Resistance of Atlantic Tomcod from the Hudson River to PCBs and TCDD, but not PAHs

Isaac Wirgin
Department of Environmental Medicine
New York University School of Medicine

SBRP Annual Meeting
Seattle, WA

Hudson River Estuary Pollutants of Concern

- PCBs- PCB Superfund Site
  - 200 miles of Hudson River
- PCDDs/PCDFs- Diamond Alkali Site
  - Passaic River, NJ
- PAHs
- Heavy Metals
  - Foundry Cove
- Pesticides

HR GE Plant

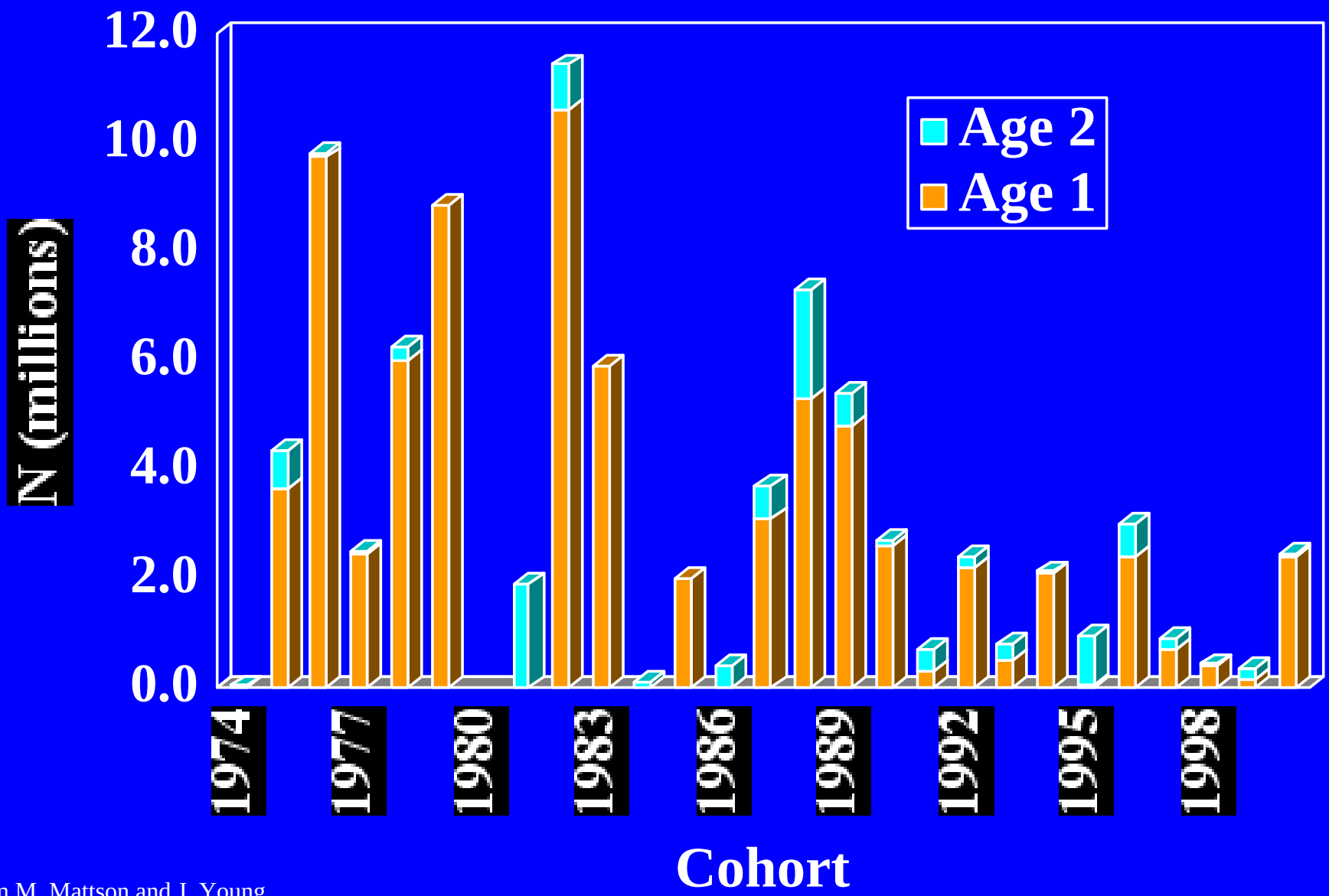
What do we know about the effects of these pollutants on Hudson River populations?

- Many years worth of data on PCB residues in fish fillets and selected benthic invertebrates
- Almost nothing on toxic effects on the Hudson River fish community

Why use Atlantic tomcod as an environmental sentinel?

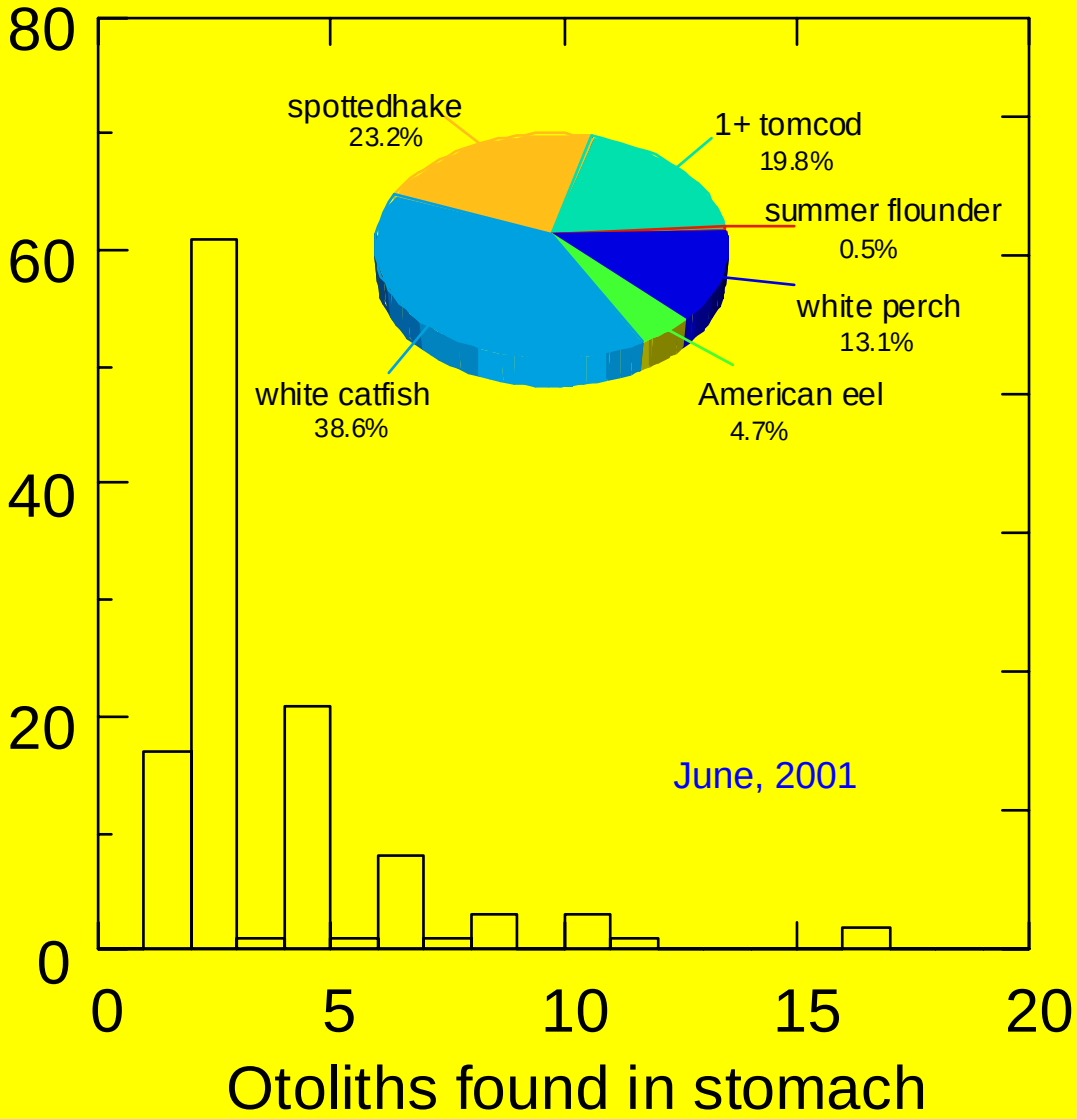
- Abundant in estuaries from the Hudson River to Labrador
- Bottom-dwelling
- Lipid-rich livers
- Complete life histories within estuaries
- Migratory within natal estuaries
- Only winter-time spawners in Estuary
- Embryo cell culture available
- Amenable to laboratory culture
  - Complete life cycle
  - Heritability studies

Adult Atlantic Tomcod Spawning Stock Abundance 1974-2000



From M. Mattson and J. Young

- Who Eats Young-of-Year Tomcod in the Hudson?



Cancer in tomcod in the early 1980s

- More than 90% of two-year-old and 40% of one-year-old tomcod from the Hudson River exhibited hepatocellular carcinomas (Dey et al. 1993)
- Hepatic tumors were in < 10% prevalence in rivers in Maine and RI-CT and absent in tomcod from Canada
- Prevalence of tumors in HR population is reduced today

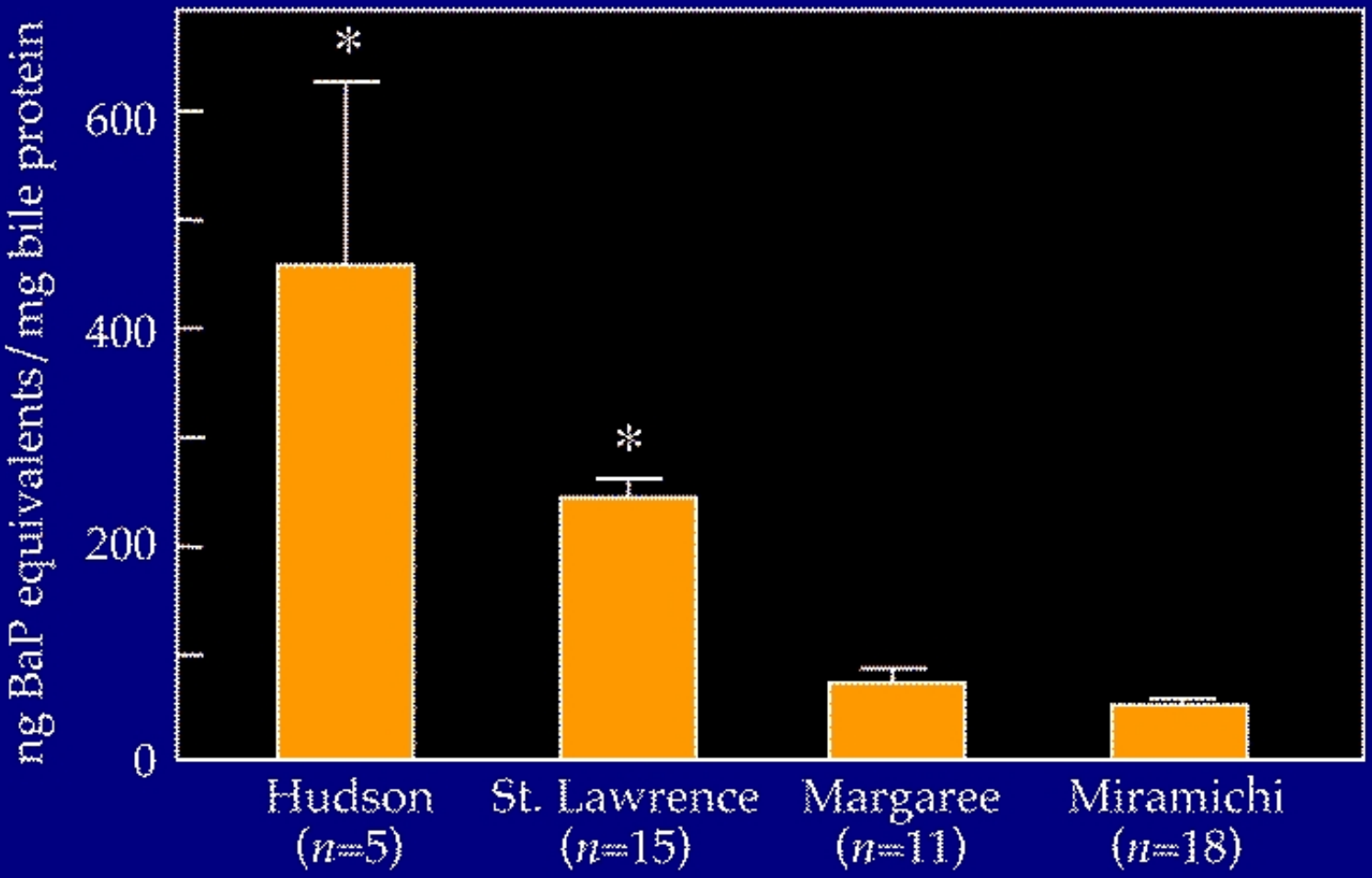
Age structure of tomcod in the early 1980s

- > 97% of Hudson River population were one-year-olds, < 3% two-year-olds, and three-year-old were almost absent
- three-and-four-year olds predominate in other populations- fish up to seven-years of age are sometimes observed
  - Dey et al. 1993

Do Hudson River Contaminants Bioaccumulate in its fish?

- Yes, sometimes to world record levels!

Concentrations of Fluorescent Aromatic Compounds in Bile of Atlantic Tomcod Collected from Four River Systems of the North American Atlantic Coast



Values are means ± SE

*Significantly different from values for tomcod from the Margaree and Miramici Rivers

Hepatic Burdens of PCDDs, PCDFs, PCBs in Adult Tomcod from the HR Estuary and Elsewhere

Wet-weight concentrations of chlorinated aromatic hydrocarbons in the livers of Atlantic tomcod collected from the the Hackensack River in the lower Hudson River estuary (Dec. 1996), and the mid-Hudson River estuary at Garrsion, NY (RM 51)(Dec. 1997). Livers were pooled from 6-10 fish/sex.

Chemical Contaminant	Hudson River (ng/kg)		Hackensack River (ng/kg)		Margaree River (ng/kg)	Miramichi River (ng/kg)	
	Males (n=10)	Females (n=10)	Males (n=6)	Females (n=10)	Males (n=10)	Males (n=8)	Females (n=15)
TCDD	46.83	10.9	553.9	207.81	0.7	48	38
TCDF	104.43	58.44	35.96	47.57	2.1	150	120
Total TCDD TEQs	98.9	31.2	673	256	1.35	67	53
3,3',4,4' TCB	17,394	6,341	17,365	14,713	87	280	260
Total Coplanar PCBs*	21,115	7,364	21,119	16,182	182	498	409

* Total coplanar PCBs=sum of concentrations of 3,3',4,4'- tetrachlorobiphenyl, 3,3',4,4',5- pentachlorobiphenyl, and 3,3',4,4',5,5'- hexachlorobiphenyl.

