Nutrient Management Webinar – February 13, 2013

Poultry Manure-Induced Endocrine Disruption: Laboratory and Field Studies

Lance Yonkos, Asst Prof

Environmental Science and Technology

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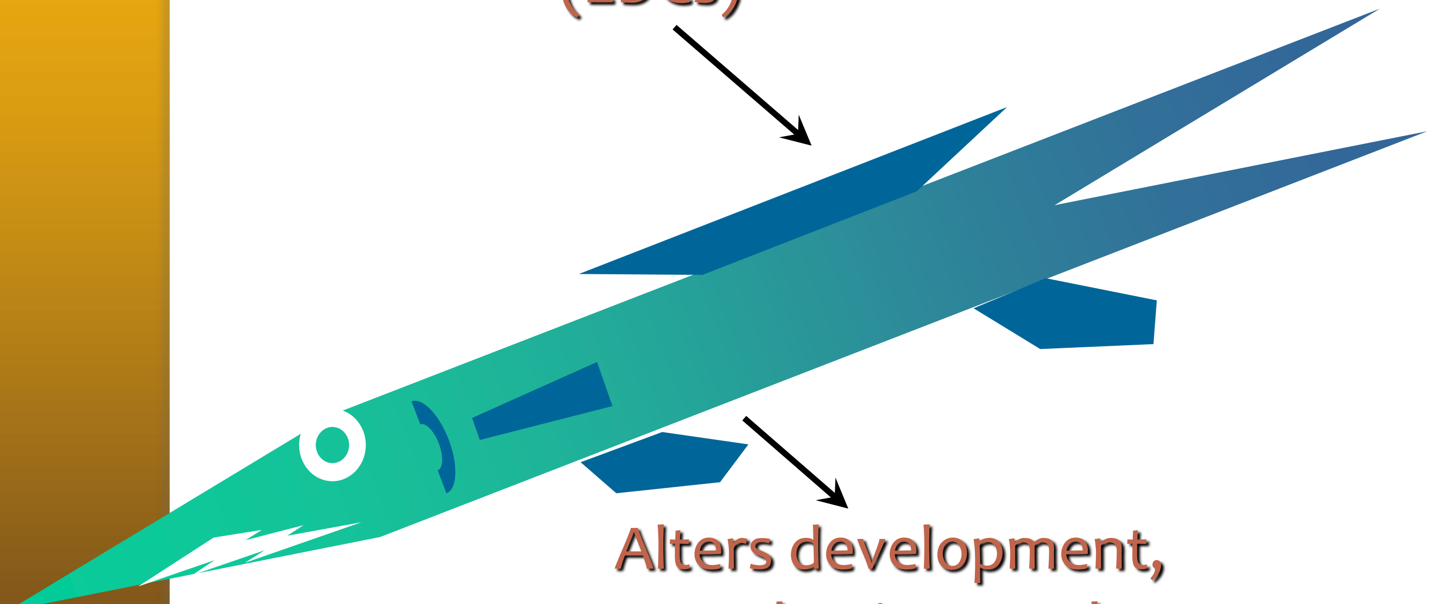
- Harry R. Hughes Center for Agro-Ecology, Inc
- Environmental Protection Agency STAR grant

So… what's an endocrine disruptor?

"An endocrine disruptor is an exogenous (external) substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny, or population."

Damstra et al. 2002
World Health Org.

Endocrine Disrupting Chemicals (EDCs)



Alters development, reproduction & other endocrine-mediated functions

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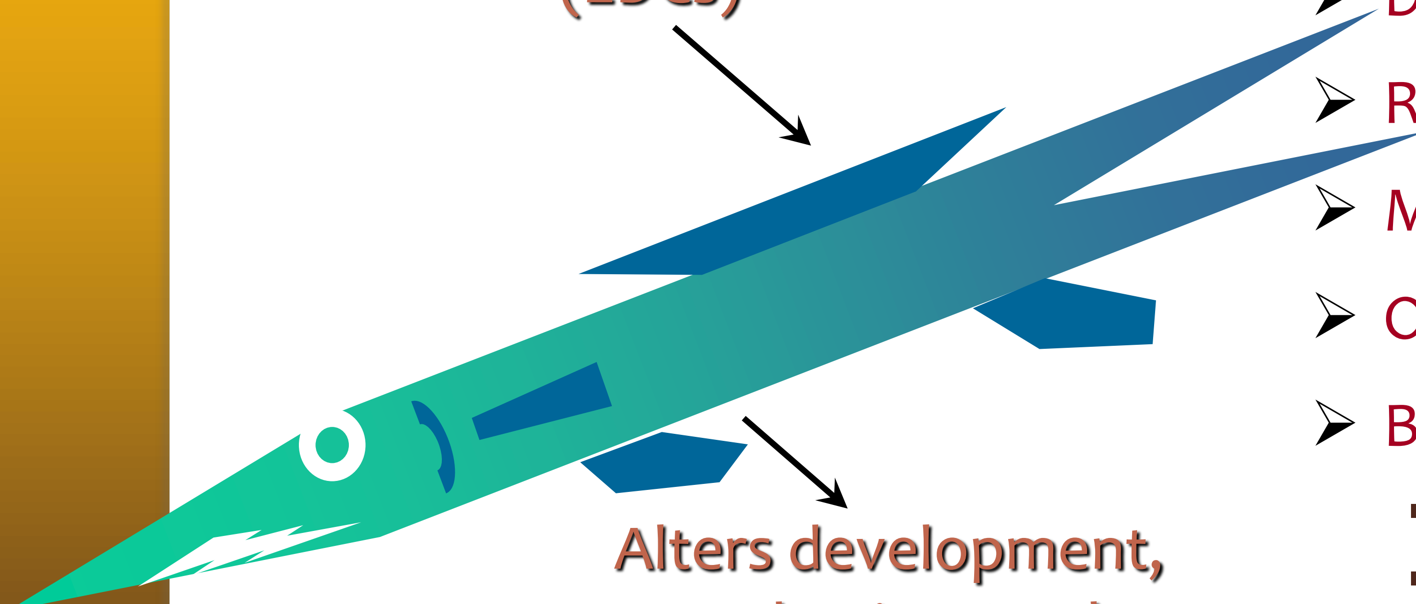


