



# Estrogenicity of tissue extracts from white bass and channel catfish caught along the Three Rivers of Pittsburgh, Pennsylvania

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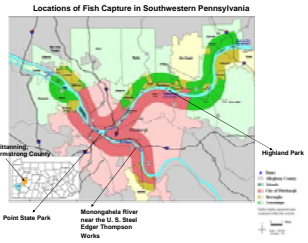
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## Introduction

- Accumulation of endocrine-disrupting substances from manufacturing, agricultural and residential sites in waterways is an enormous and universal environmental problem.
  - Such waterways are important sources of drinking water, recreation, and subsistence fishing by the local community.
  - Examples of endocrine disruption in fish include the feminization of male fish exposed to effluents from sewage treatment plants, as well as masculinization of female fish exposed to treated water from paper mills.
- Our concerns:**
- Are xenoestrogens present in fish captured in the Allegheny, Monongahela, and Ohio Rivers in Pittsburgh?
  - What are the implications for the health of the rivers, the communities that use water from these rivers, and the fishers who subsist on fish caught from these rivers?

**Aim:** to determine if estrogenic substances are present in fish caught in various parts of Pittsburgh's three rivers.



## Pollution at Fish Capture Locations

- Kittingham:** 36 miles upstream from Pittsburgh on the Allegheny River in Armstrong county. This site is relatively unpolluted by local industry. Angler focus groups consider fish from this site "safe" for eating (Volz and Christen, 2007).
- Monongahela:** Catch site at the Braddock Dam, 11 miles upstream from Pittsburgh, bordered by the Edger Thompson Works of US Steel. This plant was part of the largest integrated iron and steel making operations in the world. It remains a significant EPA Toxic Release Inventory (TRI) site (EPA, 2007). Upstream of this catch site are numerous other TRI sites, including coking operations.
- Highland Park:** Catch site at the Highland Park Bridge/Dam, on the Allegheny River. It is significantly downstream of the relatively few TRI sites on the Allegheny. Additionally, it is downstream of far fewer combined sewer overflows (CSO).
- Point:** Catch site is approximately 2 miles downstream from a US Steel Plant. It is near over 100 CSOs and aging municipal sewage infrastructures that overflow into the rivers (Volz, 2006).
- Store:** Channel catfish from a Georgia fish farm and white bass from Lake Erie were purchased from a local fish market for comparison purposes.

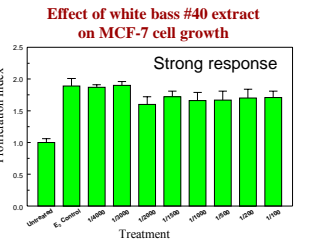
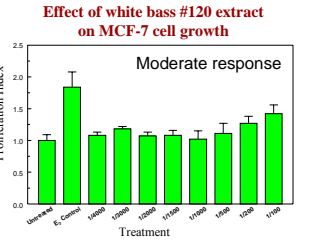
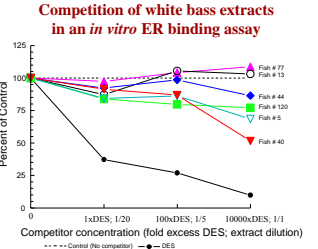
## Methods

- Fish Capture:** Fish were caught by local anglers, placed on ice, and transported to the laboratory for immediate dissection.
- Extraction of fish:** a one-gram sample including skin, muscle, and fat, was taken perpendicular to the lateral line from each white bass (n=7) and channel catfish (n=21). Each sample was homogenized and extracted with chloroform:methanol (9:1). The organic phase was evaporated under nitrogen, and the residue was stored under nitrogen at -20°C. For use in assays, residues were dissolved in EtOH:glycerol (70:30).
- In vitro competitive estrogen receptor (ER) binding assay:** Cytosol was prepared from mature rabbit uteri, and aliquots were incubated with <sup>3</sup>H-17β-estradiol (E<sub>2</sub>) in the absence and presence of test substances. Diethylstilbestrol (DES) was used as a positive control in concentrations of 1-10,000 times that of the labeled E<sub>2</sub>. Fish tissue extracts were tested at dilutions of 1/20 to full-strength. The mixtures were incubated at 4°C overnight, and bound ligand was separated from free by P6 resin spin columns.
- Cell proliferation assay (CPA):** Human breast cancer cell lines used were ER-positive MCF-7 and ER-negative BT-20. Cells were incubated for 72h with estradiol (1nM) or fish extracts diluted in medium to final concentrations of 1/4000-1/100 of the original extracts. Cell growth was detected by use of CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega). Proliferation in test wells is expressed compared to proliferation in untreated and estradiol-treated cells.