All analyses were performed through the courtesy of the Contaminants Science Section, Institute of Ocean Sciences, Sidney, B.C, Canada. Dioxin/furan analyses were performed using the Environment Canada protocol which is very similar to the equivalent EPA-1613 method. PCBs were fractionated using carbon fibre chromatography into three fractions (di-ortho, mono-ortho, and non-orthos PCBs). Each fraction was analyzed by HRGC/HRMS.

But, early life stages of fish are known to be most sensitive to contaminants

Congener specific analysis of hepatic PCBs, PCDDs, and PCDFs in young-of-year tomcod from 20 sites on main-stem Hudson River, Newark Bay, and Hackensack River

TABLE 1. Sample Collection and Meristic Data for YOY (Y) and Adult (M) Atlantic Tomcod Sampled from the Main-Stem Hudson River, NY, Newark Bay, NJ, Hackensack River, NJ, and Miramichi River

sample ID	month/year	site name	RM	composite no.	male (m)/female (f)	length (cm)	age (years)
M50(m)	12/1997	Garrison	50	10	m	23.7 ± 1.7	2
M50(f)	12/1997	Garrison	50	10	f	27.0 ± 1.4	2
M-2(m)	12/1996	Hackensack R.	1−3[a]	6	m	18.5 ± 2.7	1−2
M-2(f)	12/1996	Hackensack R.	1−3[a]	11	f	19.5 ± 1.7	1
Y-2	08/1998	Hackensack R.	1−3[a]	8			<1
Y-1	08/1998	Newark Bay	proper	10			<1
Y1	08/1998	Battery	1	9			<1
Y8	08/1998	Manhattan	8	10			<1
Y10	08/1998	Manhattan	10	10			<1
Y17	08/1998	Yonkers	17	9			<1
Y37	08/1998	Haverstraw Bay	37	5			<1
Y58	08/1998	Moodna Cr.	58	9			<1
Y67	08/1998	Poughkeepsie	67	8			<1
Y77	08/1998	Mid-Hudson	77	10			<1
Y82	08/1998	RM82	82	10			<1
M50(f)[b]	01/1999	Garrison	50	10	f	18.4−25.5	>1
Mmir(f)[b]	01/1999	Miramichi R[c]	18[d]	5 × 4−5	f	21.5−31.5	>1
Y0	09/2000	Battery	0	5			<1
Y17[e]	09/2000	Yonkers	17	1			<1
Y37	09/2000	Haverstraw Bay	37	5			<1
Y43	09/2000	RM43	43	5			<1
Y50	09/2000	Garrison	50	5			<1
Y80	09/2000	RM80	80	5			<1
Y85	09/2000	RM85	85	3			<1
Y91	09/2000	Kingston	91	1			<1
Y96	09/2000	RM96	96	1			<1
Y107	09/2000	Catskill	107	1			<1

[a] Distance from the confluence of the Hackensack River with Newark Bay. [b] Unfertilized egg samples were also collected from these fish. [c] Captured at Loggieville, NB, Canada (reference site). [d] Distance upstream from Miramichi Inner Bay barrier islands. [e] Low lipid weight.

Hepatic Burdens of PCDDs, PCDFs, and PCBs in YOY Tomcod

Wet-weight Concentrations of Halogenated Aromatic Hydrocarbons in Livers of Young-of-the-year Tomcod from Various River Miles (RM) of the Hudson River Expressed in pg/g or ng/g and TCDD Toxic Equivalent Units (TEQ) Based on Fish-specific Toxic Equivalency Factors (TEF) (Van den Berg et al. 1998)

		Newark Bay	Hackensack River	Hudson River								
				RM1	RM8	RM10	RM17	RM37	RM58	RM67	RM77	RM82
Dioxins and Furans												
2,3,7,8-TCDD	pg/g	655	897	26	28	14	27	9	4	0	1	0
	TEQ	655	897	26	28	14	27	9	4	0	1	0
Total PCDD	pg/g	728	1,039	100	84	75	98	143	101	52	38	88
	TEQ	671	921	31	36	14	35	16	8	3	4	0
2,3,7,8-TCDF	pg/g	103	99	71	98	65	146	94	51	42	39	17
	TEQ	5	5	4	5	3	7	5	3	2	2	1
Total PCDF	pg/g	380	697	129	200	93	287	282	155	149	133	49
	TEQ	50	103	13	20	12	31	27	13	9	7	1
Coplanar PCBs												
Total Coplanars	pg/g	100,472	118,085	36,418	58,234	29,528	73,301	111,759	70,627	57,364	52,301	22,668
PCB77	pg/g	32,224	37,886	12,747	17,970	10,067	25,256	31,674	23,167	16,549	14,855	6,459
	TEQ	3	4	1	2	1	3	3	2	2	1	1
PCB81	pg/g	1,945	2,628	514	810	355	1,031	1,957	1,354	714	653	351
	TEQ	1	1	0	0	0	1	1	1	0	0	0
PCB126	pg/g	2,251	2,945	824	1,371	599	1,790	2,282	1,271	817	765	593
	TEQ	11	15	4	7	3	9	11	6	4	4	3
PCB169	pg/g	91	110	42	61	31	82	88	45	30	28	28
	TEQ	0	0	0	0	0	0	0	0	0	0	0

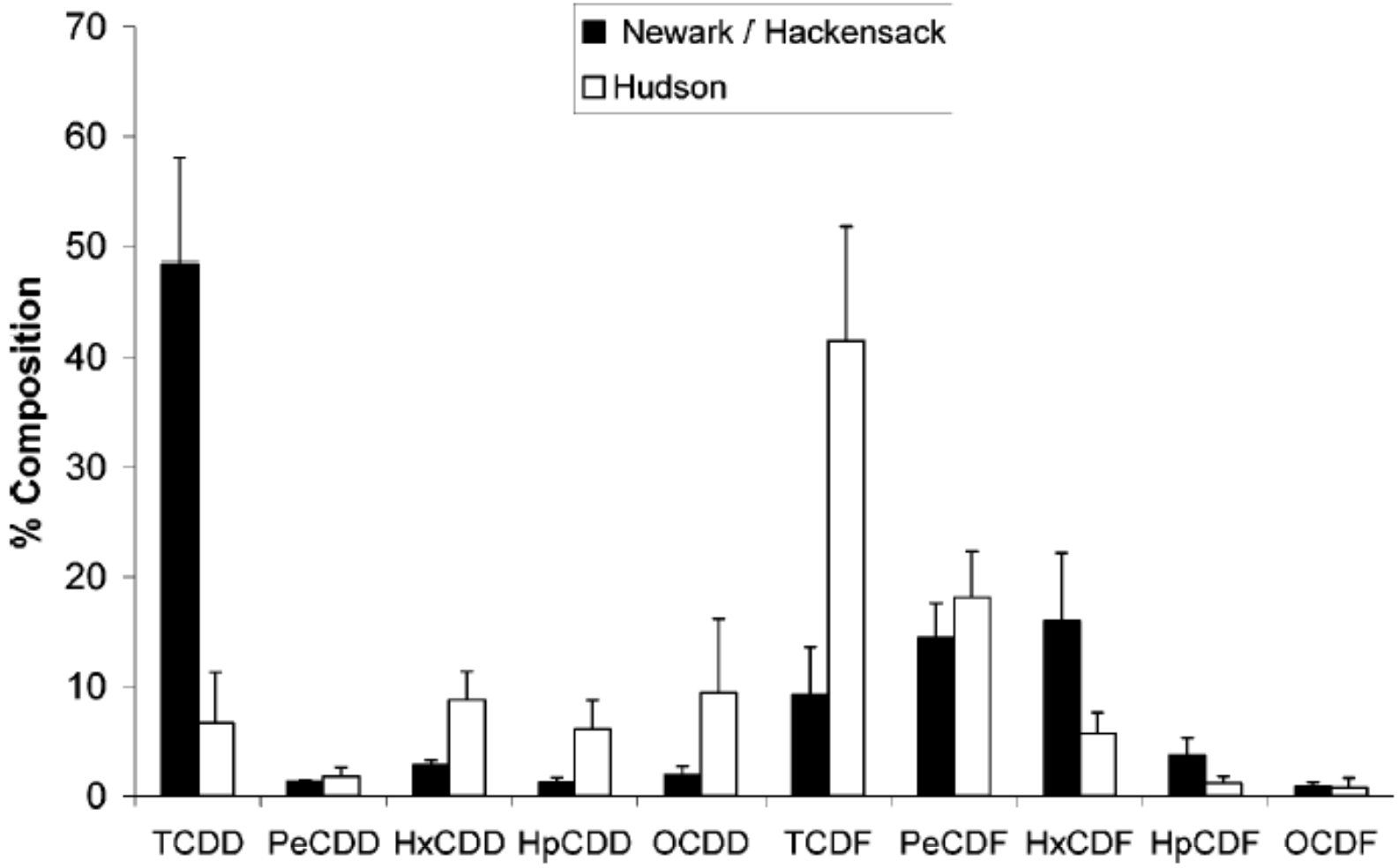


FIGURE 5. Normalized hepatic PCDD/F chlorohomolgue patterns in male/female, adult, and YOY pooled tomcod from the main-stem Hudson River ($n = 22$) and Newark Bay/Hackensack River complex ($n = 4$). Error bars are in units of standard deviation.

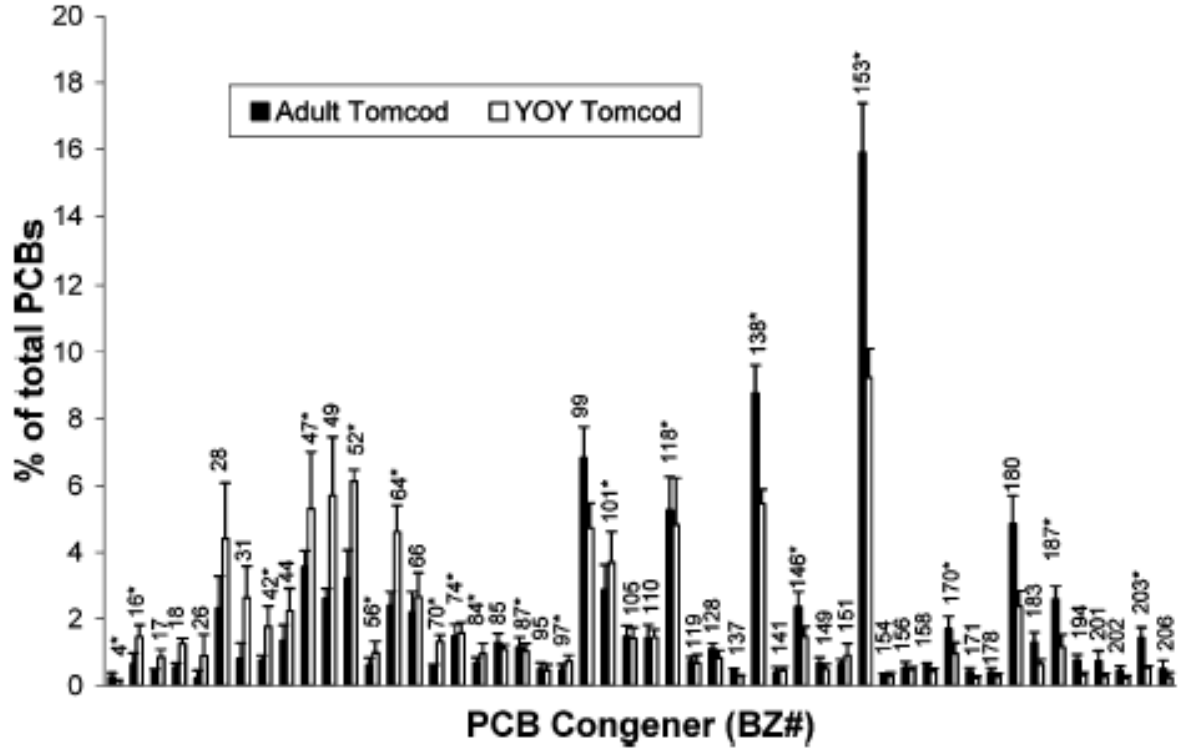


FIGURE 3. Normalized patterns of the 50 most prevalent PCB congeners in YOY tomcod livers ($n = 5$) compared to that in adult tomcod livers ($n = 5$) collected at similar locations in the lower Hudson River and adjacent Newark Bay/Hackensack River between 1997 and 2000. Error bars are in units of standard deviation. Asterisk (*) denotes coeluting congener group with most abundant congener listed based on preferential substitution patterns in Aroclor production via electrophilic aromatic substitution mechanisms (*38*).

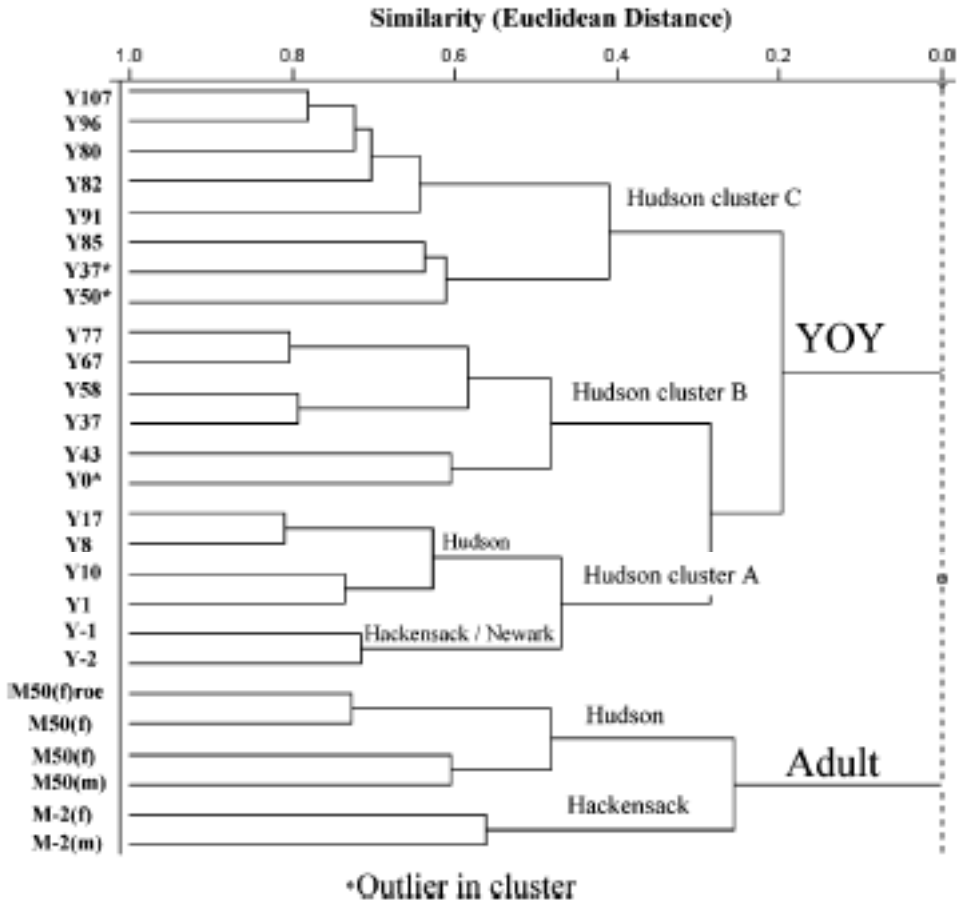


FIGURE 2. Hierarchical cluster analysis dendrogram using farthest-neighbor linkage method on normalized YOY and adult hepatic PCB compositions. Y17[e] was excluded from this analysis.