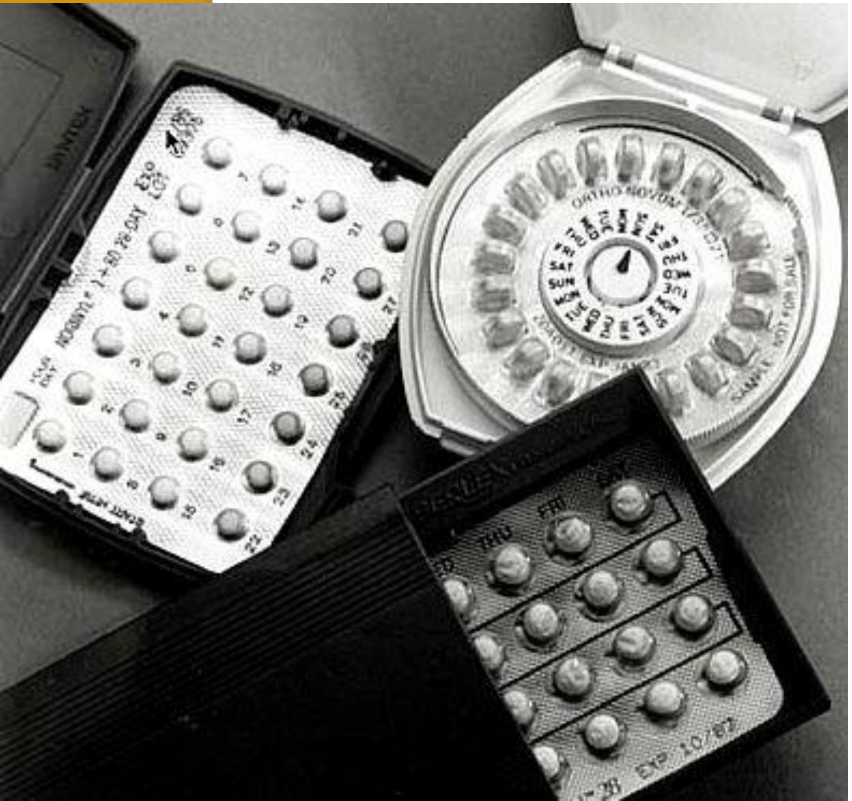
Alters development, reproduction & other endocrine-mediated functions

- Growth
- Development
- Reproduction
- Metabolism
- Osmoregulation
- Behavior
  - Breeding
  - Predator avoidance
- Etc.

Is endocrine disruption real?

Female birth control

Synthetic estrogen works by disrupting normal functioning of female reproductive system
- Prevents release of egg



