Early Life-Stage Toxicities in PCB and TCDD-Treated Atlantic and Shortnose Sturgeons

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Objectives

- To develop protocols for experimental methodologies in support of toxicological studies on early life-stages (ELS)
- To determine if sturgeons express toxicities in ELS to environmentally relevant doses of dioxin-like compounds
- To identify phenotypic (sublethal) responses to these mixtures and congeners of PCBs at the organismal and molecular levels that might be used as biomarkers of exposure in ELS

Atlantic and shortnose sturgeons

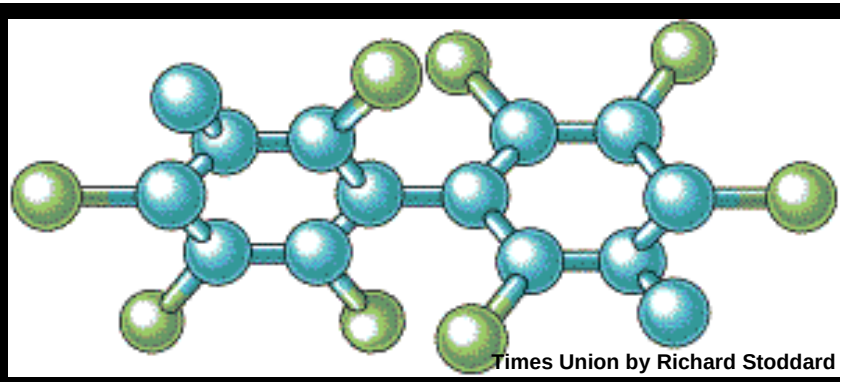
Shortnose sturgeon 56 hrs post-fertilization

Key biological features

- History of commercial exploitation and habitat loss / degradation
- Long-lived and anadromous
- Benthic eggs with protracted developmental period
- Bioaccumulate lipophilic toxicants

Polychlorinated biphenyls
(PCBs)

- **Industrial contamination of the Hudson River**
- **Stable, persistent organic chemicals**
- **~ 1,.3 million lbs dumped into the Hudson by GE over 30 years**


Times Union by Richard Stoddard

Frank Whitney

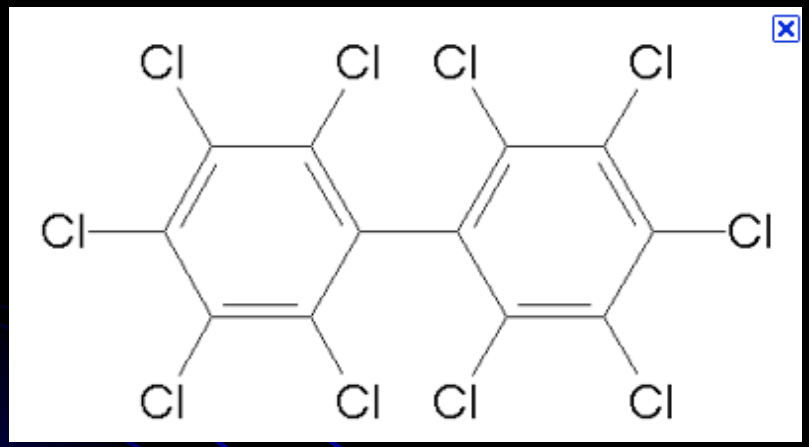
Jos. T. Fewkes & Co.

EonFusion.com

ENS Newswire

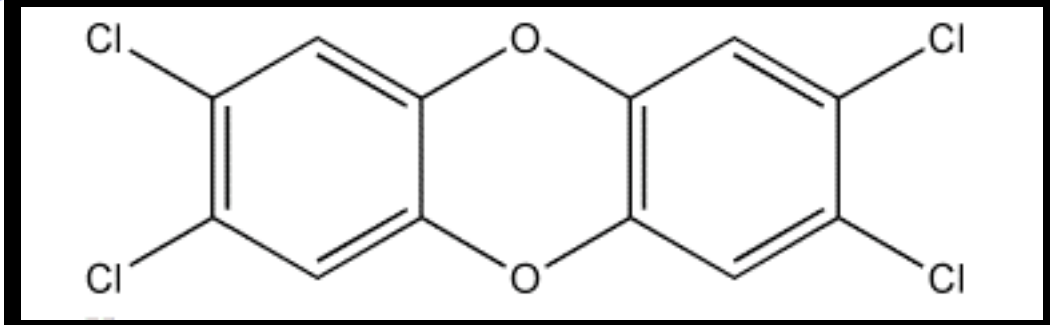
Structures of AHR Agonists

PCBs

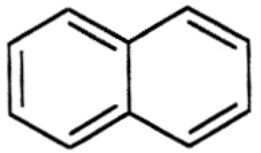


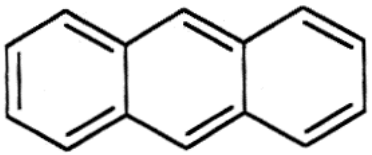
PCDDs

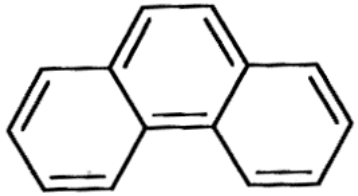
(TCDD)

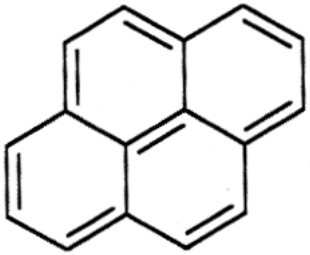


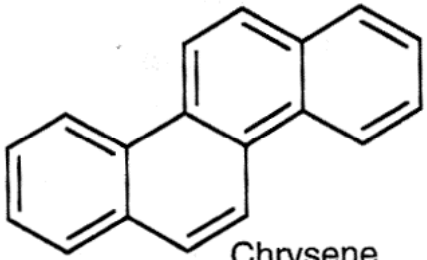
Polycyclic aromatic hydrocarbons
(PAHs, constituents of oil)