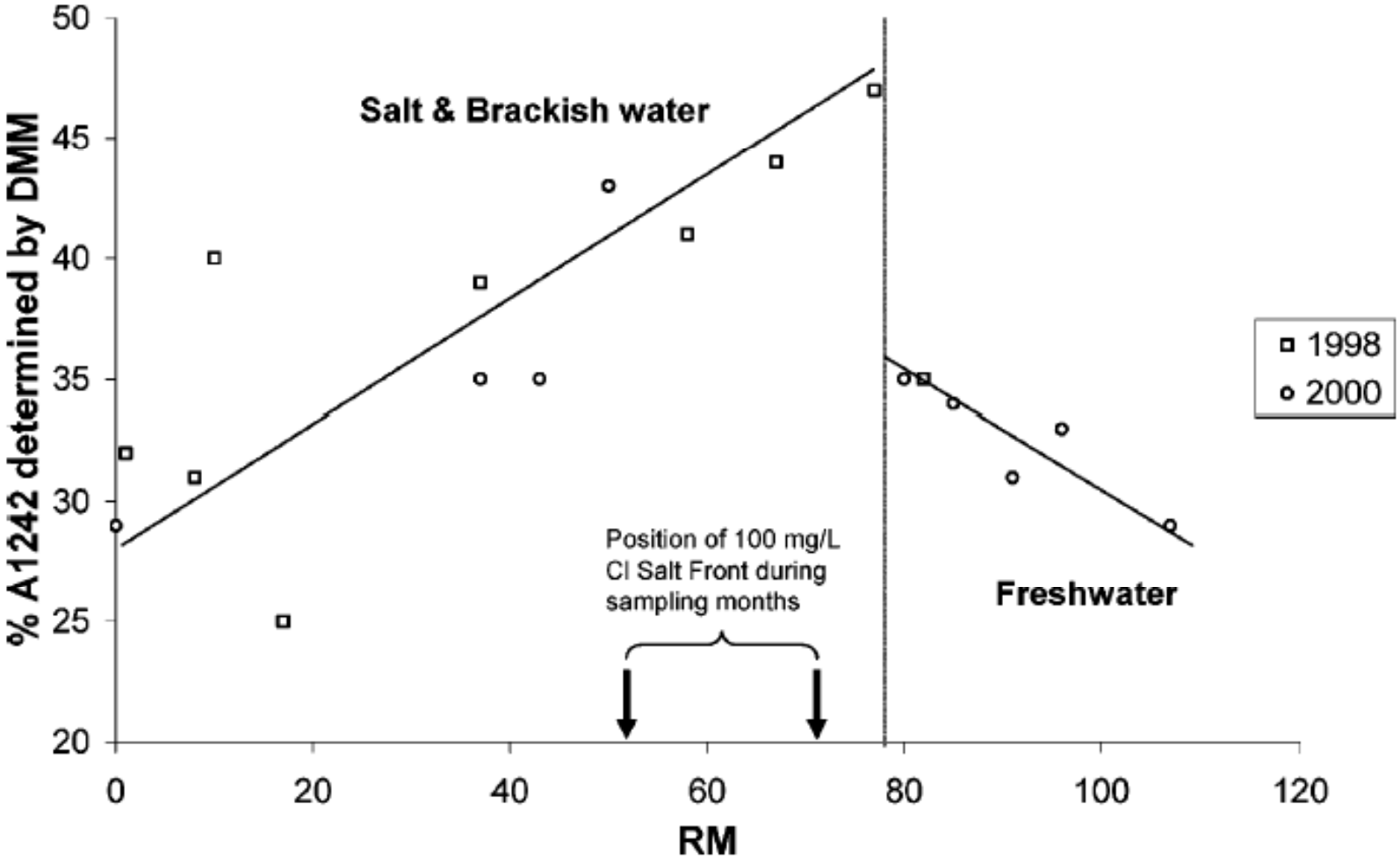
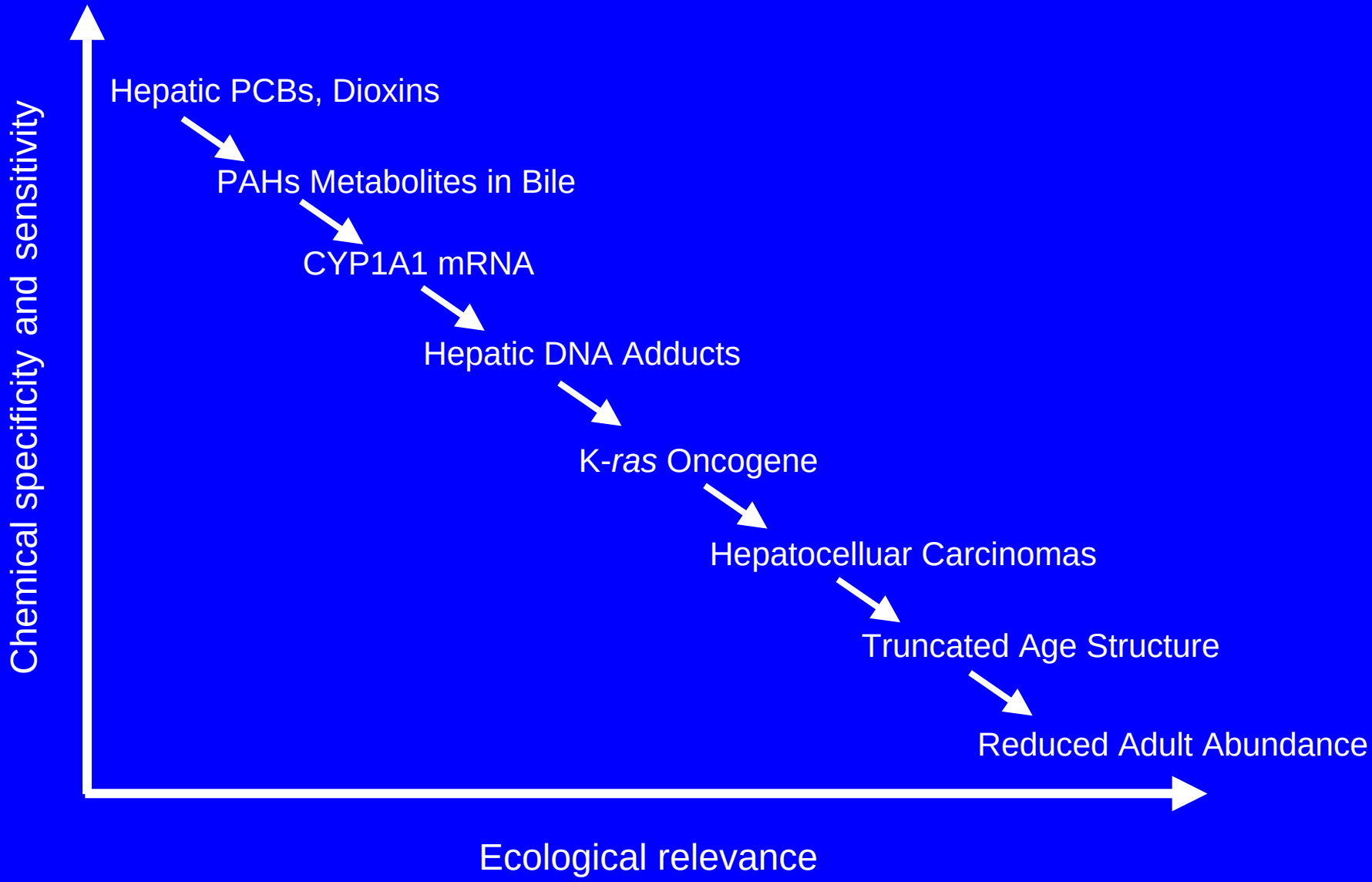


FIGURE 4. Percent A1242 characteristic as determined by a direct mixing model (DMM) from the normalized PCB congener pattern in YOY tomcod from various locations on the main stem of the Hudson River (coefficient of determination (r^2): salt and brackish = 0.471; freshwater = 0.618).

St. Lawrence River
Québec
Miramichi River
New Brunswick
Margaree River
MA.
Vt.
N. H.
N. Y.
Saco River
Mass.
Conn.
R. I.
N.J.
Hudson River

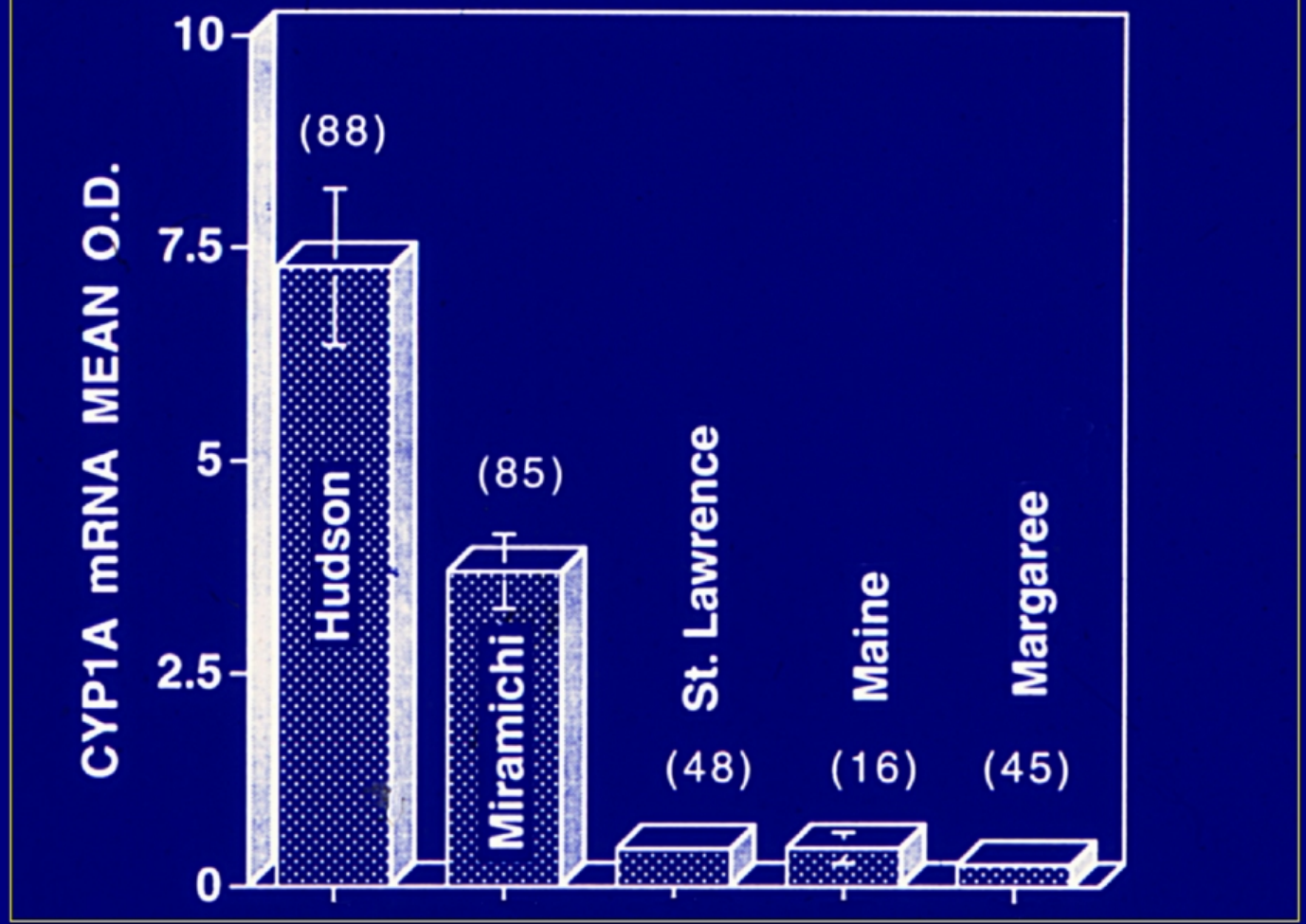
Biomarker Responses in HR Tomcod



CYP1A1 Transcription

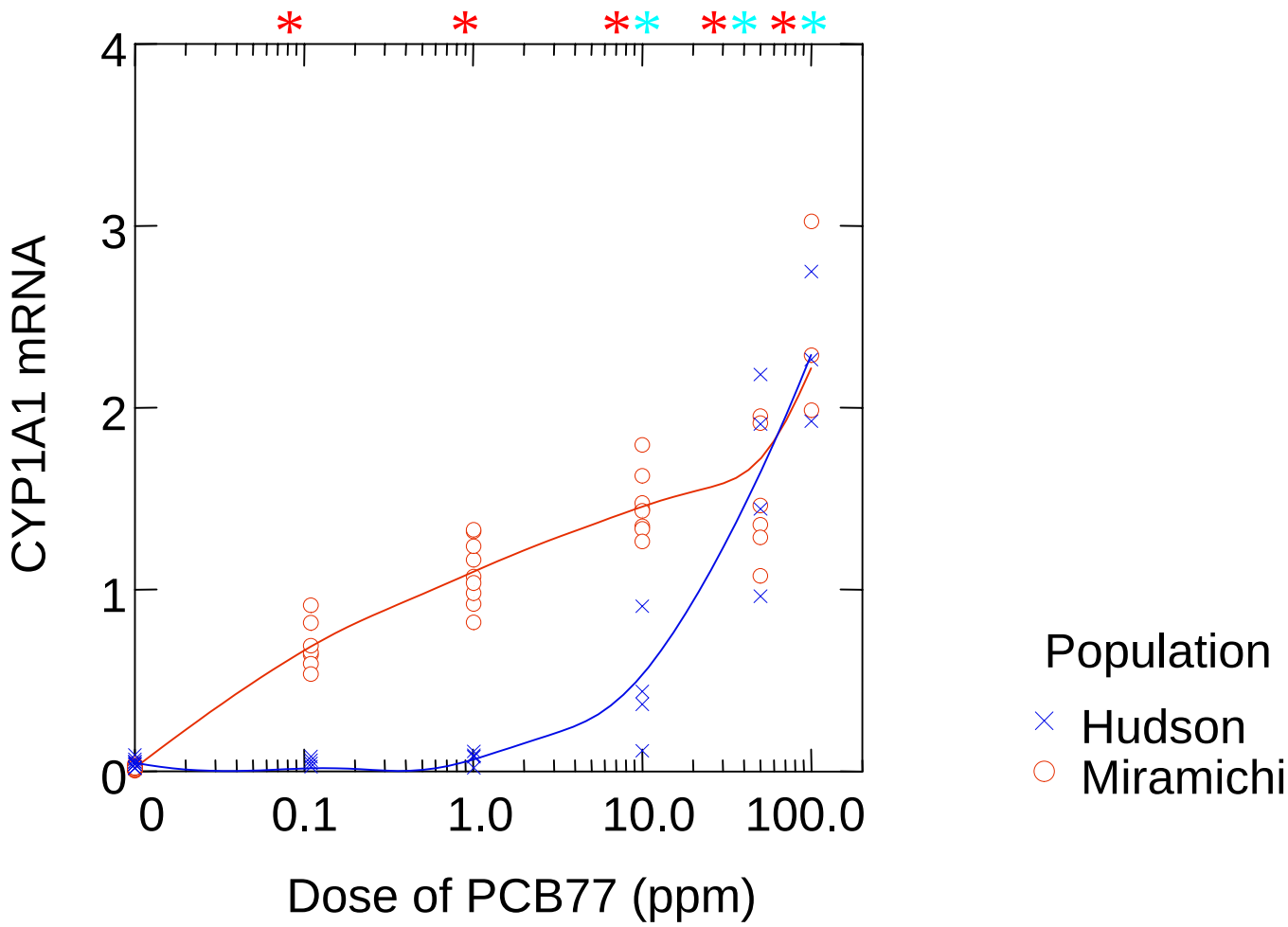
- is highly induced in fishes by PCDD/Fs, coplanar PCBs, and PAHs
- induction is activated by the Aryl Hydrocarbon Receptor (AHR) pathway
- most toxic responses to AH contaminants are mediated through AHR
- CYP1A1 expression is probably predictive of most toxic responses to AH contaminants