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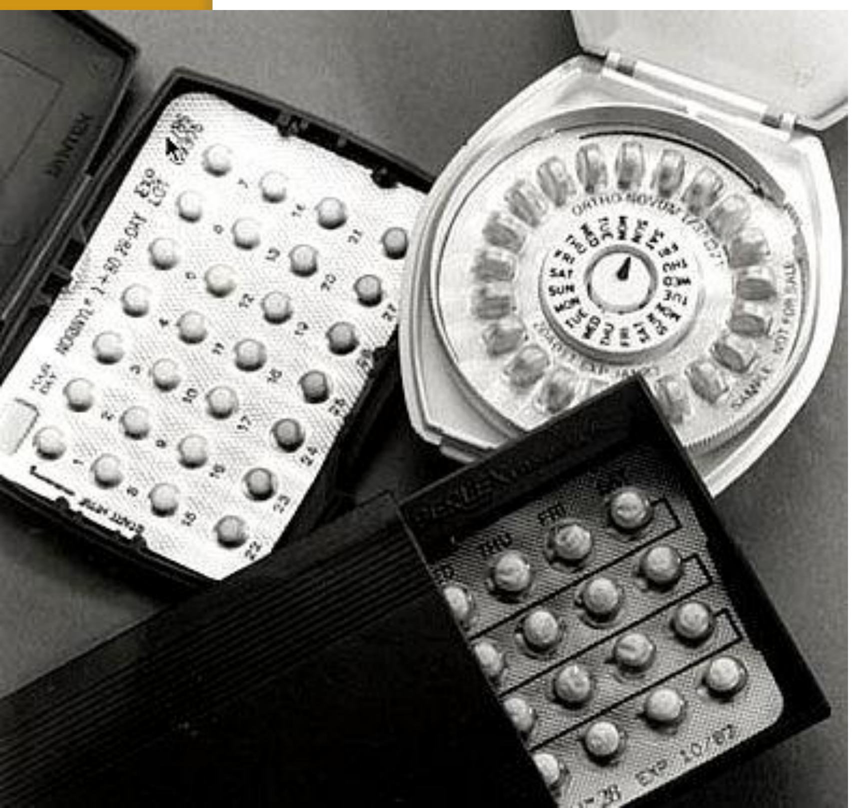
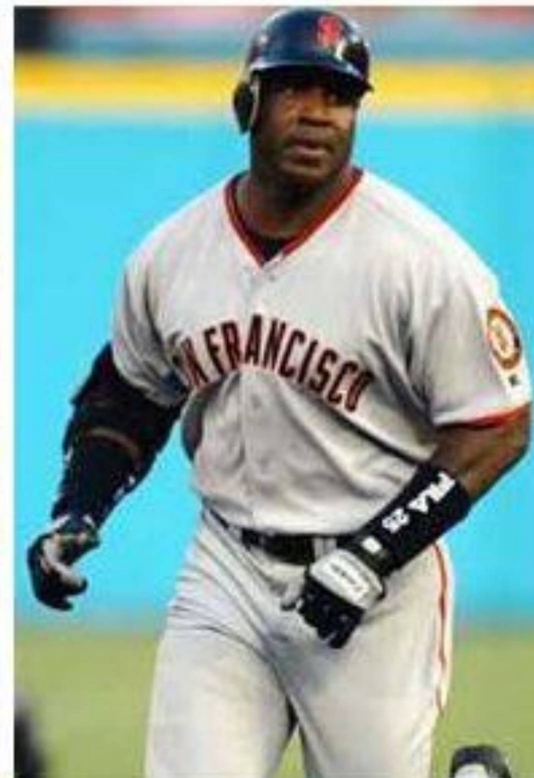


Synthetic androgens =

more home runs!



EDCs in the environment…

- Sewage treatment plant effluents (natural and synthetic steroids) – England; birth control in effluents;
  - Ethynylestradiol – synthetic estrogen (feminized male fish) below outfalls

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- Manufacturing & process wastes (detergents / plasticizers)
- Landfill leachates / Mine tailings (heavy metals)
- Agricultural pesticide residues (MOA of many insecticides)
- Plastic water bottles, baby bottles, etc, etc (Bisphenol A?)
  - Canada and EU have declared it a toxic substance – banned from baby bottles

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- Plastic water bottles, baby bottles, etc, etc (Bisphenol A?)
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- *Concentrated animal feeding operations — CAFOs*
  - Swine lagoons – NC & SC, AK
  - Beef – Midwest /West (Trenbolone - synthetic androgen)
  - Poultry on Eastern Shore – Delmarva (& Shenandoah)

Delmarva poultry facts

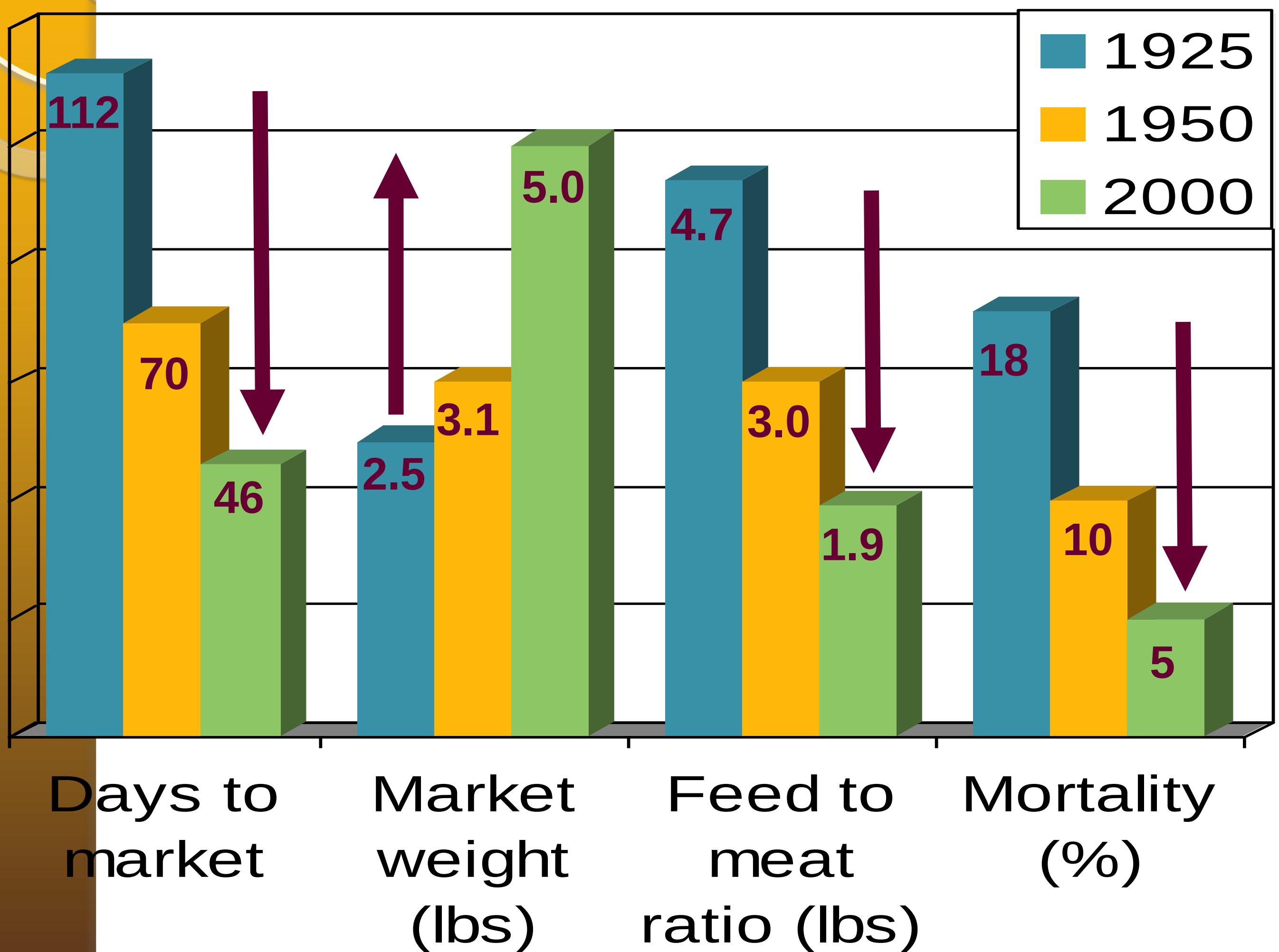
Poultry Litter = Waste & bedding material