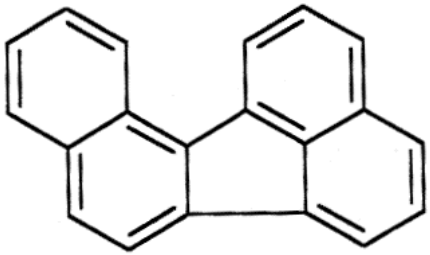

Naphthalene

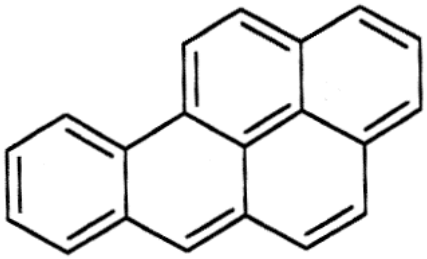

Anthracene

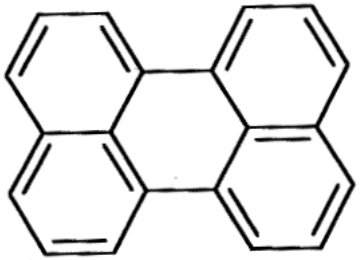

Phenanthrene

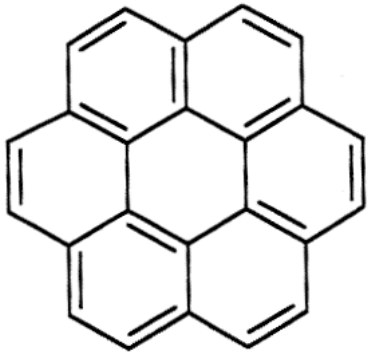

Pyrene


Chrysene


Benzo[j]fluoranthene


Benzo[a]pyrene

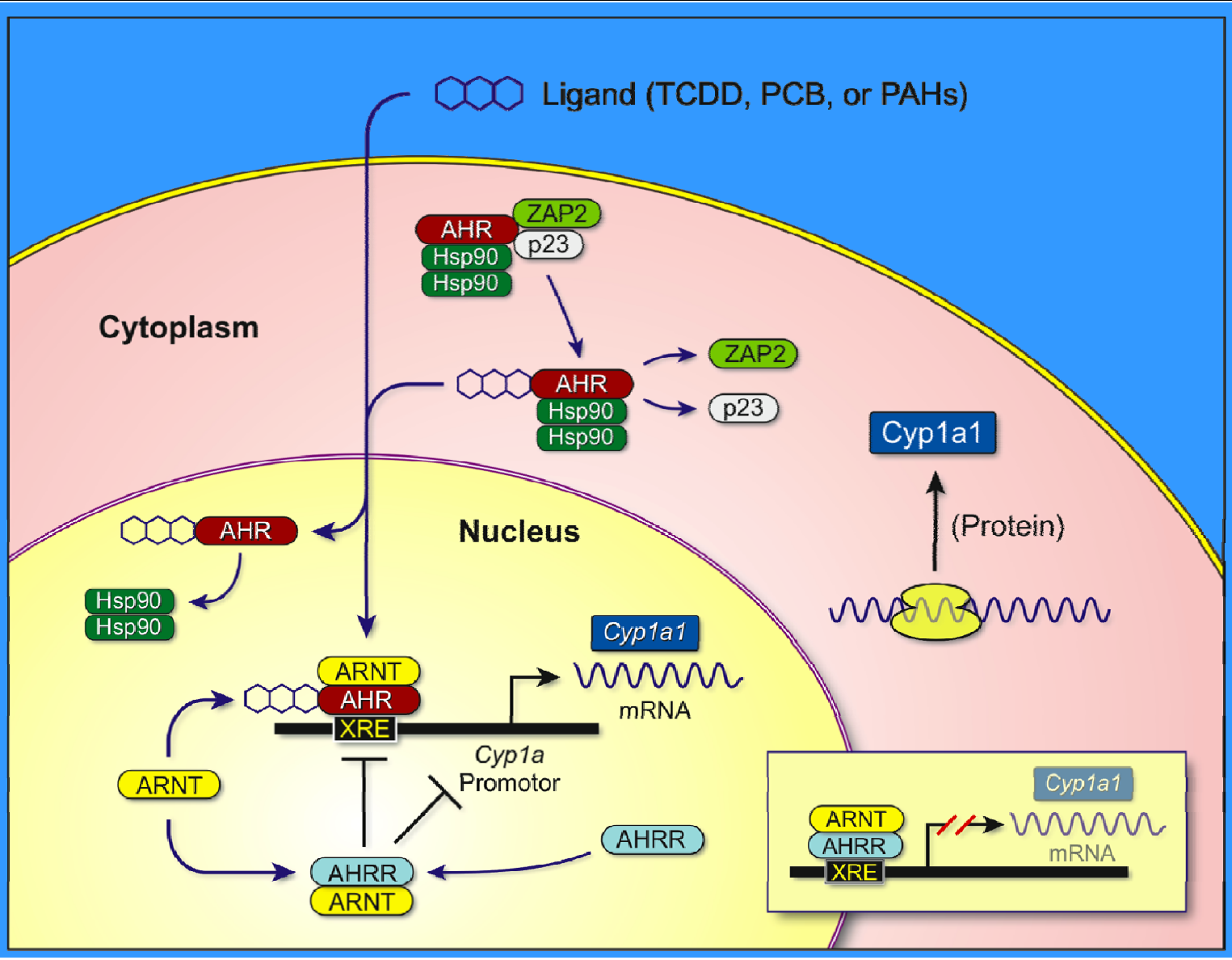

Perylene


Coronene

Early life-stage toxicities in other taxa
cytochrome P4501A (CYP1A)