COMPARISON OF CYP1A mRNA LEVELS IN ATLANTIC TOMCOD FROM DIFFERENT SITES

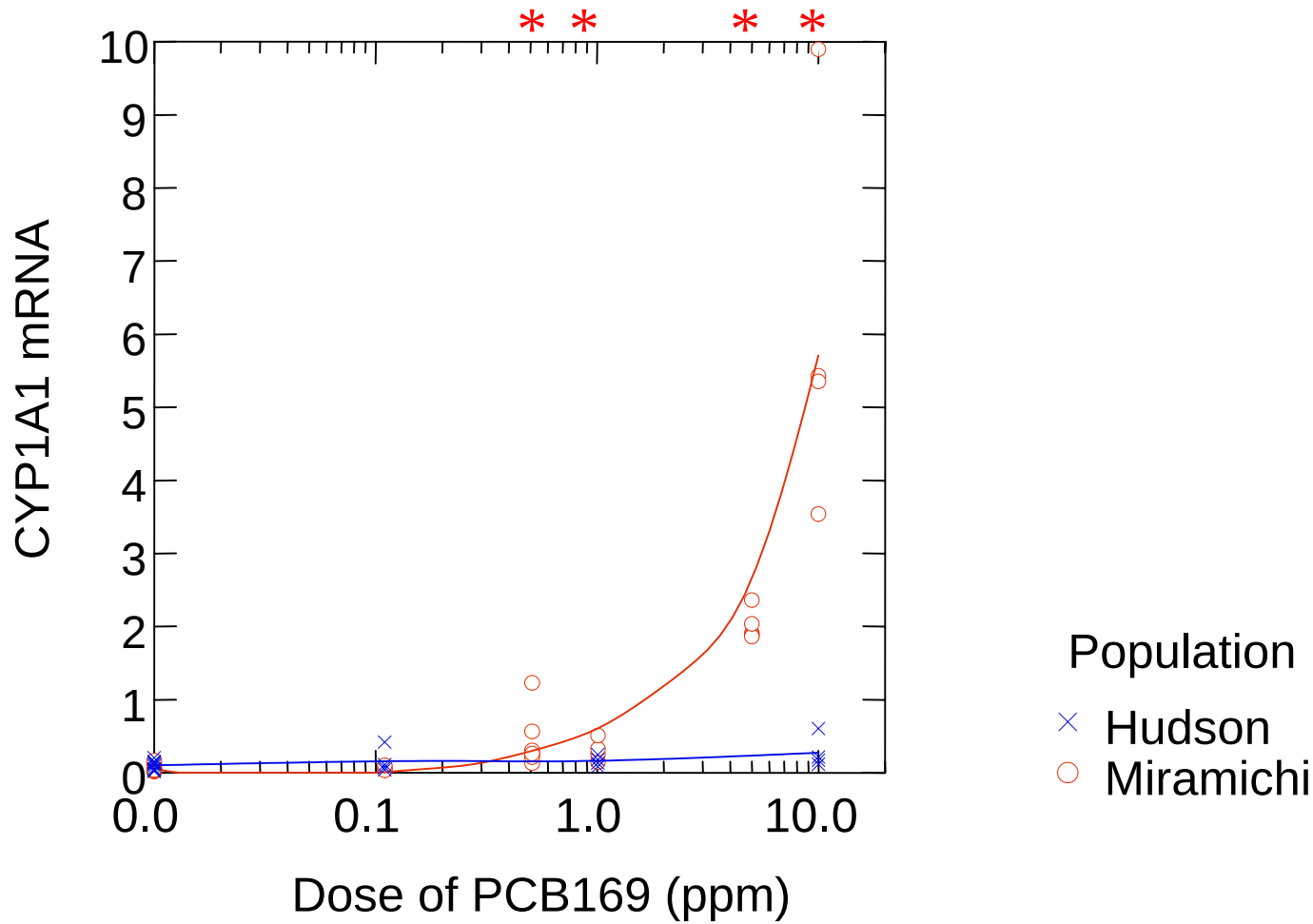


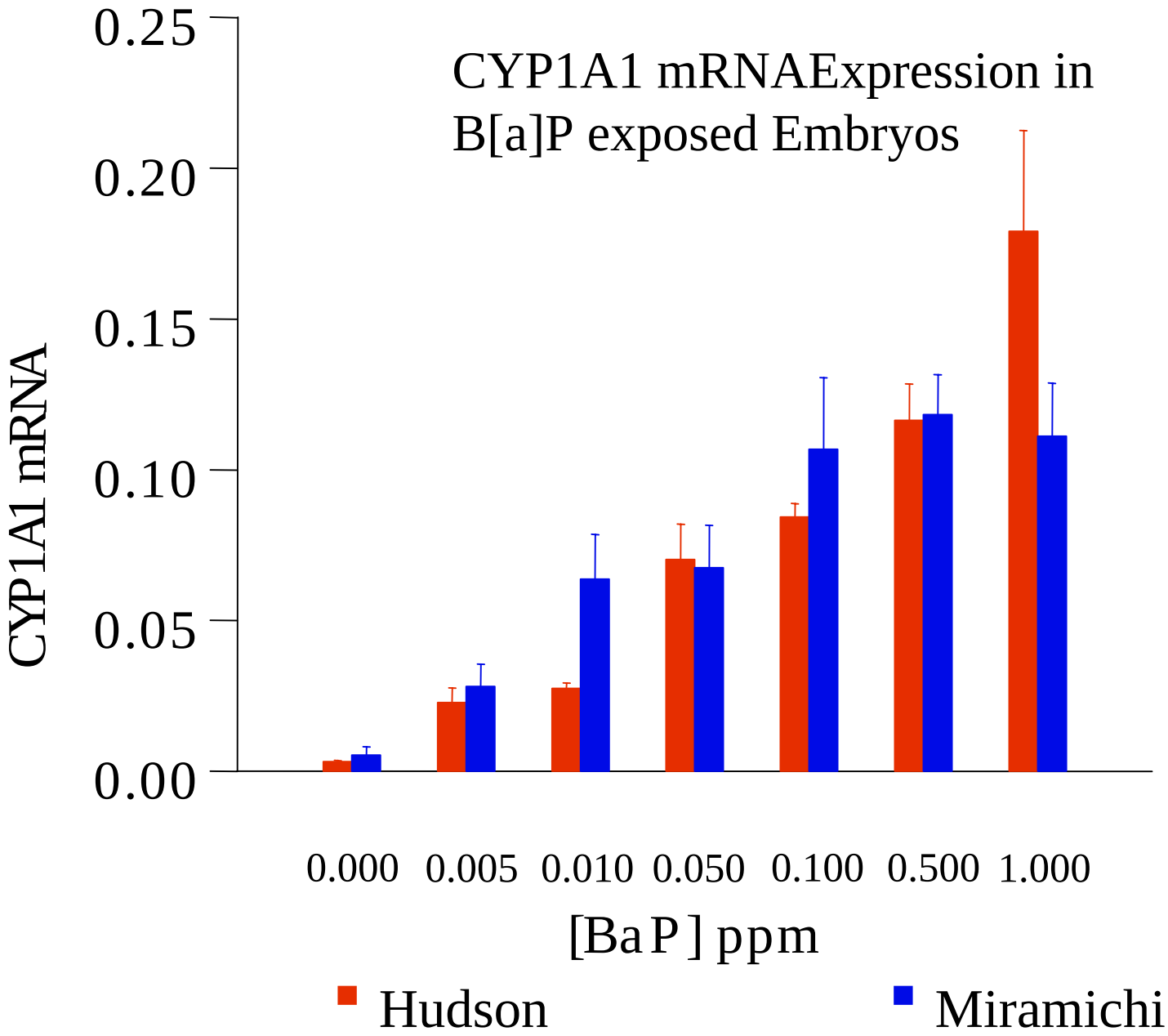
Adult tomcod collected from the Hudson River and Miramichi River were depurated for 20-300 days in clean water and treated with TCDD, four coplanar PCBs, or three PAHs, and CYP1A1 mRNA expression was measured

CYP1A1 mRNA Expression in Adult tomcod i.p. Injected with PCB77



CYP1A1 mRNA Expression in Adult tomcod i.p. Injected with PCB169




CYP1A1 mRNAExpression in
B[a]P exposed Embryos
CYP1A1 mRNA
0.25
0.20
0.15
0.10
0.05
0.00
0.000
0.005
0.010
0.050
0.100
0.500
1.000
[BaP] ppm
Hudson
Miramichi

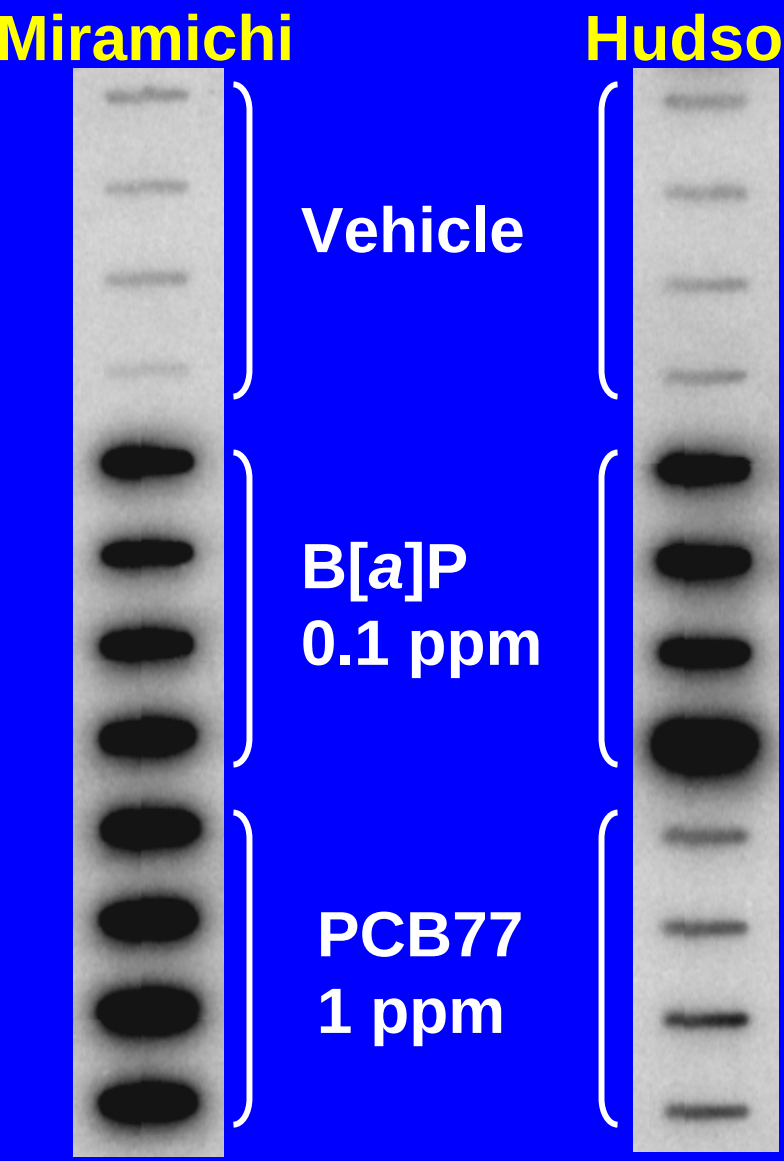
Reduced CYP1A1 inducibility in Hudson River tomcod

- For TCDD and three coplanar PCB congeners, but not PAHs
- In all tissues tested
- In tomcod adults, juveniles, larvae, embryos

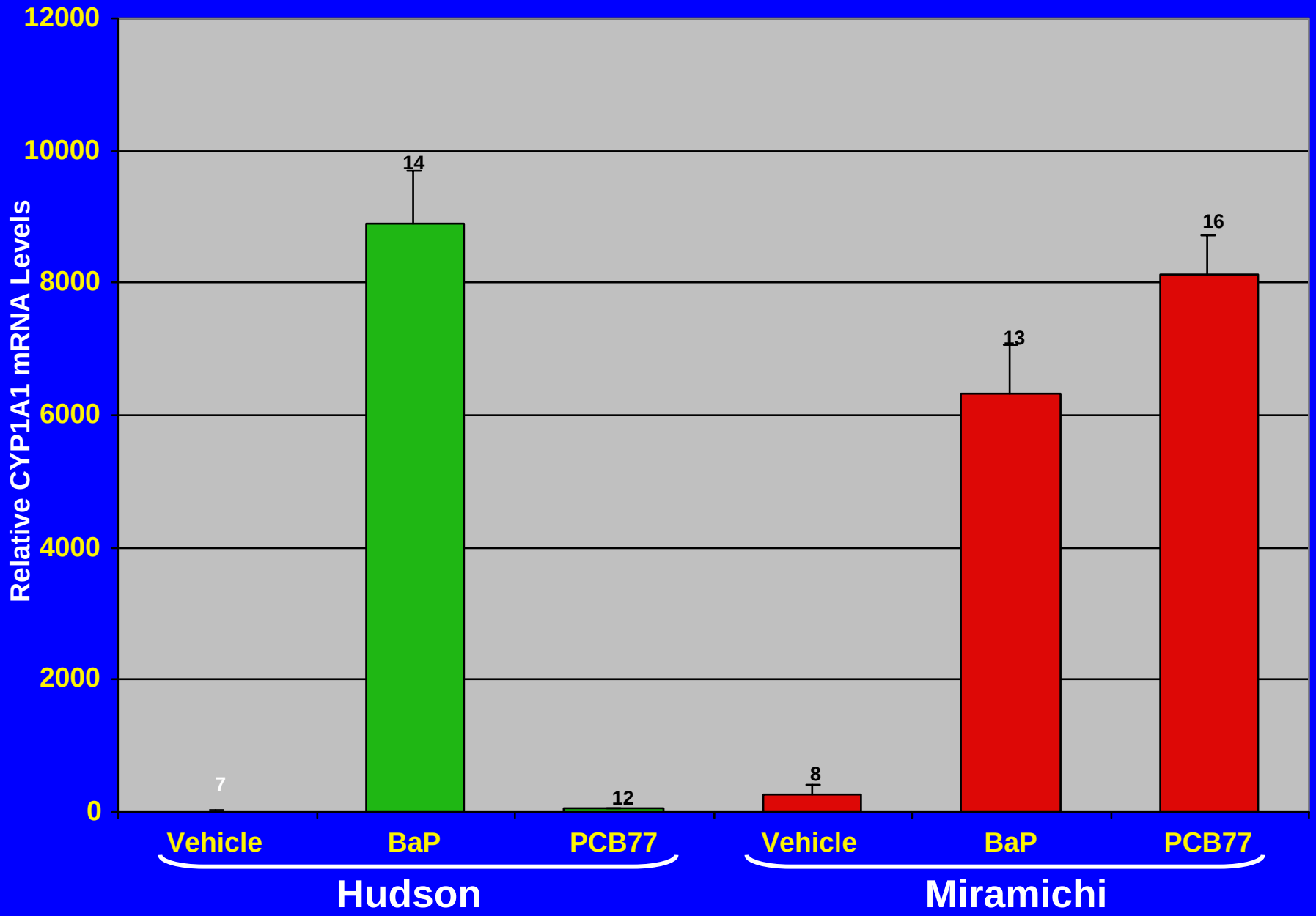
Genetic or Physiological?