Approximately 25,000 – 37,000 birds/house at any one time

Approximately 5 – 6 flocks per house per year

Up to 2 years between clean-outs

US POULTRY FACTS



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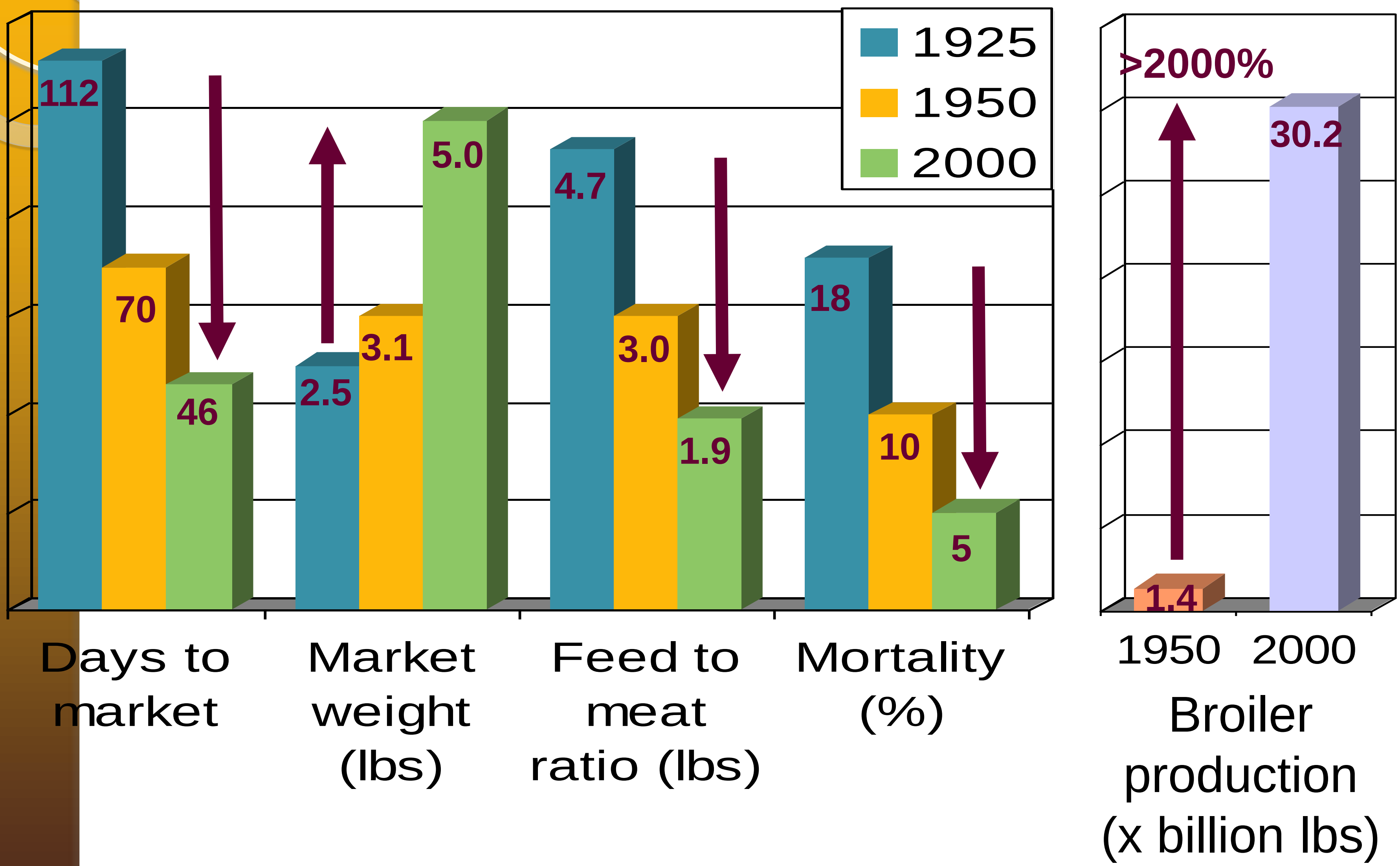