- CYP1A expression is increased by exposure to PCDD/Fs > coplanar PCBs > PAHs
- CYP1A transcription is activated by the Aryl Hydrocarbon Receptor (AHR) pathway
- Early life-stage (ELS) toxicities are activated by AHR and co-occur with induced CYP1A

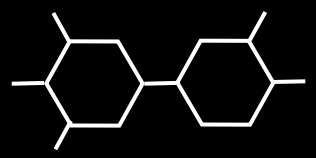
AHR pathway


Ligand (TCDD, PCB, or PAHs)
Cytoplasm
AHR
ZAP2
p23
Hsp90
Hsp90
AHR
Hsp90
Hsp90
ZAP2
p23
Cyp1a1
(Protein)
Nucleus
AHR
Hsp90
Hsp90
Cyp1a1
ARNT
AHR
XRE
mRNA
Cyp1a
Promotor
ARNT
AHRR
AHRR
ARNT
Cyp1a1
ARNT
AHRR
XRE
mRNA

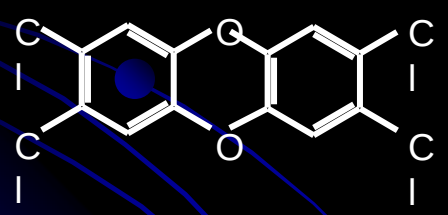
Early life-stage toxicities in other taxa
organismal level

- Lower survival
- Altered developmental rates
- Morphological abnormalities predictive of reduced fitness
  - Craniofacial alterations, pericardial and yolk-sac edema, abnormal spinal curvature (consequences of structural and functional aberrations of heart)
- Reduced activity

Methods
contaminants used – CYP1A



congener 126

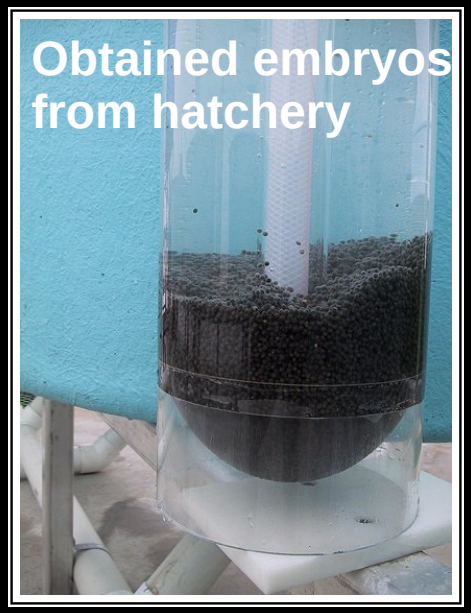


TCD-dioxin

- 2 toxins
  - PCB126 and TCDD
- 5 concentrations of each toxin:
  - PCB: 0.01, 0.1, 1, 10 and 100 ppb
  - TCDD: 0.001, 0.01, 0.1, 1, and 10 ppb

Methods

exposure protocol

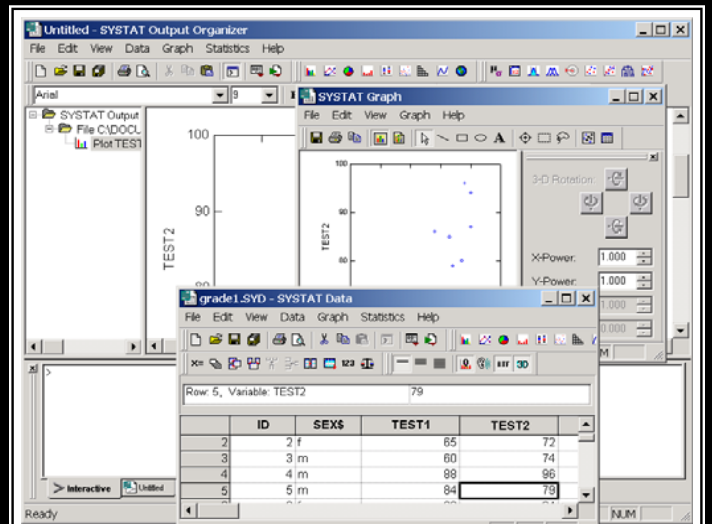


Exposed embryos for 26 hrs

(radiolabeled uptake estimates - ^{3}H PCB126)

Recorded survival, % hatch, and size (at 24 – 48 hr post-hatch)