- Tomcod from the Hudson River and Miramichi River mated, F_1 and F_2 embryos and larvae exposed to B[a]P (0.1 and 10 ppm) or PCB 77 (1 and 10 ppm) and levels of CYP1A1 mRNA measured

CYP1A1 mRNA in F_1 Early Larvae



CYP1A1 mRNA in F_2 Embryos



Early Life-Stage Toxicity

- Very sensitive response in fish to PCDD/Fs and PCBs
- Relevance at the population level
- Toxicities
  - cardiovascular dysfunction, craniofacial malformations, reduced survivorship
- Are early life-stages of tomcod sensitive to environmentally relevant levels of PCBs and TCDD?

Early Life Stage Protocol

1. Graded-dose experiment
   - 2x2x5 factorial design with replication and controls
   - Test all main effects of, and interactions between
     - Source population (Hudson vs. Miramichi)
     - Toxicant (PCB mix vs. TCDD)
       - PCB mix contained PCBs 77, 81, 126, 169
         - x = hepatic burden measured in HR TC
         - x = 28,000 ng PCBs/kg BW)
     - Dose (5 levels)
       - 0 x, 0.1 x, 1 x, 10 x, 100 x
   - F_1 generation MR and HR tomcod-winter 2002
   - F_2 generation MR and HR tomcod- winter 2003
   - Principle component analysis
2. Uptake and clearance (^3H labeled PCB mix and ^3H TCDD)

14 Response Variables Assayed in Early Life-Stages of F_1 and F_2 Tomcod

Morphological Measurements

Total length and curvature of larvae

Yolk quantity

Yolk-sac major & minor axes

Body length & depth

Jaw length

Eye diameter

Rate Measurements

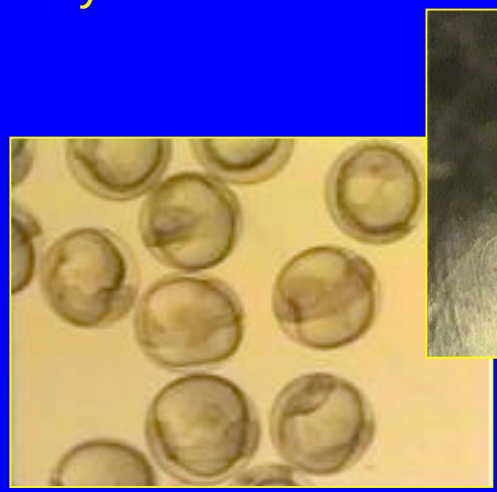
Mortality in embryonic period

Late embryo heart beat frequency

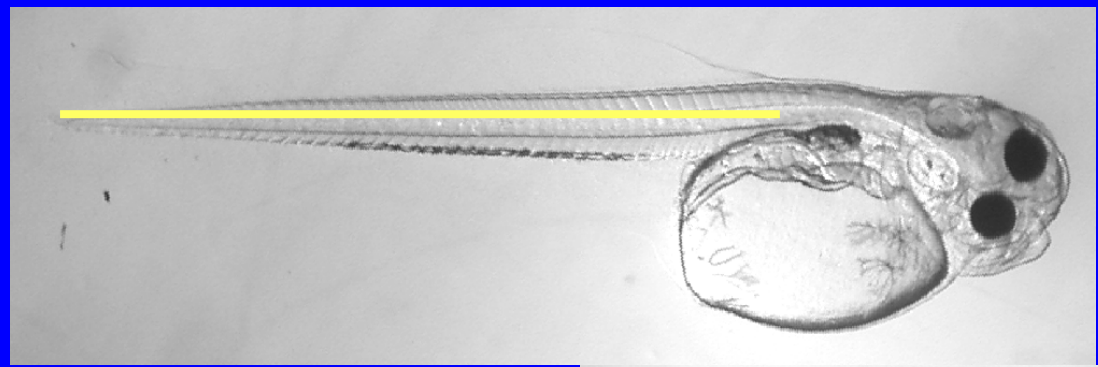
Yolk-sac larvae survival

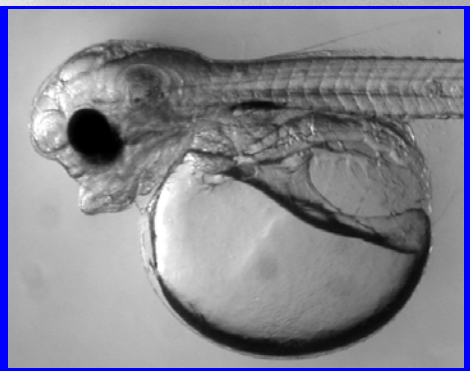
Yolk-sac larvae activity

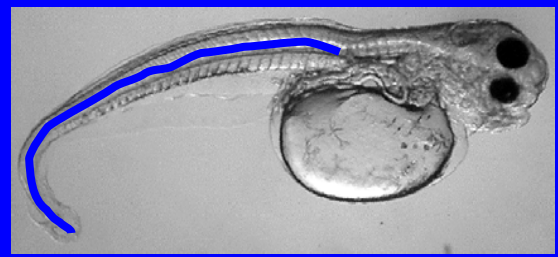
Growth and condition of feeding larvae and juveniles

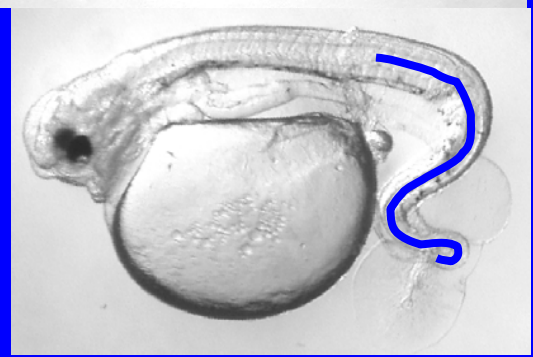


PCBs and TCDD Induce Malformations in MR, but not HR larvae









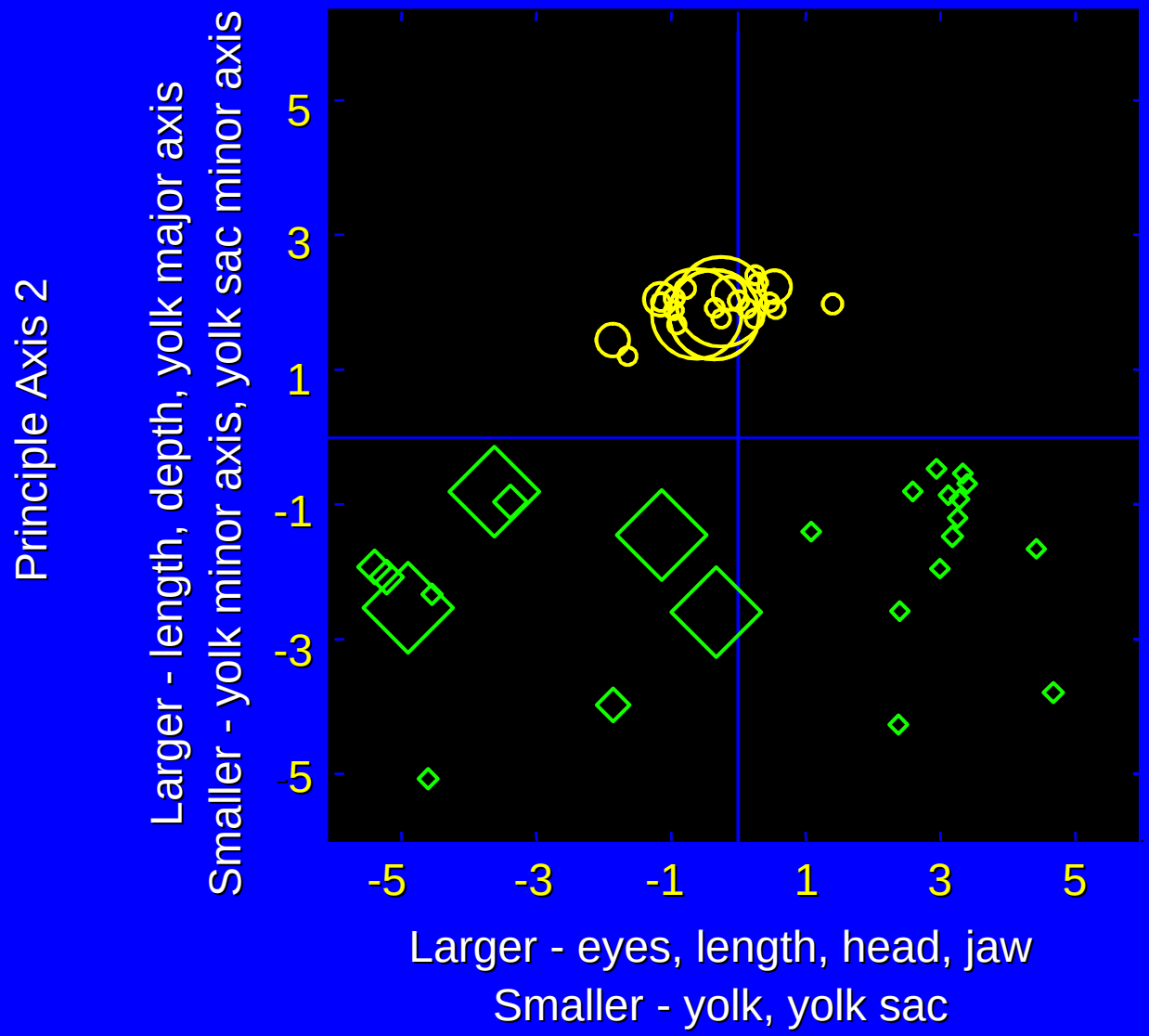
Results:

Multivariate variation in morphology

(contribution to principal axes)

	Principal axis	
Variable	PCA 1 (47%)	PCA 2 (33%)
TL-running	0.35 ***	0.10 ns
TL-minimum spanning distance	0.15 ***	0.41 **
body length	0.24 ***	-0.25 ***
body depth-total	0.10 ns	0.42 ***
body depth-musculature	0.15 **	0.35 ***
head length	0.33 ***	-0.03 ns
head depth	0.34 ***	-0.05 ns
jaw length	0.35 ***	0.06 ns
eye diameter-horizontal axis	0.31 ***	-0.25 ***
eye diameter-vertical axis	0.33 ***	-0.18 **
yolk major axis	-0.11 ns	0.40 ***
yolk minor axis	-0.31 ***	-0.16 *
yolk sac major axis	-0.18 **	0.33 ***
yolk sac minor axis	-0.27 ***	-0.26 ***