Delmarva poultry facts

- ~600 millions birds are produced annually (~7% of US total)
- ~1.6 billion lbs (>700 million kg) of poultry manure are produced annually
- litter is land applied (primarily to satisfy nutrient requirements of corn production)
- excessive / inappropriate land application can have severe impacts on regional surface and ground water quality

Poultry litter-associated contaminants (PLACs)

- nutrients (e.g., nitrogen & phosphorus)
- bacterial & viral pathogens
- feed additives / therapeutics
  - Arsenic
  - Antibiotics

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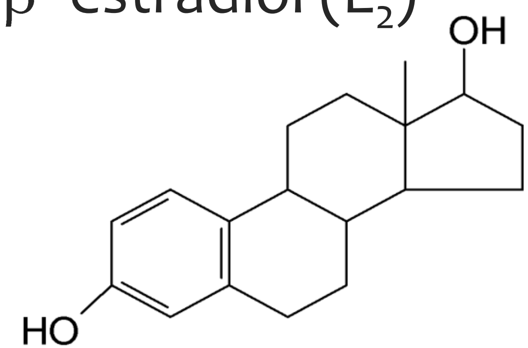
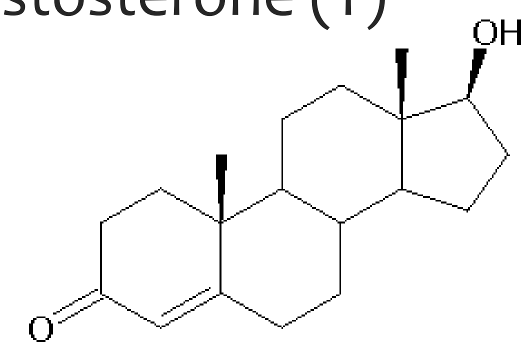
- natural fecal steroids
  - Estradiol (E_2)
  - Estrone (E_1)
  - Testosterone (T)

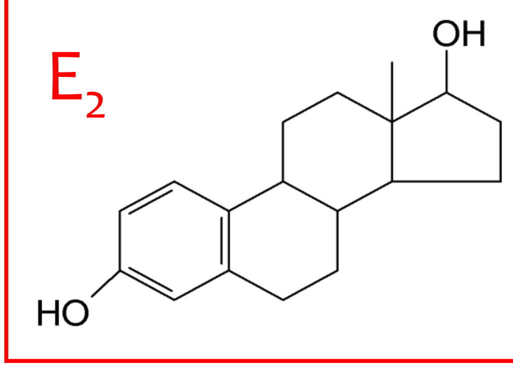
17 β–estradiol (E_2)

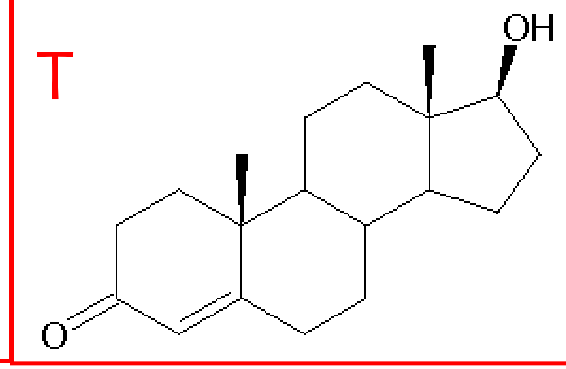


Testosterone (T)



- **Natural** fecal steroids in poultry litter
  - Estradiol (E2)
  - Estrone (E1)
  - Testosterone (T)





Chickens not fed steroids!!
Produced naturally

Steroids (from 8 litter sources)

- *17 β-Estradiol (~ 125 ng/g dry litter)*
- *Testosterone* (~ 50 *ng/g* dry litter)

These ng/g or parts per billion (ppb) concentrations in litter can lead to ng/L or parts per trillion (ppt) concentrations in water

