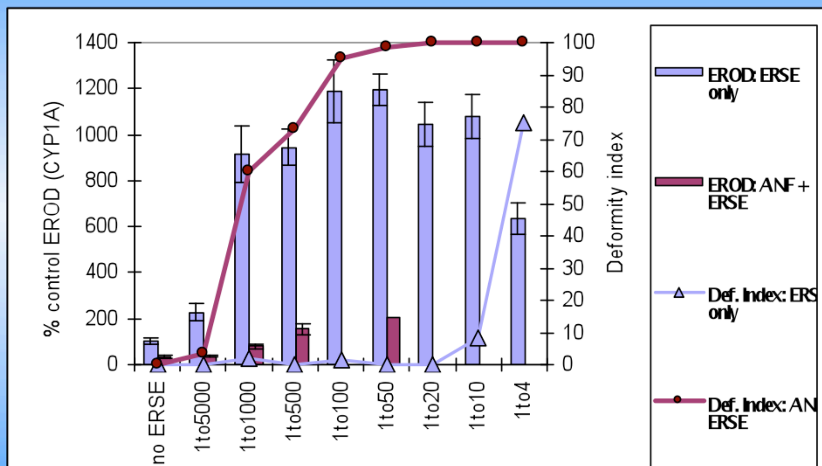


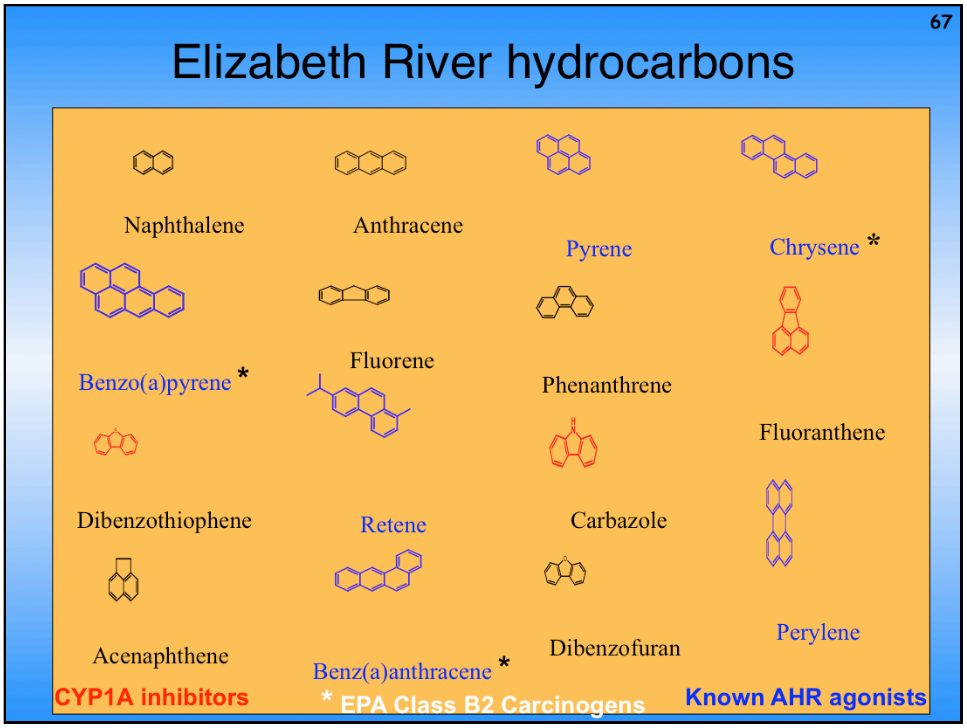
Wassenberg and Di Giulio, 2004, *Mar Env Res* 58:163-168

Effects of 100 μg/L ANF on ERSE induced EROD and deformities



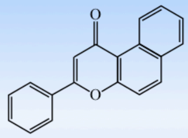
Effects of 100 μg/L ANF on ERSE induced EROD and deformities




67
Elizabeth River hydrocarbons
Naphthalene
Anthracene
Pyrene
Chrysene *
Benzo(a)pyrene *
Fluorene
Phenanthrene
Fluoranthene
Dibenzothiophene
Retene
Carbazole
Perylene
Acenaphthene
Benz(a)anthracene *
Dibenzofuran
CYP1A inhibitors
* EPA Class B2 Carcinogens
Known AHR agonists