Measured YSL for morphometric variation

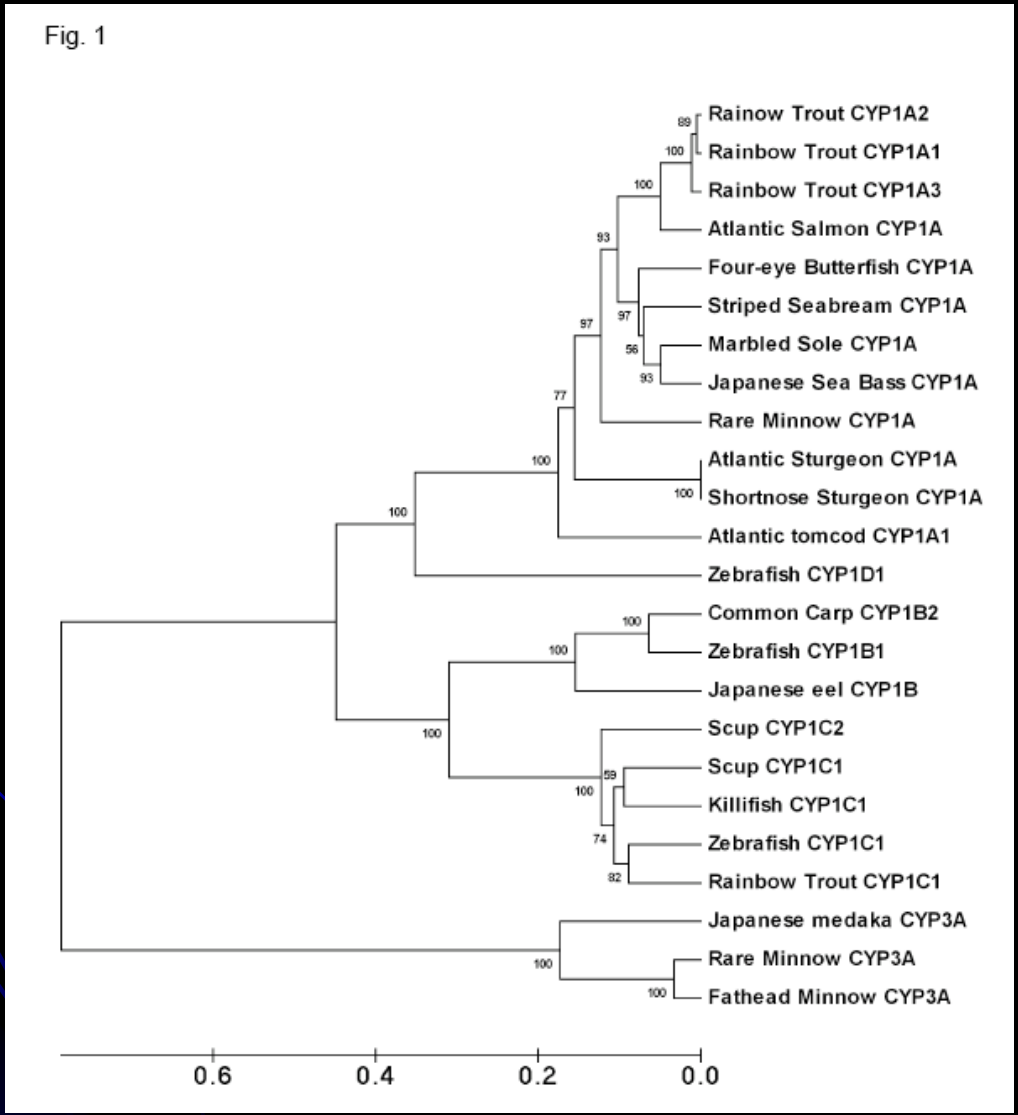


Methods
CYP1A

- Clone and characterize CYP1A in Atlantic and shortnose sturgeons using conserved fish CYP1A sequences
- Develop semi-quantitative and real-time RT-PCR assays to quantify CYP1A mRNA in both species
- Measure CYP1A expression in PCB126 and TCDD-exposed larvae