Multivariate Variation in Morphology of F_1 Larvae

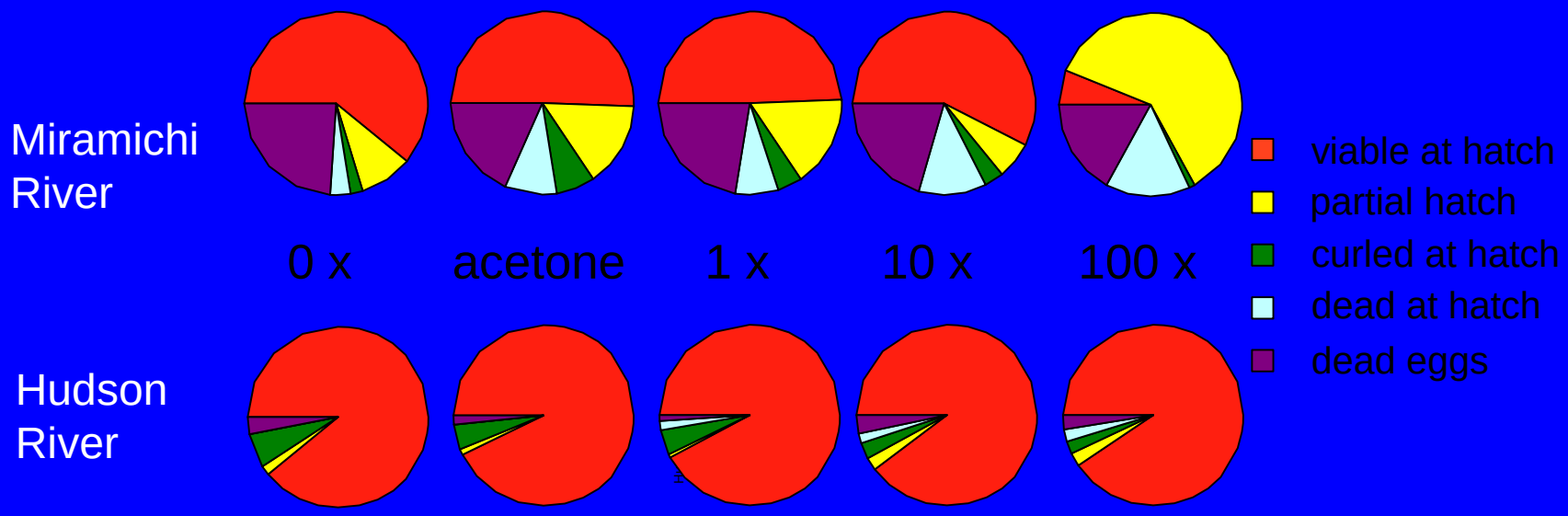


Hudson

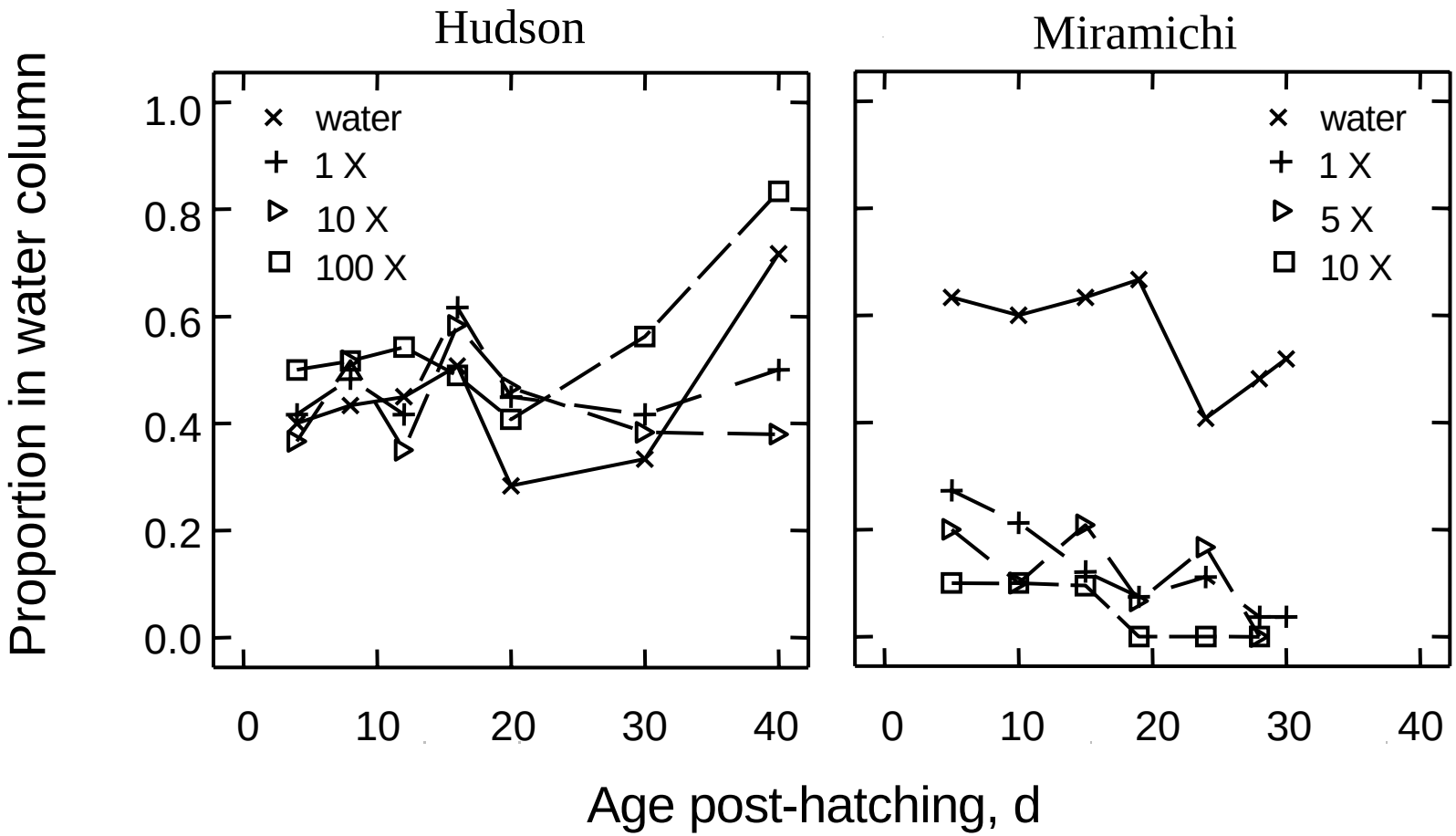
Miramichi

F_1 and F_2 Hudson River larvae were unaffected by environmentally relevant doses of either PCBs or TCDD--Miramichi by both chemicals

Hatching Success of Tomcod Embryos when Exposed to PCBs at 14 d Post-Fertilization



Activity of PCB-Exposed F_2 Yolk-Sac Larvae



Summary of Results

- Early life-stages of TC of MR or SB origin are very sensitive to coplanar PCBs or TCDD at environmentally relevant concentrations at many early life-stage toxic endpoints
- Early life-stages of TC of HR origin are unaffected by these treatments at any endpoint, at any dose tested

Conclusions

- Tomcod from the HR are highly resistant to PCBs and TCDD
  - Population level alteration
  - Community effect?
- PCBs, PCDD/Fs,or other contaminants may have incurred significant evolutionary change in the HR population

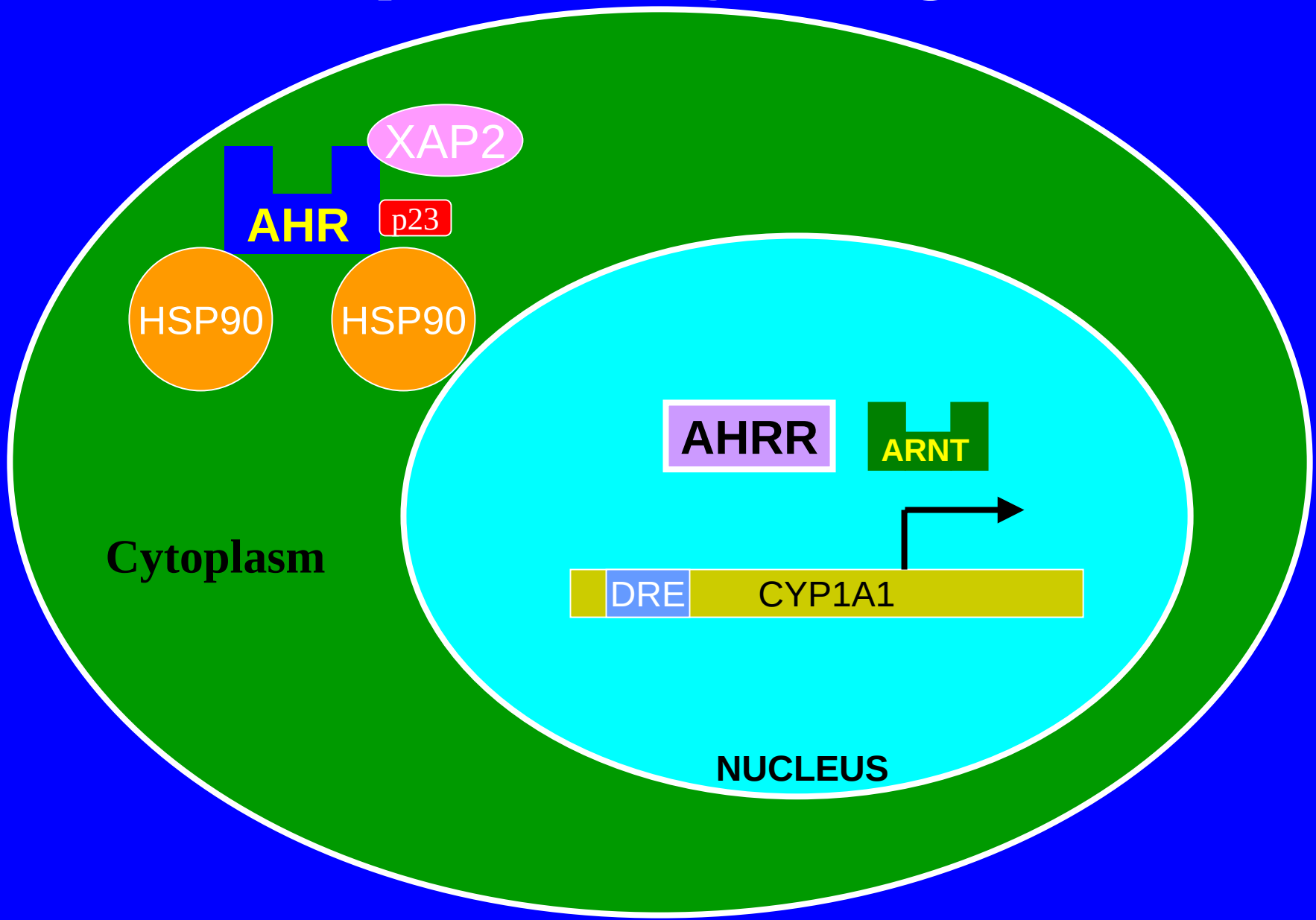
Conclusions

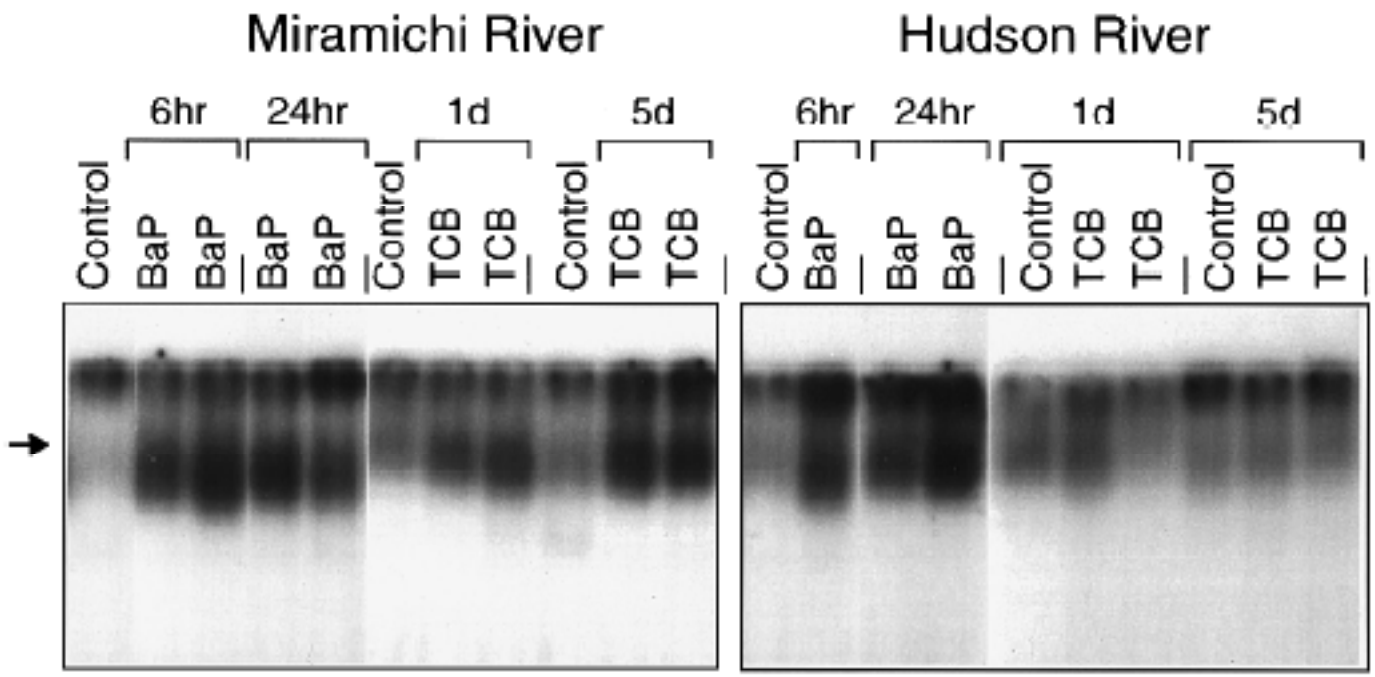
- More than more one molecular pathway may mediate AH toxicities in tomcod from the HR
  - More than one AHR2
    - Atlantic salmon (Hansson et al. 2004)
  - AHR-independent

Why are tomcod from the Hudson River resistant?

- **Hypothesis**
  - resistance results from altered expression of genes in the AHR pathway

AHR pathway targets

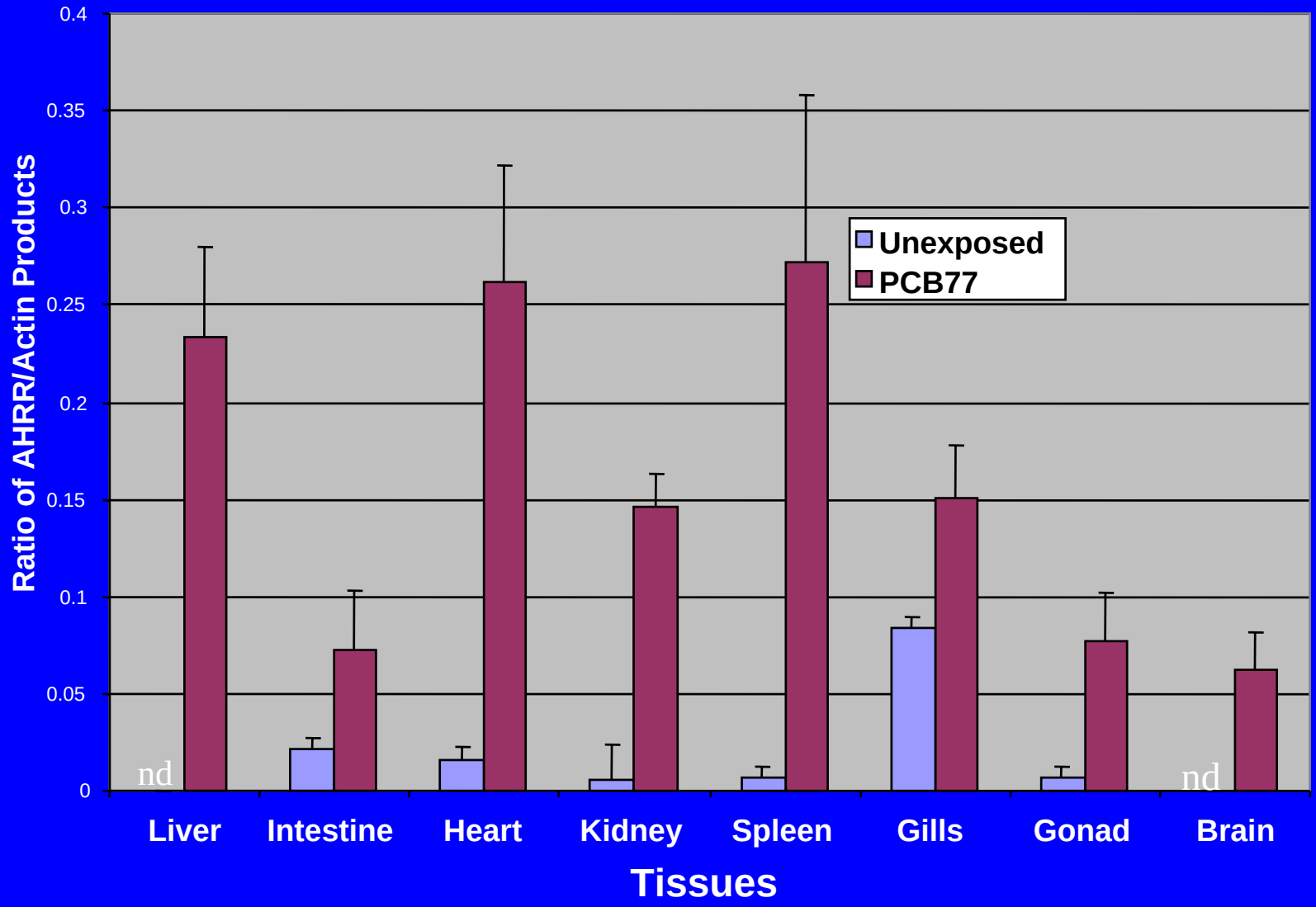




lity shift assays of inducible hepatic protein extracts prepared from Atlantic tomcod from the Miramichi River were treated with B[a]P (for 6 and 24 h), or TCB (for 1 and 5 days) or that were treated with vehicle control (e respectively) and incubated with ^{32}P-radiolabeled oligonucleotides containing tomcod DREs. Arrow indicates shift