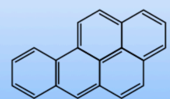
Synergistic developmental toxicity observed with a variety of AhR agonists + CYP1A inhibitors combinations

AHR Agonists

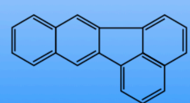


β-naphthoflavone
BNF

Environmentally Relevant

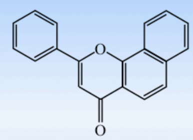


benzo[a]pyrene
BaP



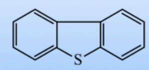
benzo[k]fluoranthene
BkF

CYP1A Inhibitors

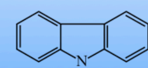


α-naphthoflavone
ANF

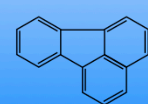
Environmentally Relevant



dibenzothiophene



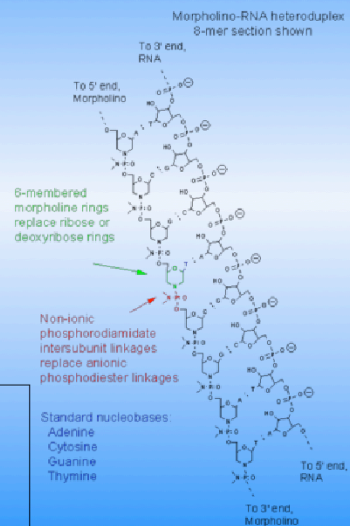
carbazole



fluoranthene
FL

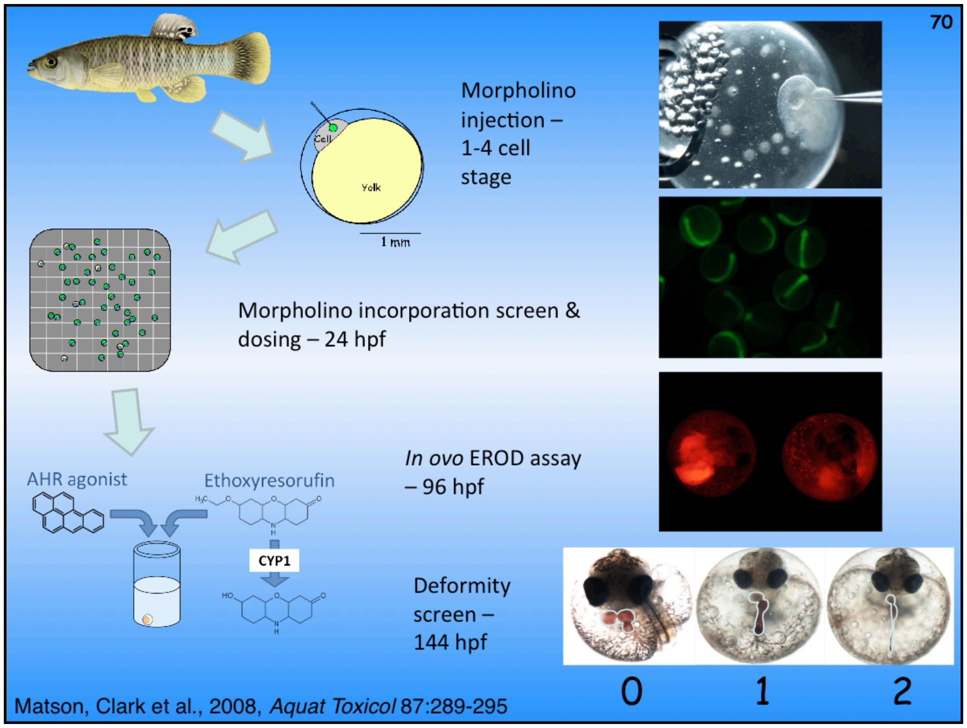
Approach: Antisense morpholinos

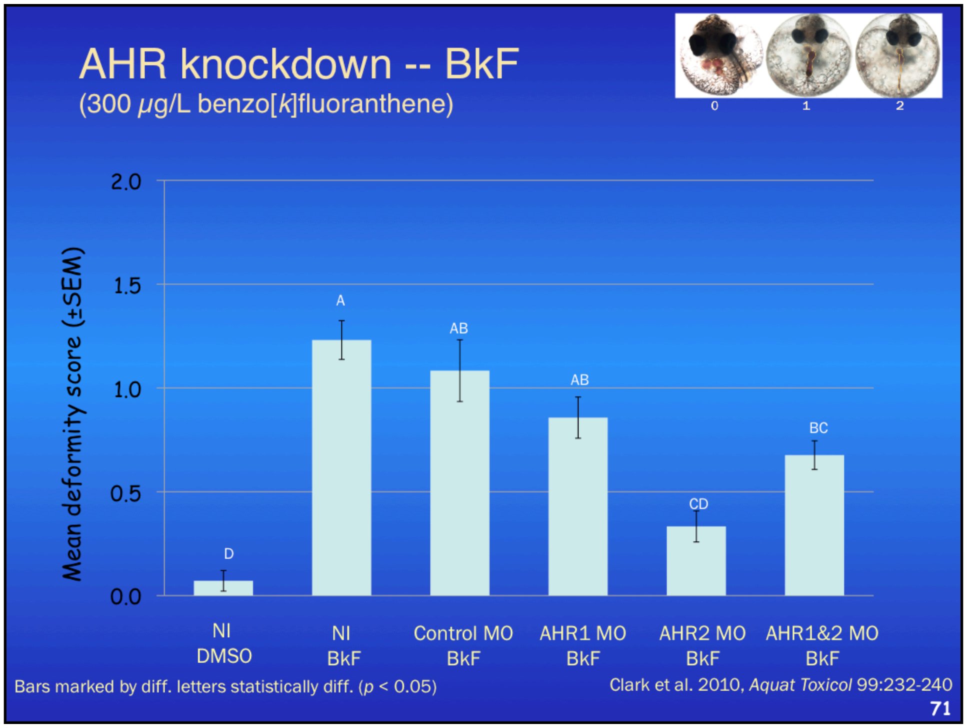
- Antisense oligonucleotides
- Target 5' UTR or splice junctions
- *In vivo* knockdown (not knock-out) of gene of interest
- Best for use during development