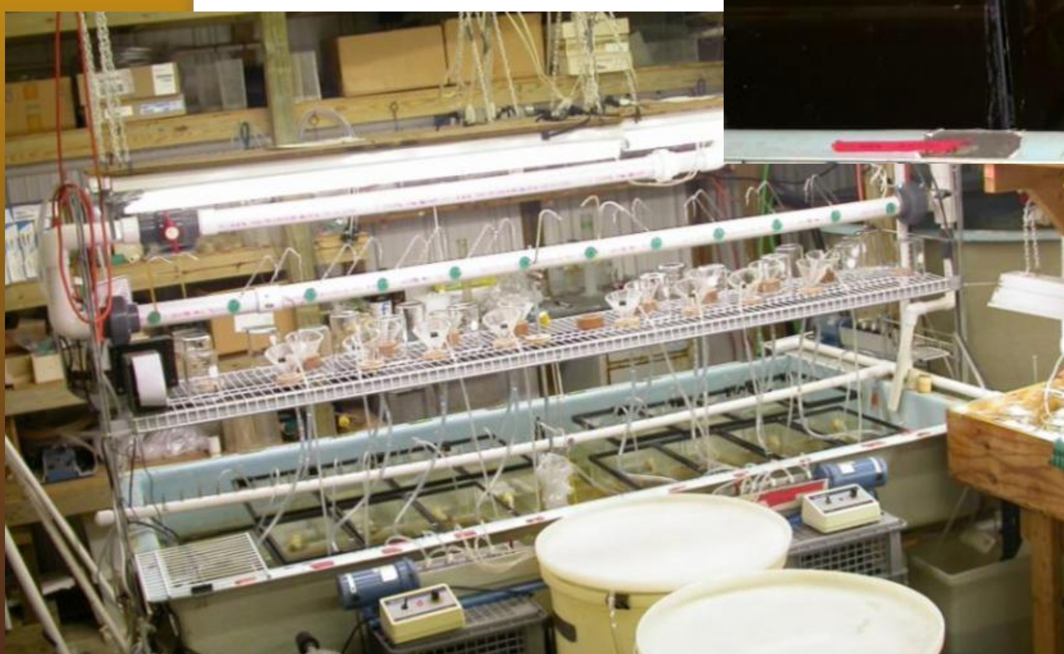
Important, natural hormones normally work at very low levels, in the low parts per trillion range

TEST EXPOSURE SCENARIOS

- 2 dozen bioassays
- 3 fish species
- 4, 7, 9, 14, 21-day

- Flow-through
- Static with daily renewal
- Static without renewal

- Larvae/juveniles
- Mature males
- Breeding groups



Test material: Whole-house clean-out

- used in lab exposures
- used in controlled field applications

Waste & bedding material

TEST SPECIES:

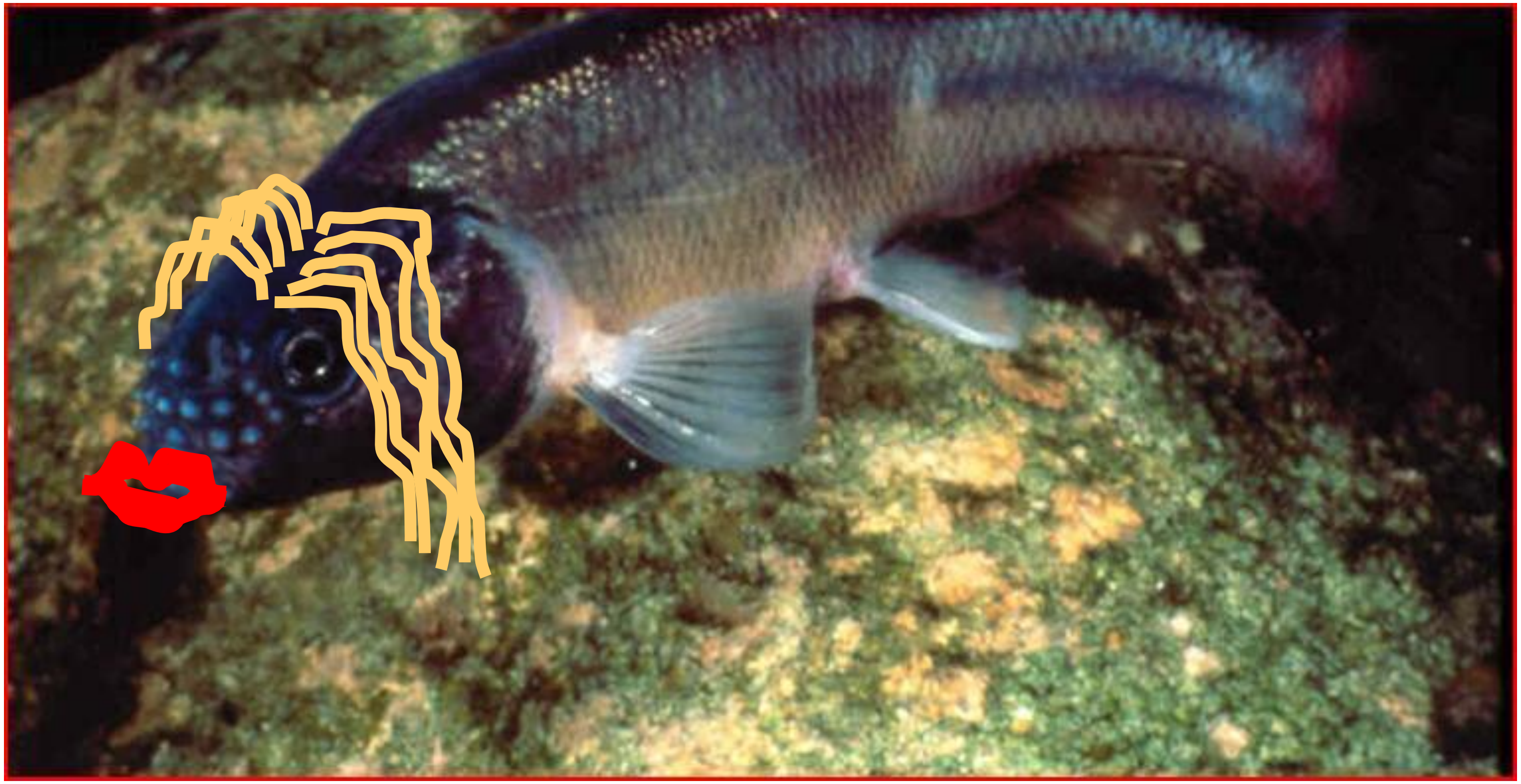
FATHEAD MINNOW *PIMEPHALES PROMELAS*

Biological indicators

Test species:

Fathead minnow *Pimephales promelas*

Biological indicators



Biological indicators

Vitellogenin (Vtg): high molecular weight lipoprotein produced in liver and taken up by ovary during gonadal maturation – becomes primary egg yolk nutrient for developing eggs /embryos.

Important for females and larval fish!!!!

Biological indicators

Vitellogenin (Vtg) – Males maintain genetic capacity to produce Vtg in response to stimulation from external estrogens or estrogen agonists. Therefore detection of Vtg in males is a sensitive biomarker of exposure to estrogenic compounds.

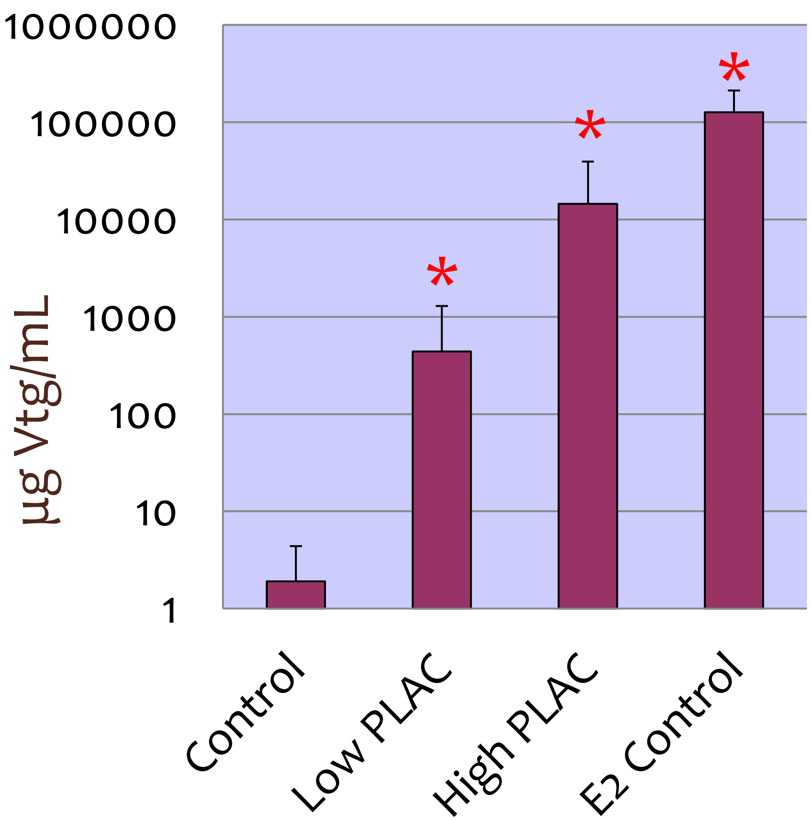
How measured:

- Measured in blood by Enzyme Linked Immunosorbent Assay (ELISA)