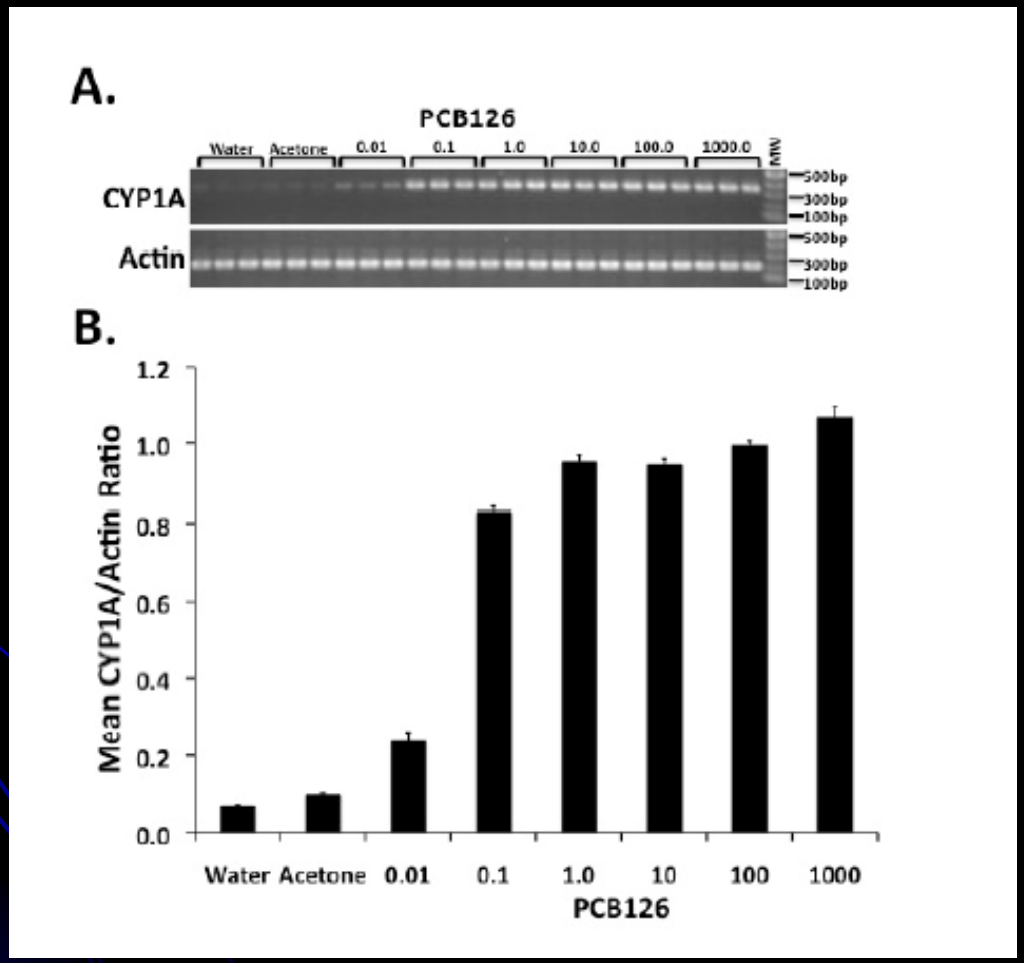
Results

UPGMA dendrogram of fish CYP cDNA sequences



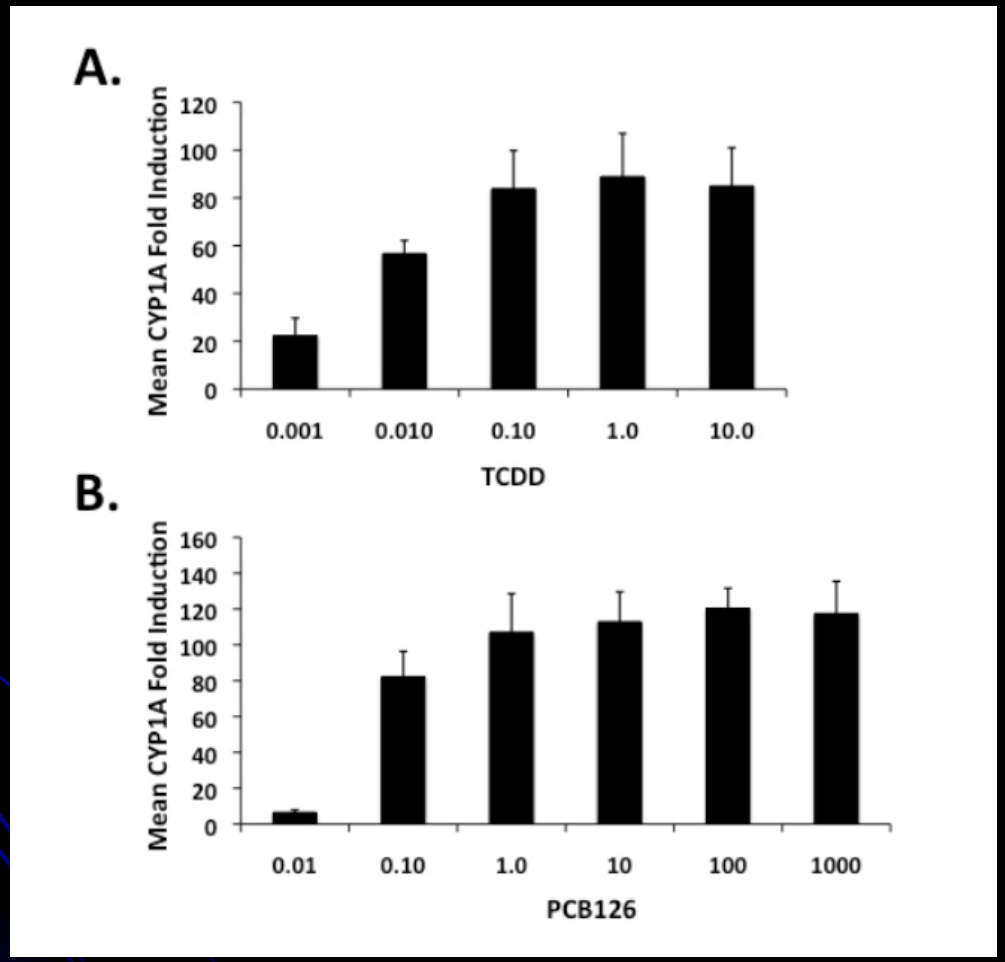
Results

Semi-quantitative RT-PCR of CYP1A in Atlantic sturgeon larvae exposed to PCB126

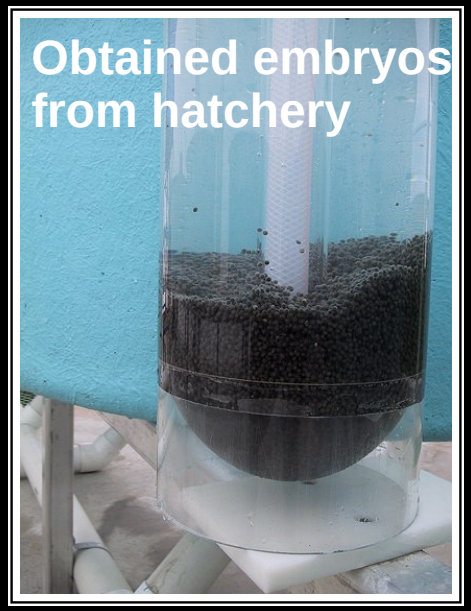


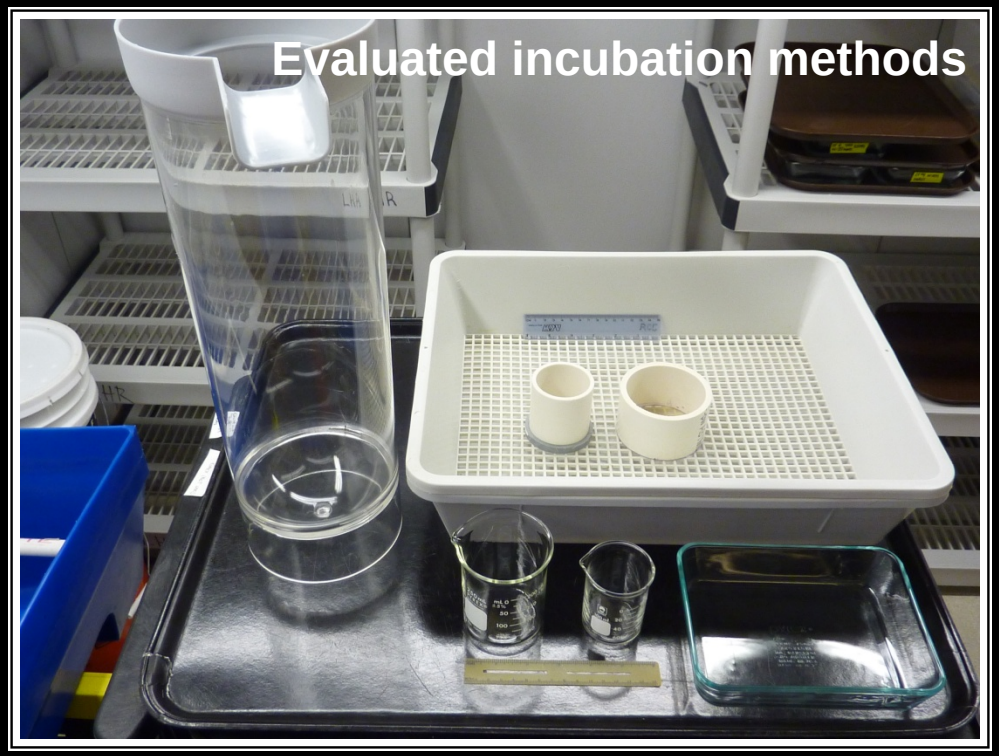
Results

Real-Time RT-PCR of CYP1A in shortnose sturgeon larvae exposed to TCDD and PCB126

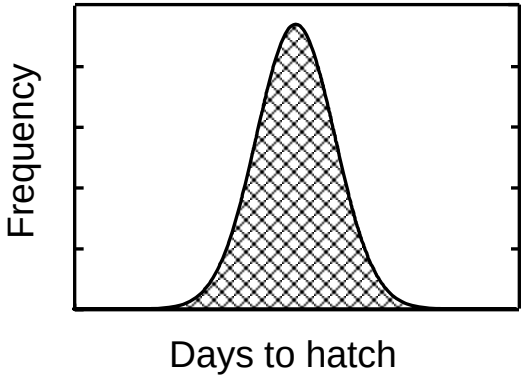


Methods - rearing protocol



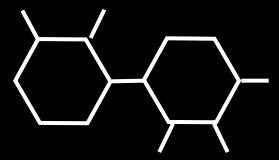