Three Genes in the AHR Pathway (AHR, ARNT, AHRR) Were Cloned in Tomcod and Their Structure and Levels of Expression Were Compared Between Tomcod from the Hudson and Miramichi Rivers

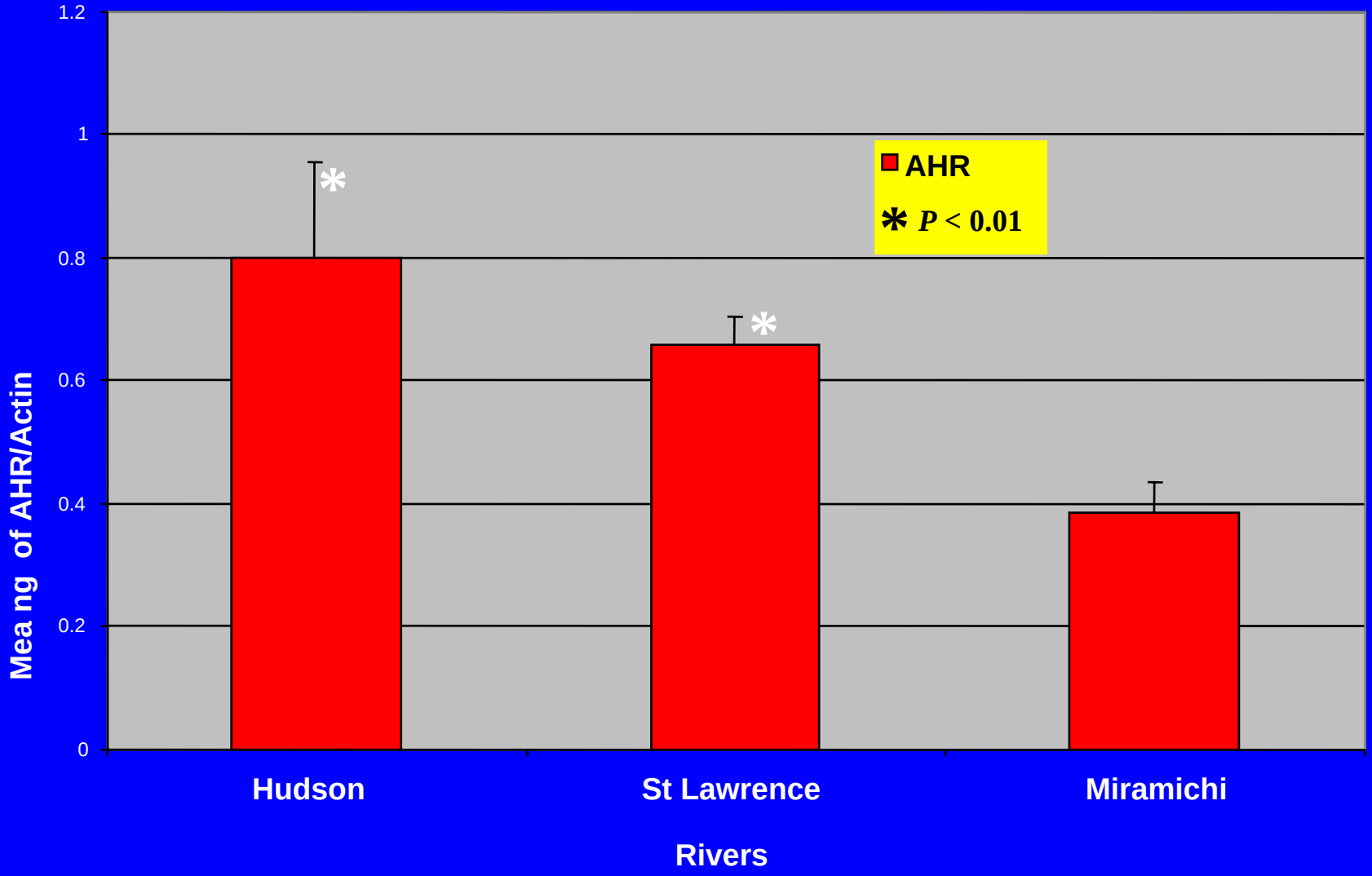
Comparison of AHRR Expression in Tissues of PCB77 (1 ppm) and Untreated F_1 Juvenile Miramichi Tomcod (n=6-8/tissue)



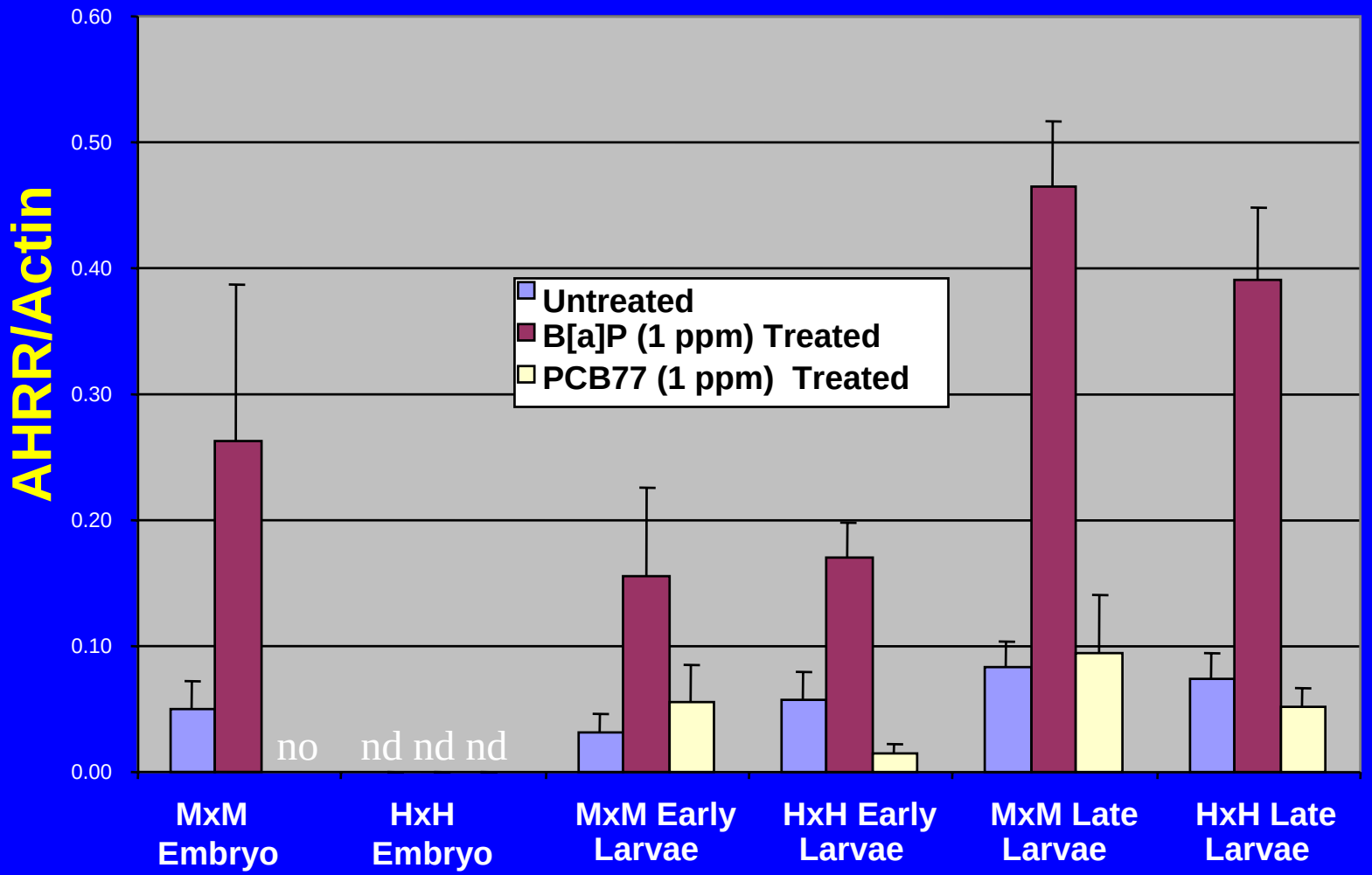
nd= not detectable

Comparison of Hepatic AHR Expression in Environmentally-Exposed Adult Tomcod from Three Rivers

(n=12 fish/river)



AHRR Expression in Treated and Untreated Early Life Stages of Tomcod from the Hudson and Miramichi



nd = not detectable
no = no data

Life-Stages and Pop

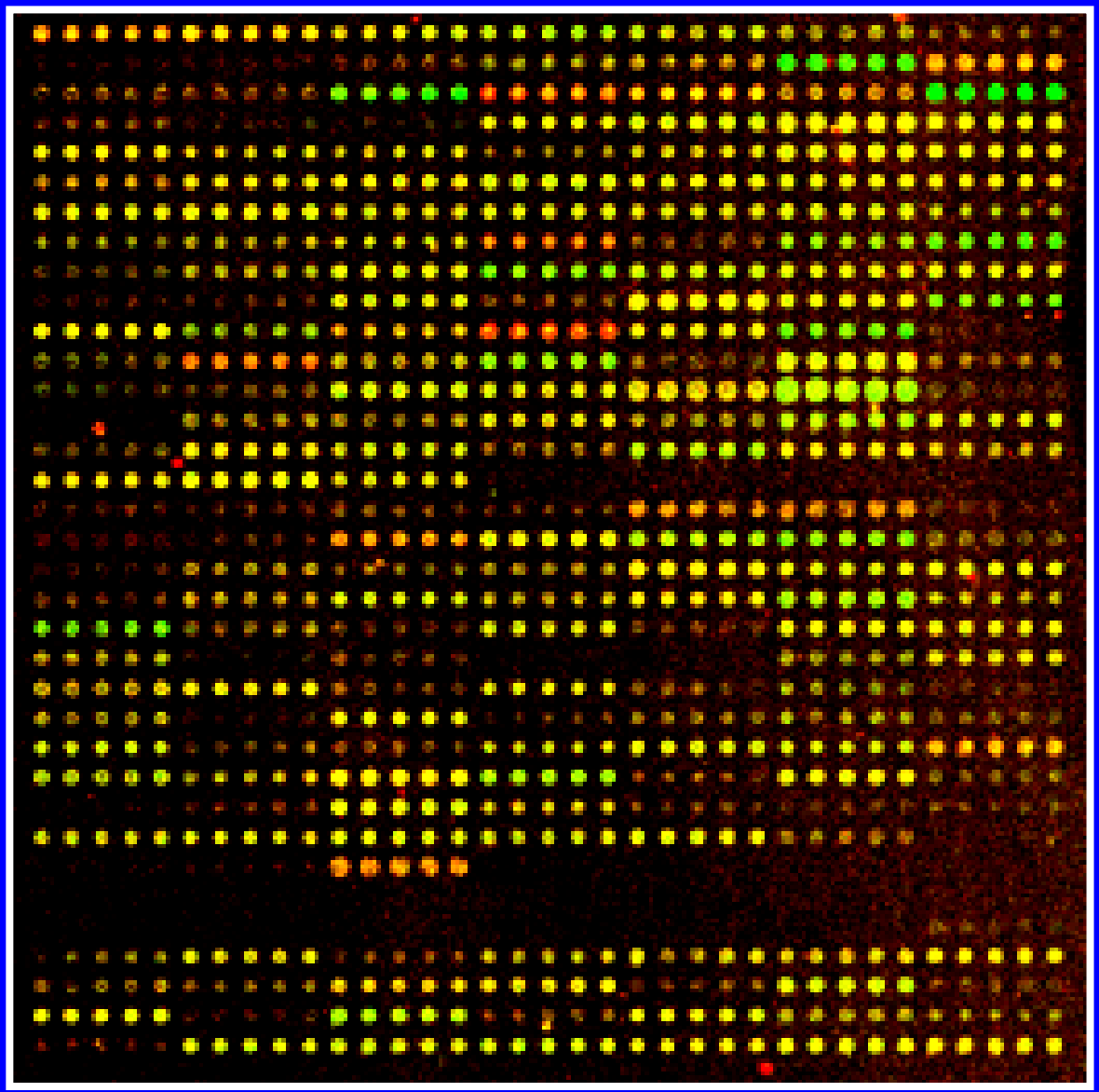
Comparison of Gene Expression

- Differences between the two populations in expression of any of the components of the AHR pathway are not large and do not correlate with presence of resistance
  - HR environment- AHR ↑
  - HR embryos - AHRR ↓
- However, all analyses were at the mRNA, rather than protein levels

Develop Tomcod Microarrays to

- Identify novel genes whose expression is altered by PCBs exposure
- Identify genes/pathways associated with early life-stage cardiovascular dysfunction
- Version 2
  - 4,800 anonymous clones from tomcod heart cDNA library