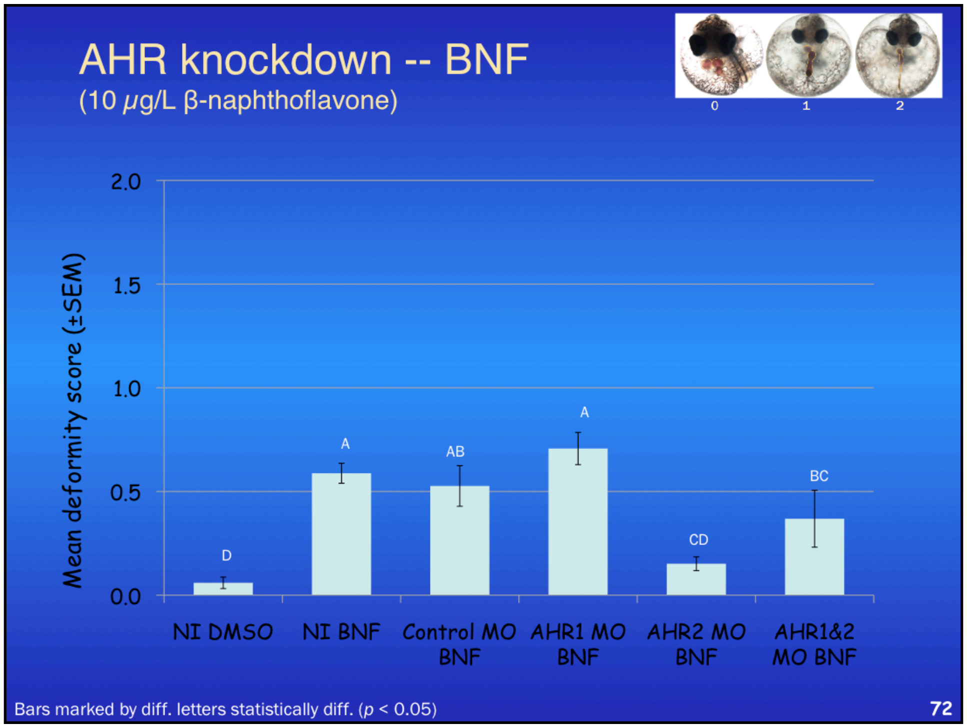


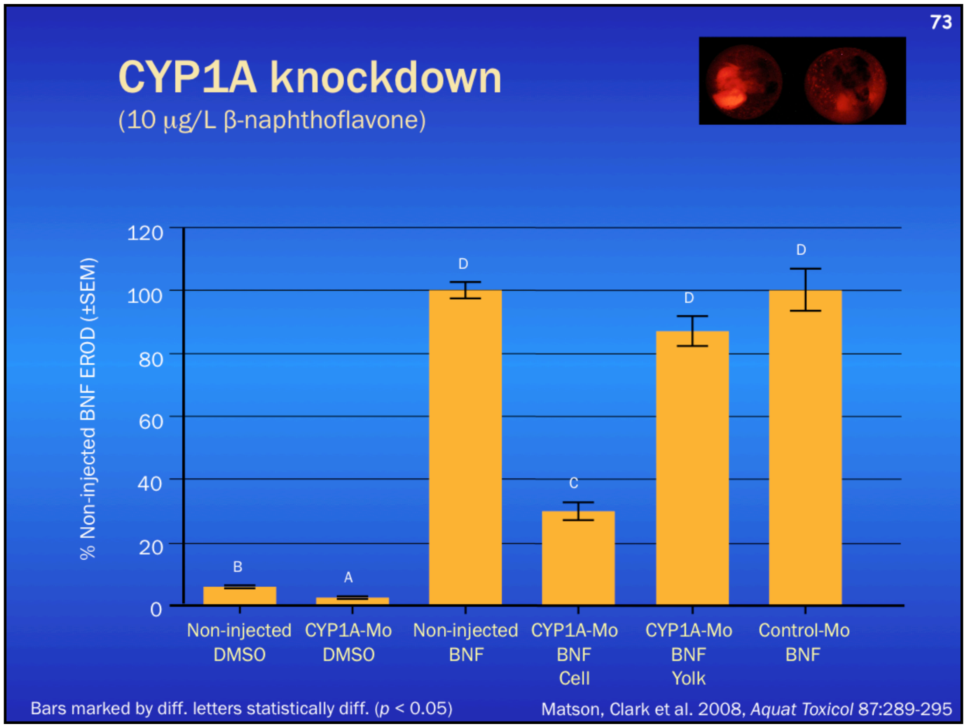
GeneTools, LLC

Our related zebrafish studies:
- -Billiard et al., 2006, *Toxicol Sci* 92:526-536
- -Timme-Laragy et al., 2007, *Aquat Toxicol* 85:241-250
- -Timme-Laragy et al., 2008, *Mar Env Res* 66:85-87
- -Timme-Laragy et al., 2009, *Toxicol Sci* 109:217-227


70
Cell
Yolk
1 mm
Morpholino injection – 1-4 cell stage
Morpholino incorporation screen & dosing – 24 hpf
AHR agonist
Ethoxyresorufin
CYP1
In ovo EROD assay – 96 hpf
Deformity screen – 144 hpf
0
1
2
Matson, Clark et al., 2008, *Aquat Toxicol* 87:289-295

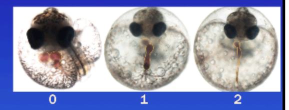

AHR knockdown -- BkF
(300 μg/L benzo[k]fluoranthene)
0
1
2
Mean deformity score (±SEM)
2.0
1.5
1.0
0.5
0.0
D
A
AB
AB
CD
BC
NI
DMSO
NI
BkF
Control MO
BkF
AHR1 MO
BkF
AHR2 MO
BkF
AHR1&2 MO
BkF
Bars marked by diff. letters statistically diff. (p < 0.05)
Clark et al. 2010, Aquat Toxicol 99:232-240
71