Recorded survival, % hatch, and size at hatching



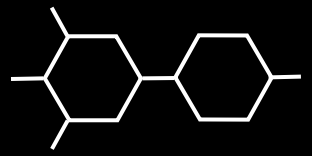
Methods

contaminants used – lethal and sublethal phenotypic effects

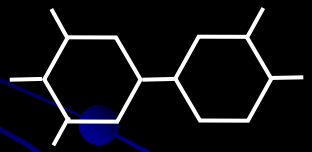


Aroclor 1254

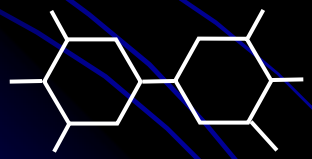
congener 77



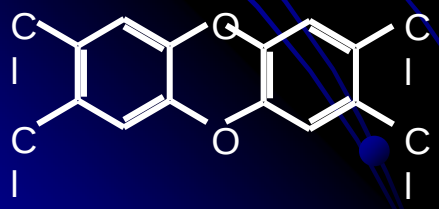
congener 81



congener 126



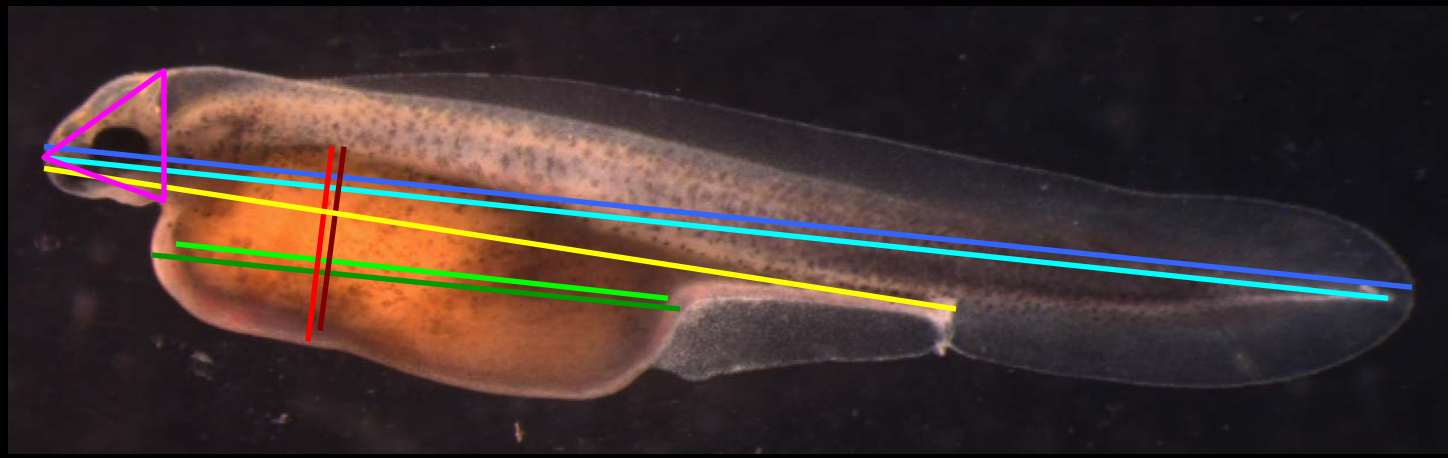
congener 169



TCD-dioxin

- 6 Toxins
  - PCB77, PCB81, PCB126, PCB169, Aroclor 1254, and TCDD
- 6 Concentrations
  - 0.001, 0.01, 0.1, 1, 10 and 100ppb