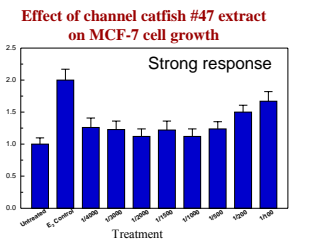
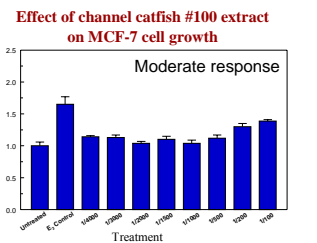
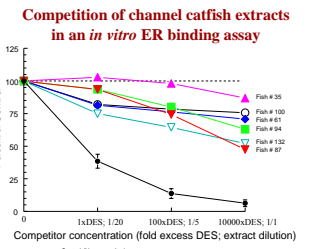
**White Bass**  
(*Morone chrysops*)

White bass are silvery in color with a milky white belly. In Pennsylvania the white bass is native to the western counties, especially Lake Erie and the Ohio River watershed. They prefer large open clear water with a firm bottom and water depths of less than 30 feet. Adult white bass feed primarily on small fishes and larger insects. Life expectancy is usually 3 to 5 years.



**Channel catfish**  
(*Ictalurus punctatus*)

The channel "cat" has a slender body with a deeply forked tail. The upper jaw is longer than the lower jaw, with long, black barbels around the mouth. They are found statewide. They prefer areas with deep water, i.e. clear, warm lakes and moderately large to large rivers, with clean sand, gravel or rock-rubble bottoms. Adult channel catfish are bottom feeders that use small and taste to locate food. They are omnivorous and will eat insect larvae, crayfish, mollusks, small fish, and dead fish.



## Results

- In general, extracts that compete for ER binding are also stimulators of cell proliferation.
- The sex of the fish was not a factor in predicting response in either assay.
- Estrogenicity of fish extracts differed with location of fish capture.

## Results: ER binding assays

- Bass extracts:** Of 6 bass extracts tested, 4 exhibited moderate or strong competition for ER binding in a dose-dependent manner; 2 enhanced binding.
- Catfish extracts:** Of 21 catfish extracts tested, 2 were strong and 7 were moderate competitors, 10 were weak competitors, and 1 was non-competitive.

## Results: CELL PROLIFERATION ASSAYS

- Some extracts resulted in strong cellular responses in ER-positive MCF-7 cells, comparable to their response to physiological levels of estradiol.
- Effects of fish extracts differed with location of fish capture:
  - Bass extracts (n=7):**
    - 2 produced cell growth: one from the Monongahela, one from Store.
    - Extracts of bass from other sites did not exhibit proliferative responses.
  - Catfish extracts (n=21):**
    - 1 was strongly stimulatory, 4 moderate, 10 weak, and 6 had no response.
    - Of interest, catfish extracts with strong and moderate proliferative responses were from fish caught at polluted sites.
- The ER-negative BT-20 cells exhibited no growth stimulation when treated with the fish extracts or with E<sub>2</sub>.

## Acknowledgements

This study is part of the Community Based Participatory Research Project: Partners include Venner Outdoors, Clean Water Action, Basomasters, and individual local anglers. All fish were caught by angler participants. Information derived from this study will be shared with all participants and the general public.

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## References

## Summary of ER binding and cell proliferation assays

Species	Location	Sex	ER Binding	MCF-7	BT-20
White Bass	Kittingham	Male	Weak	None	None
White Bass	Kittingham	Female	Weak	None	None
White Bass	Point State Park	Male	Weak	None	None
White Bass	Point State Park	Female	Weak	None	None
White Bass	Monongahela	Male	Strong	Strong	None
White Bass	Monongahela	Female	Strong	Strong	None
White Bass	Highland Park	Male	Weak	None	None
White Bass	Highland Park	Female	Weak	None	None
White Bass	Store	Male	Weak	Weak	None
White Bass	Store	Female	Weak	Weak	None
Channel Catfish	Kittingham	Male	None	None	None
Channel Catfish	Kittingham	Female	None	None	None
Channel Catfish	Point State Park	Male	None	None	None
Channel Catfish	Point State Park	Female	None	None	None
Channel Catfish	Monongahela	Male	None	None	None
Channel Catfish	Monongahela	Female	None	None	None
Channel Catfish	Highland Park	Male	None	None	None
Channel Catfish	Highland Park	Female	None	None	None
Channel Catfish	Store	Male	None	None	None
Channel Catfish	Store	Female	None	None	None
Channel Catfish	Georgia Fish Farm	Male	None	None	None
Channel Catfish	Georgia Fish Farm	Female	None	None	None
Channel Catfish	Lake Erie	Male	None	None	None
Channel Catfish	Lake Erie	Female	None	None	None

ER binding assay:  
 -/0 = no competition  
 +/- = weak competition  
 + = moderate competition  
 ++ = strong competition  
 +++ = enhancement of binding

Cell proliferation assay:  
 -/0 = no response  
 +/- = weak response  
 + = moderate response  
 ++ = strong response  
 +++ = very strong response