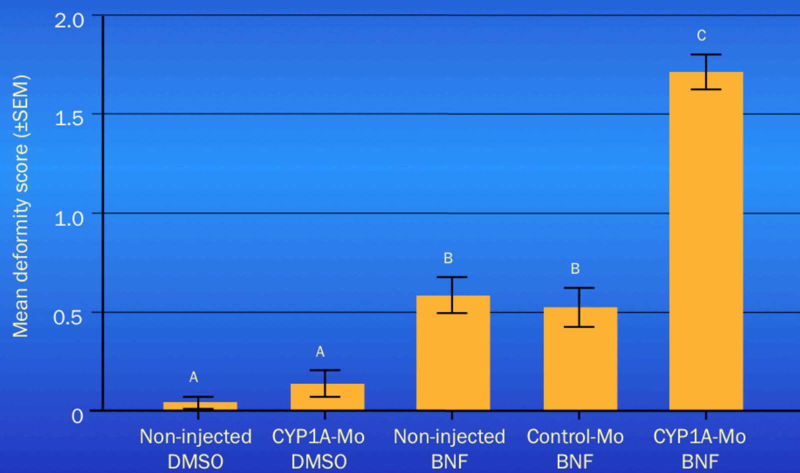

AHR knockdown -- BNF
(10 μg/L β-naphthoflavone)
0
1
2
Mean deformity score (±SEM)
2.0
1.5
1.0
0.5
0.0
D
A
AB
A
CD
BC
NI DMSO
NI BNF
Control MO BNF
AHR1 MO BNF
AHR2 MO BNF
AHR1&2 MO BNF
Bars marked by diff. letters statistically diff. (p < 0.05)
72


73
CYP1A knockdown
(10 µg/L β-naphthoflavone)
% Non-injected BNF EROD (±SEM)
120
100
80
60
40
20
0
B
A
D
C
D
D
Non-injected DMSO
CYP1A-Mo DMSO
Non-injected BNF
CYP1A-Mo BNF Cell
CYP1A-Mo BNF Yolk
Control-Mo BNF
Bars marked by diff. letters statistically diff. (p < 0.05)
Matson, Clark et al. 2008, Aquat Toxicol 87:289-295

CYP1A knockdown

(10 μg/L β-naphthoflavone)





Bars marked by diff. letters statistically diff. ($p < 0.05$)

Matson, Clark et al. 2008, *Aquat Toxicol* 87:289-295

Mechanisms of PAH Adaptation (that inform toxicity)

<u>AHR pathway responses</u>

(Van Veld et al. 1995; Meyer et al. 2002; 2003; Wills et al. 2010; Clark 2010

Phase II and III detoxification responses

(Armknecht et al., 1998; Cooper et al. 1999; Gaworecki et al. 2004)

Antioxidant defenses

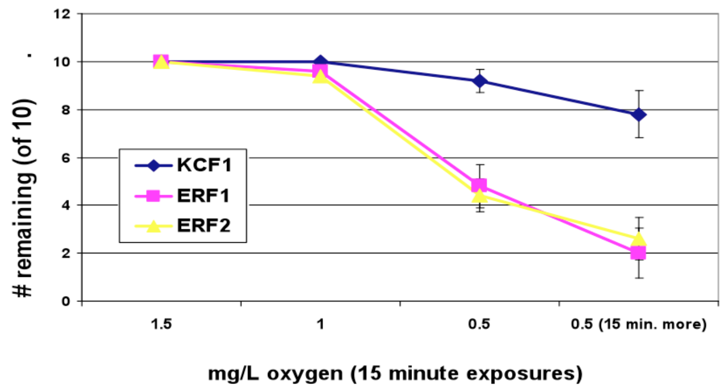
(Meyer et al. 2003b, Bacanskas et al. 2003)

"The Resistant Elizabeth River Killifish Phenotype"

Associated with the adapted phenotype, are there:

- Fitness costs?
- Cross-resistances to other chemicals?