Methods
morphological measurements



1

2

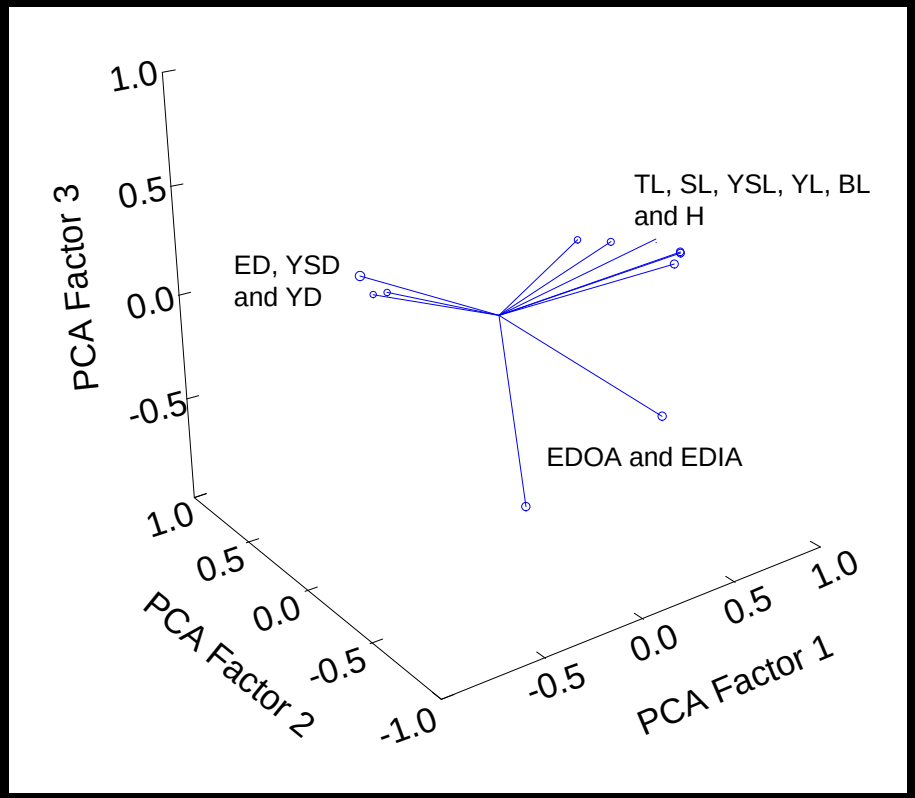
3

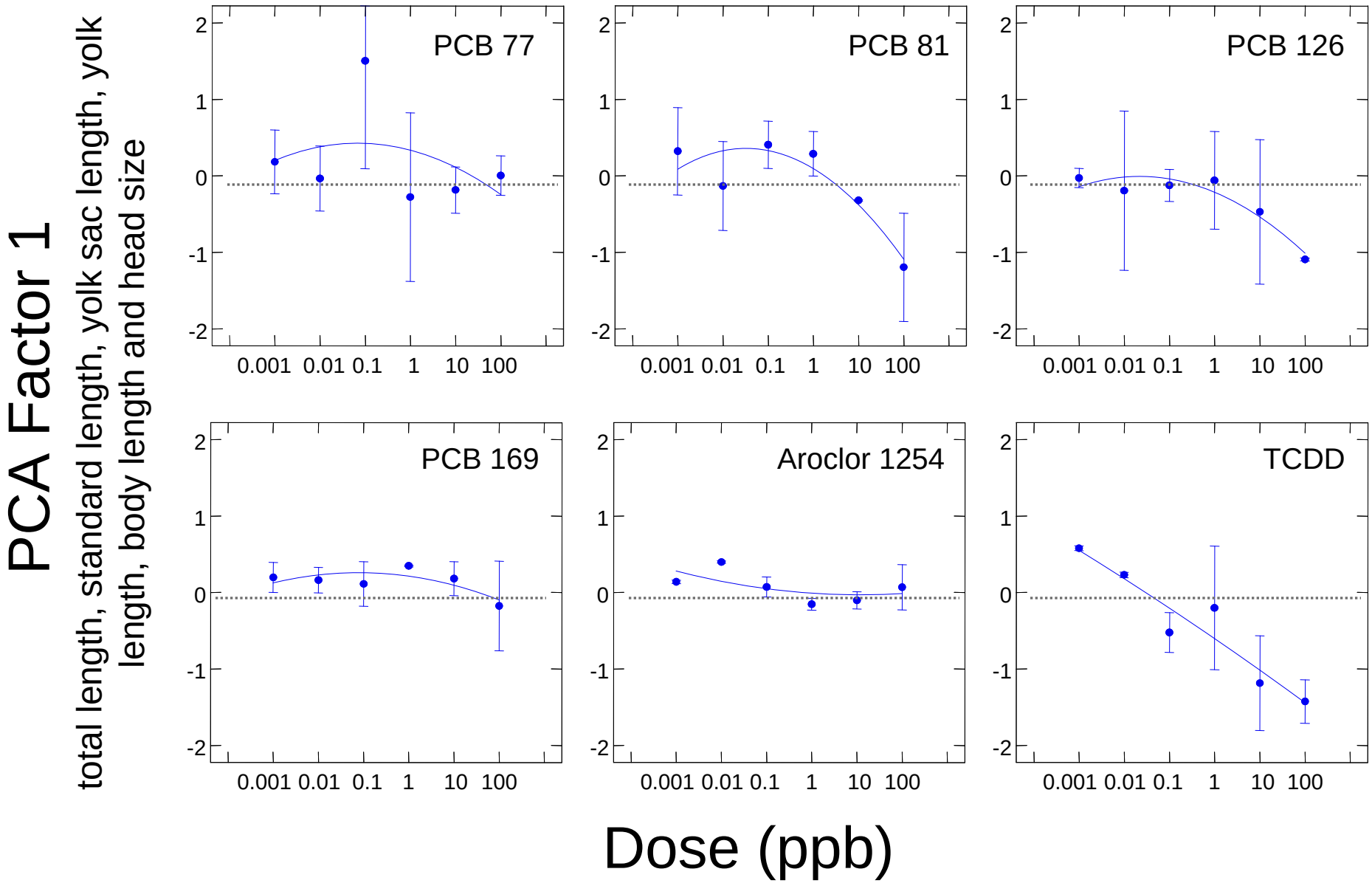
4

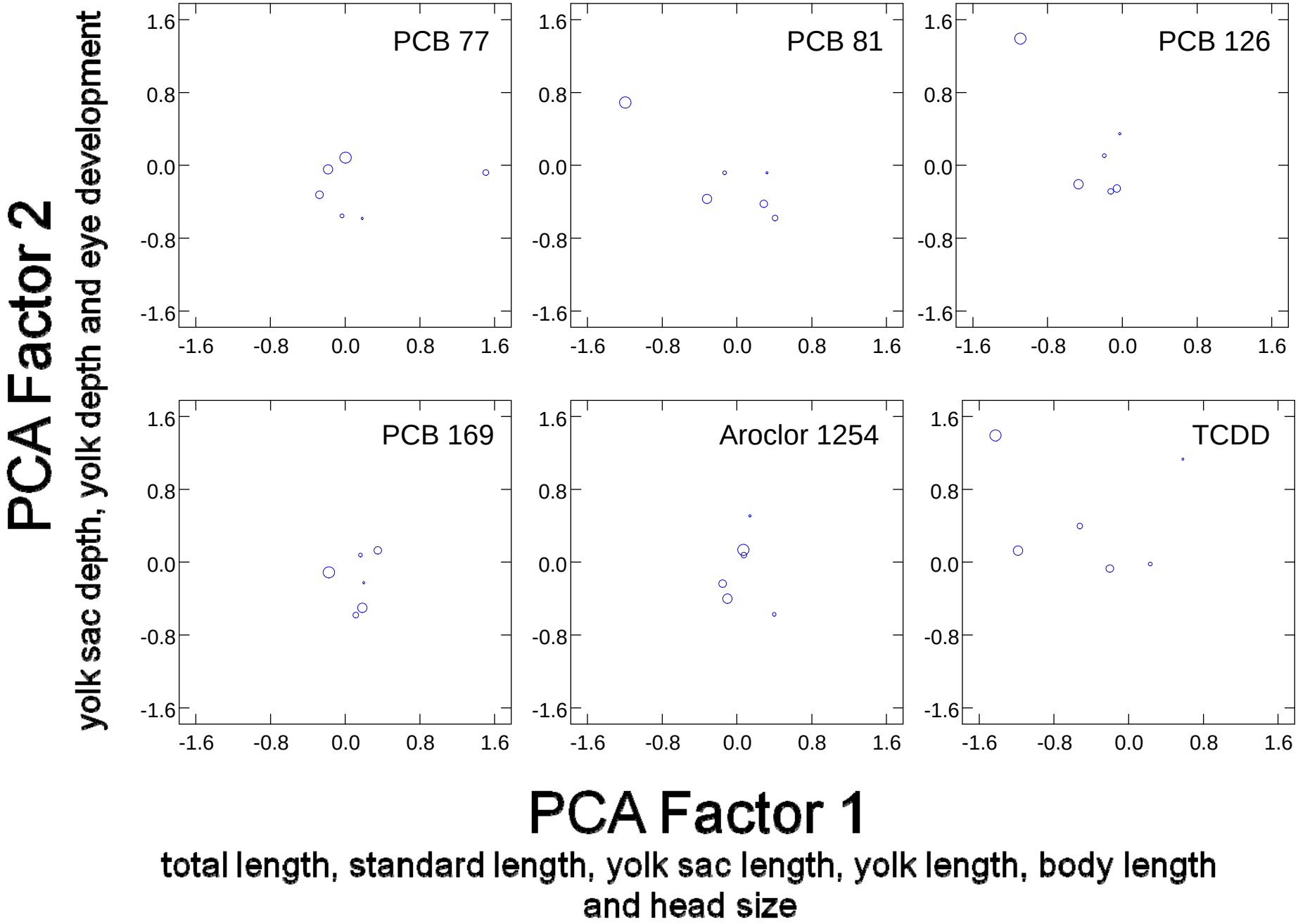
5

Results
Morphometric responses

- PC axis 1 *(49.0%)*
  - Total length, standard length, yolk sac length, yolk length, body length and head size
- PC axis 2 *(21.6%)*
  - Yolk sac depth, yolk depth and eye development
- PC axis 3 *(10.8%)*
  - eye diameters




PCA Factor 1
total length, standard length, yolk sac length, yolk length, body length and head size
PCB 77
PCB 81
PCB 126
PCB 169
Aroclor 1254
TCDD
2
1
0
-1
-2
0.001 0.01 0.1 1 10 100
Dose (ppb)


PCA Factor 2
yolk sac depth, yolk depth and eye development
PCB 77
PCB 81
PCB 126
PCB 169
Aroclor 1254
TCDD
1.6
0.8
0.0
-0.8
-1.6
PCA Factor 1
total length, standard length, yolk sac length, yolk length, body length and head size

Results
Morphometric responses (shortnose sturgeon)

Acetone

TCDD at 0.001ppb

PCB126 at 10ppb

Summary

- Protocols for toxicological exposure and maintenance through early life-stages were successful for Atlantic and shortnose sturgeons
- CYP1A cDNA cloned and partially characterized
- CYP1A dose-sensitive to AHR agonists at environmentally relevant concentrations
- Preliminary data on effects of PCB congeners, Aroclor 1254, and TCDD on shortnose showed reduced early life survival and sublethal responses (developmental, morphological, and behavioral)
- A syndrome of morphological toxic responses included shorter fish, yolk-sac edema, and retarded eye development at higher contaminant concentrations

Acknowledgments

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