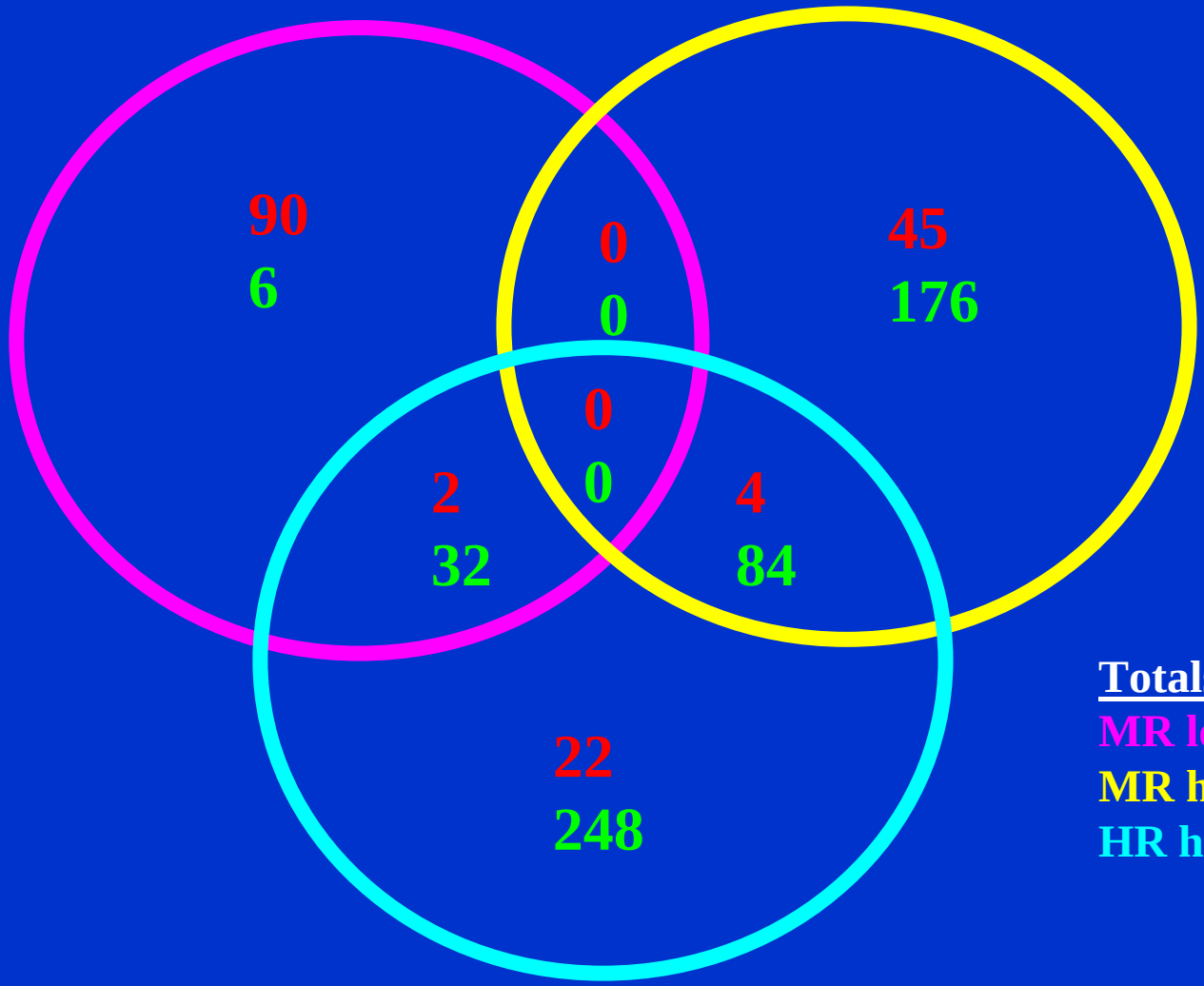
9,120 Element Microarray Produced from HR Tomcod Heart cDNA Library



*Subarray hybridized with control and PCB mix exposed MR tomcod heart targets (48 h post-injection)

Similarities in Cardiac Gene Expression Profiles Between PCB-Exposed Adult F_1 MR and HR Fish*

Red Values are ≥ 2 Fold Induced and Green Values are ≥ 2 Fold Suppressed



MR low (1X) PCB dose vs. MR control

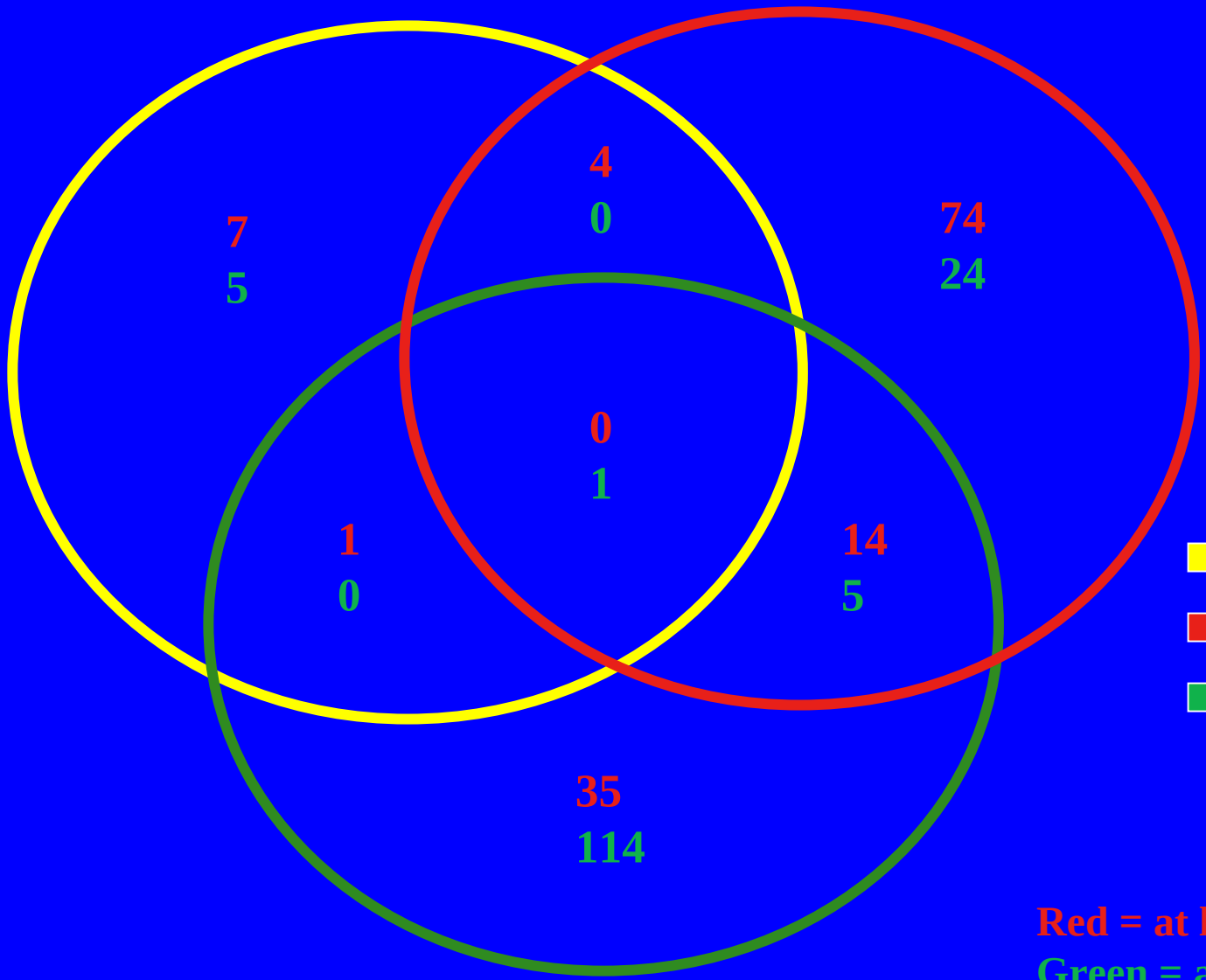
MR high (10X)PCB dose vs. MR control

HR high (10X) PCB dose vs. HR control

Totals	Up 2 Fold	Down 2 Fold
MR low	92	38
MR high	49	260
HR high	28	364

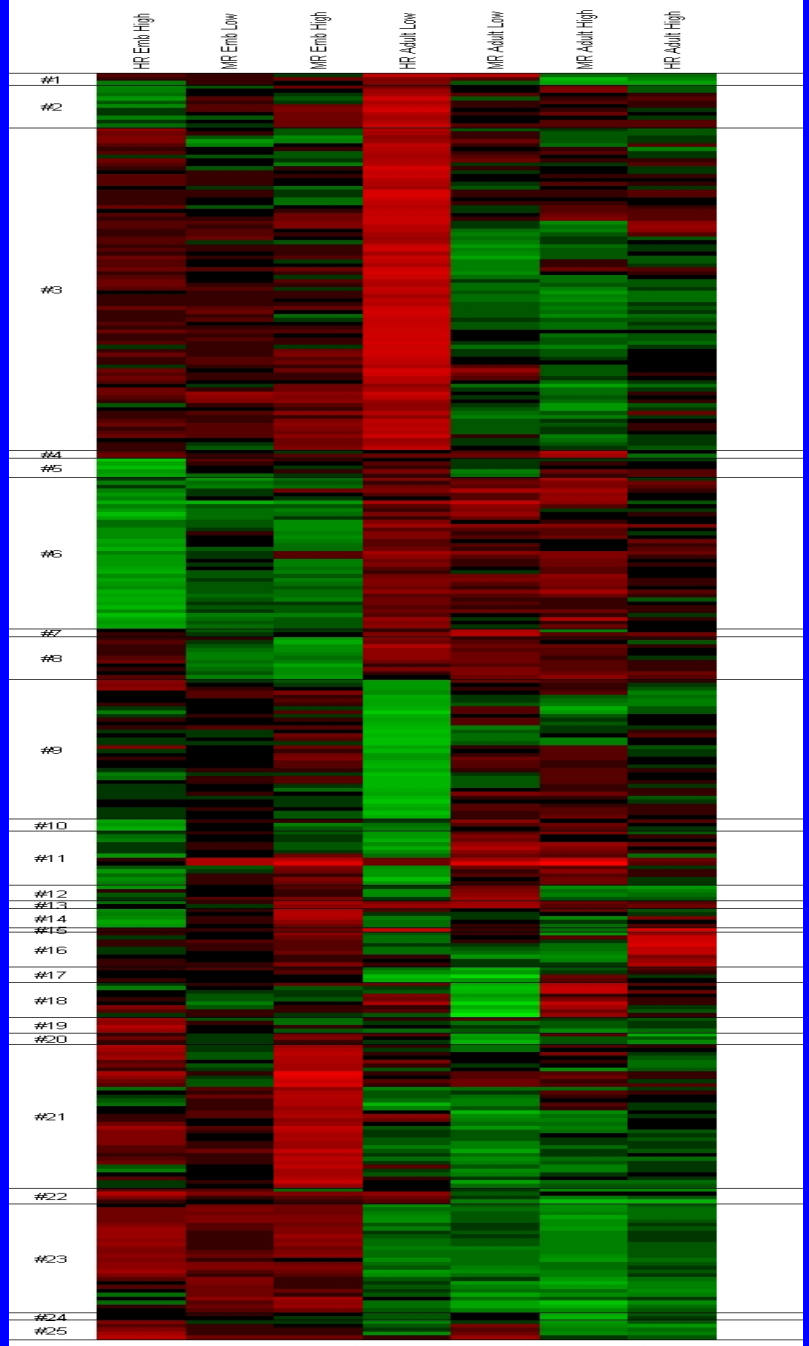
* Arrays hybridized with targets derived from total heart RNA isolated 48 h post-injection

Similarities in Gene Expression Profiles Between PCB-Exposed MR and HR Embryos

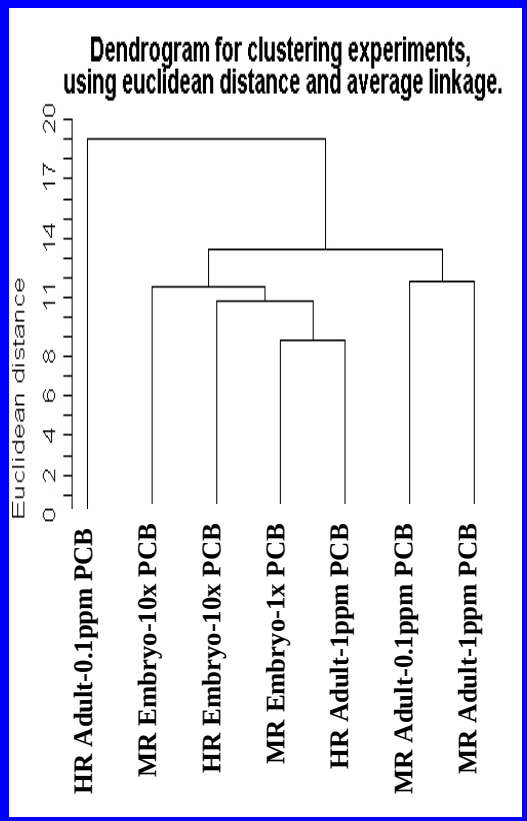


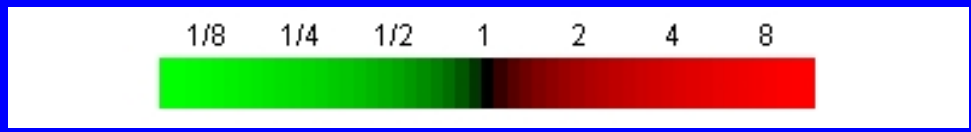
Red = at least 2 fold induced

Green = at least 2 fold repressed



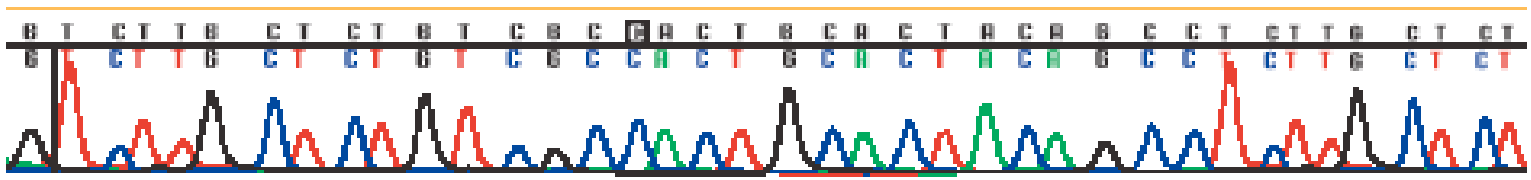
Hierarchical cluster analysis of cDNA clones that expressed across all replicate biological samples (n = 2-3).





Collaborators and Support

- N.K. Roy, E. Carlson, C. Grunwald, Z. Yuan, and C. Sorrentino
  - Environmental Medicine, NYU School of Medicine
- R. C. Chambers
  - Howard Marine Sciences Laboratory, NMFS
- M. Ikonomou, M. Fernandez
  - Institute of Ocean Sciences, Fisheries and Oceans Canada
- S. Courtenay
  - Gulf Fisheries Centre, Fisheries and Oceans, Canada
- Supported by SBRP and Hudson River Foundation

What are these Clones?

- Several genes have been identified:
  - Cathepsin L
  - Nemo-like Kinase
  - Fibrinogen
  - 70kDa Heat Shock Protein
  - Myosin Light Chain
  - Proteasome 26S Subunit
  - H+ transporting F1 ATP Synthase epsilon
  - Y-Box Binding Protein, Y-B1