Larval tolerance of low oxygen conditions

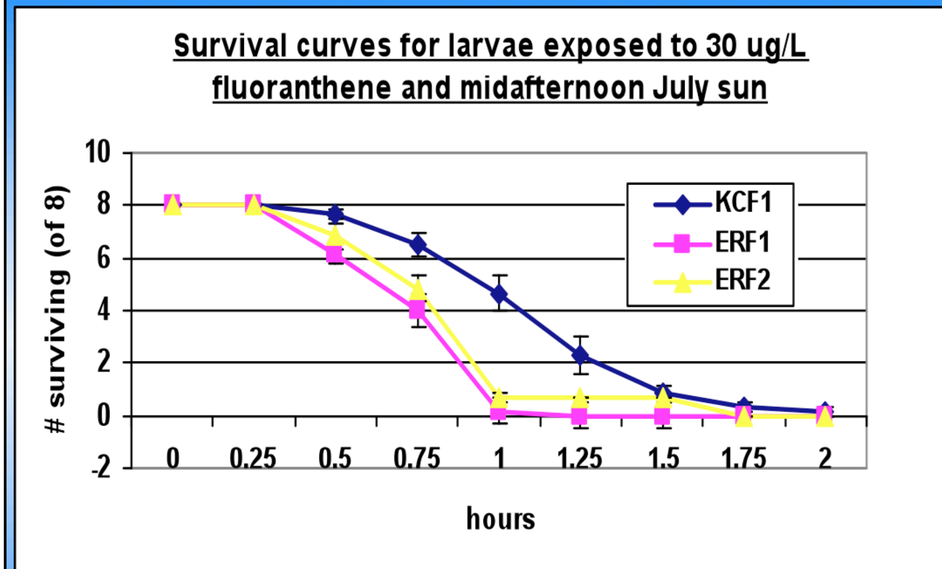


2-way ANOVA: p<.0001 for dose, population, and interaction

FLPD: p < .0001 for ERF1 vs KCF1 and ERF2 vs KCF1

p = 0.375 for ERF1 vs ERF2

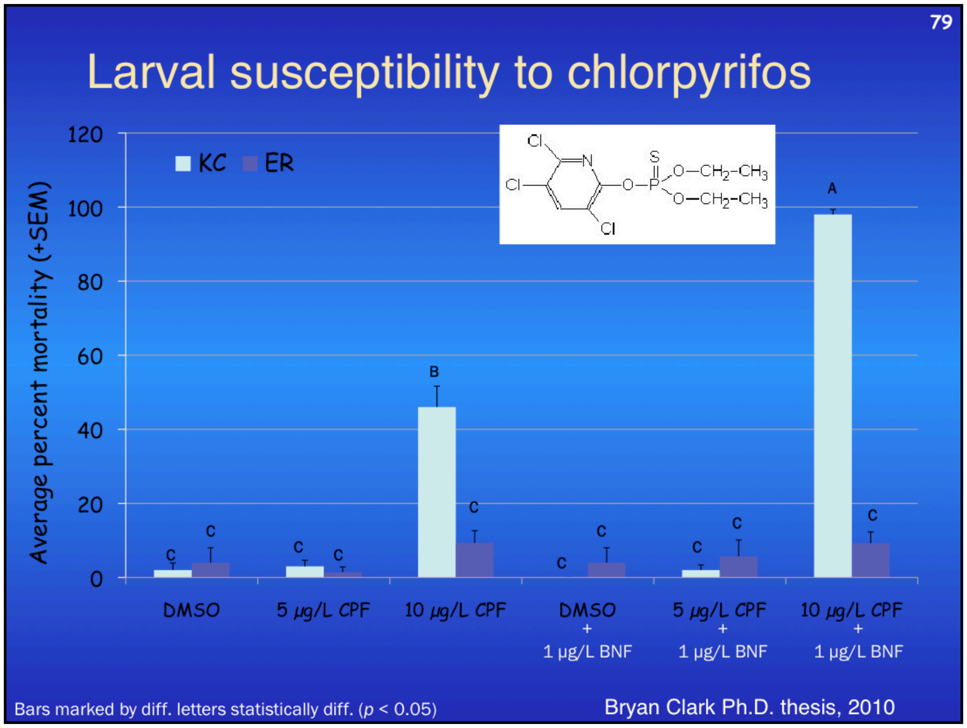
Meyer and Di Giulio, 2003, *Ecol Appl* 13:490-503



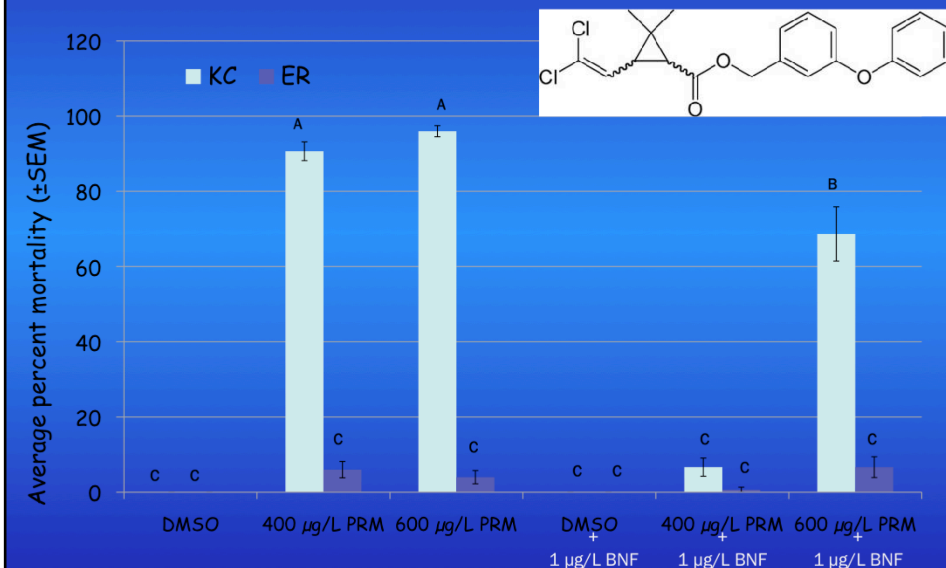
2-way ANOVA: p<.0001 for treatment, population, and interaction

FLPD: p < .0001 for ERF1 vs KCF1 and ERF2 vs KCF1

p = .023 for ERF1 vs ERF2


79
Larval susceptibility to chlorpyrifos
Average percent mortality (+SEM)
KC
ER
0
20
40
60
80
100
120
DMSO
5 µg/L CPF
10 µg/L CPF
DMSO + 1 µg/L BNF
5 µg/L CPF + 1 µg/L BNF
10 µg/L CPF + 1 µg/L BNF
A
B
C
Bars marked by diff. letters statistically diff. (p < 0.05)
Bryan Clark Ph.D. thesis, 2010

Larval susceptibility to permethrin

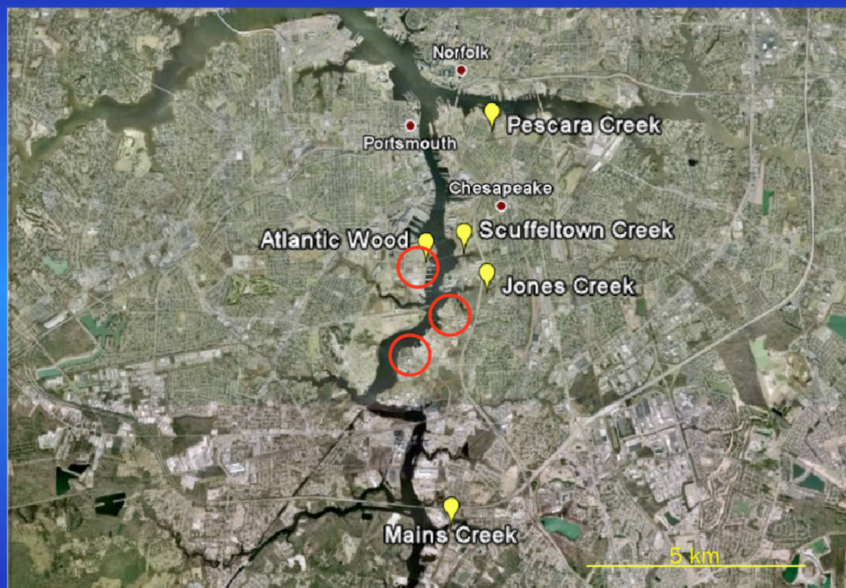


Bars marked by diff. letters statistically diff. ($p < 0.05$)

The Elizabeth River Resistant Phenotype

- Based upon studies of Atlantic Wood Superfund Site killifish.
- How widespread is it in the Elizabeth River system?
- How effective will proposed remediation efforts of the Atlantic Wood site be?

Elizabeth River subpopulations



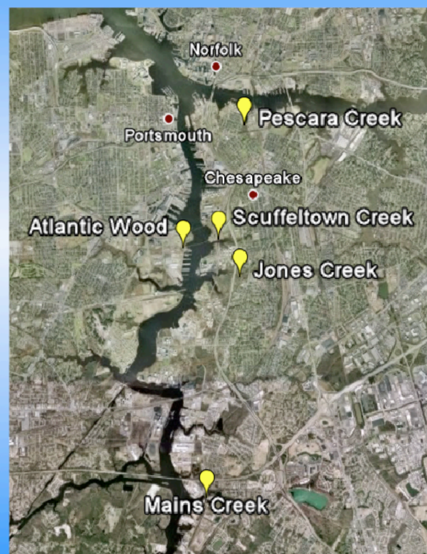
Bryan Clark Ph.D. thesis, 2010

Total PAH concentrations

King's Creek (reference)
526 ± 624 ng/g

Atlantic Wood
122,665 ± 16,854 ng/g

Mains Creek
186 ± 201 ng/g

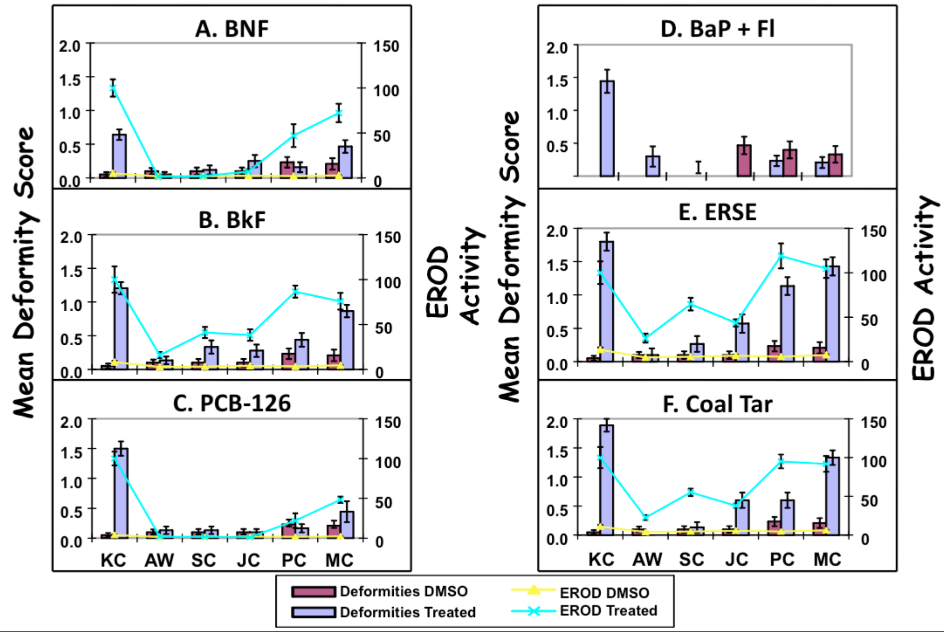


Pescara Creek
4,493 ± 557 ng/g

Scuffeltown Creek
6,328 ± 1,253 ng/g

Jones Creek
1,910 ± 518 ng/g

Elizabeth River Subpopulations Response to PAHs and PCB-126



Conclusions concerning the PAH-resistant phenotype:

- The phenotype is in part genetic
- Fitness costs incurred, also genetic
- Cross-resistance also observed
- Adaptation involves downregulation of AHR pathway (and upregulation of antioxidant defense systems and phase II/III components)
- Studies of this phenotype help inform 'traditional' dose-response studies
- The "Atlantic Wood" resistant phenotype is widespread in the Elizabeth River system

Conclusions & implications from PAH studies:

- PAH mixtures can display marked synergies (AHR agonists + CYP inhibitors)
- This is in contrast to dioxin-like compounds
- Default assumptions of additive toxicity of PAHs may be under-protective (and PAHs are increasing in the environment)
- Implications for human health?

"All things are connected. Whatever befalls the earth befalls the children of the earth."
Chief Seattle

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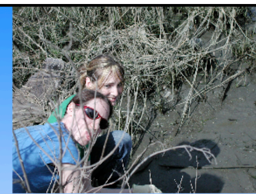
Joel Meyer, Ph.D., 2003

Deena Wassenberg, Ph.D., 2004



Alicia Timme-Laragy, Ph.D., 2007
Sonya Billiard, PDF, 2006

Lauren Wills, Ph.D., 2008

Life's a beach!

Dawoon Jung, Ph.D., 2009

Bryan Clark, Ph.D., 2010 (PDF)

Cole Matson, PDF

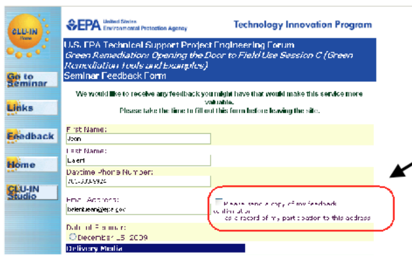
Lindsey Van Tiem, Ph.D., 2011 ???

Questions?


HEY VINNIE! I HEARD THEY'RE GONNA CLEAN UP THE ELIZABETH RIVER! VINNIE? VIN? PAL?

Resources & Feedback

- To view a complete list of resources for this seminar, please visit the **Additional Resources**
- Please complete the **Feedback Form** to help ensure events like this are offered in the future



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