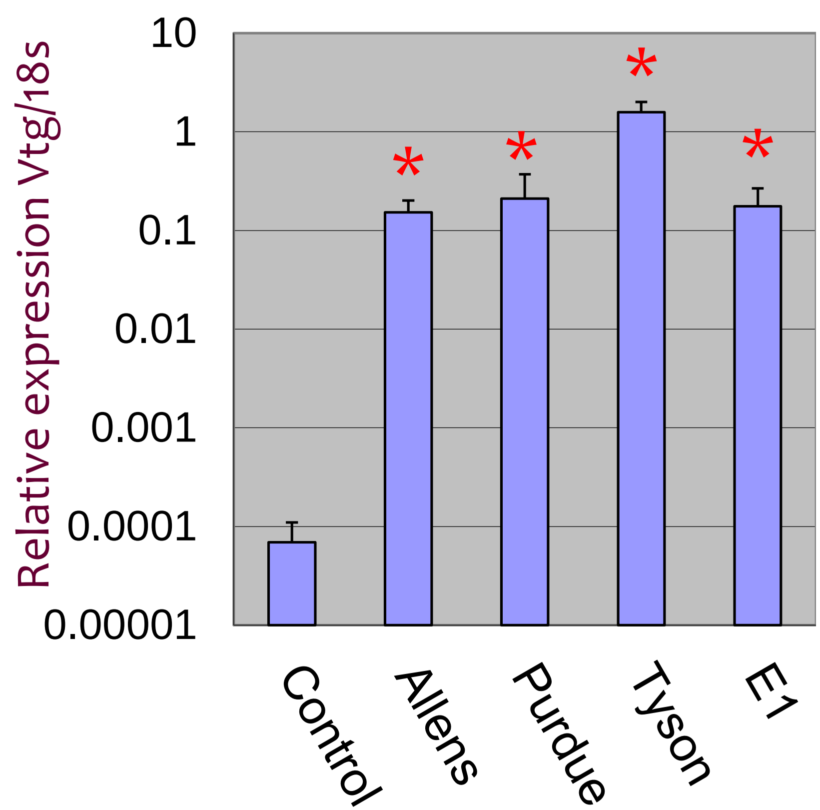
Biological indicators

- Vitellogenin (Vtg) Induction

Plasma Vtg in Mature Male FHM

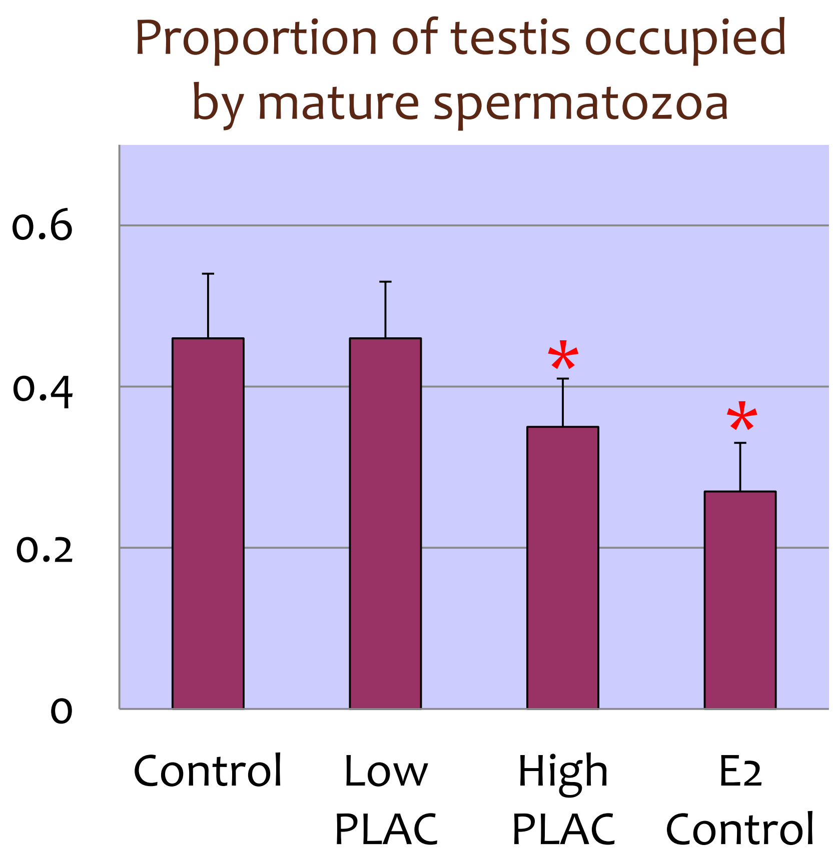


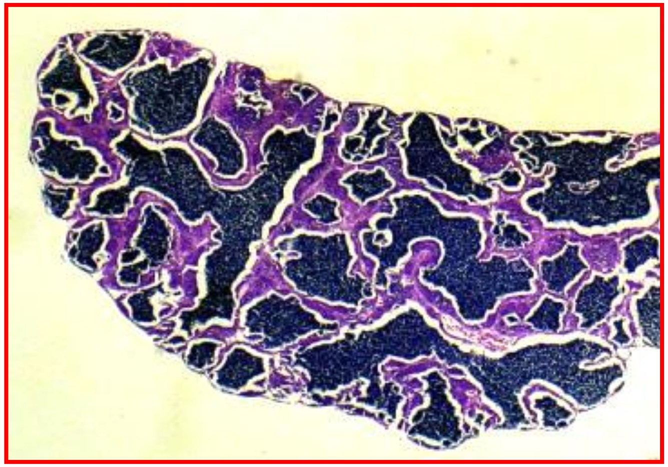
Vtg mRNA expression in Liver of Mature Male FHM



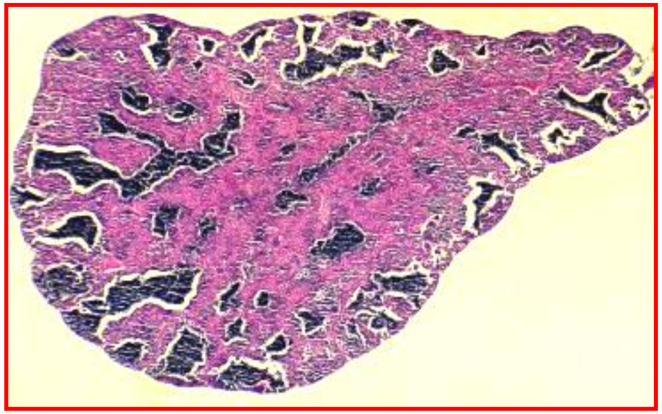
Biological indicators

- Staging of gonad maturity (testis of adult males)





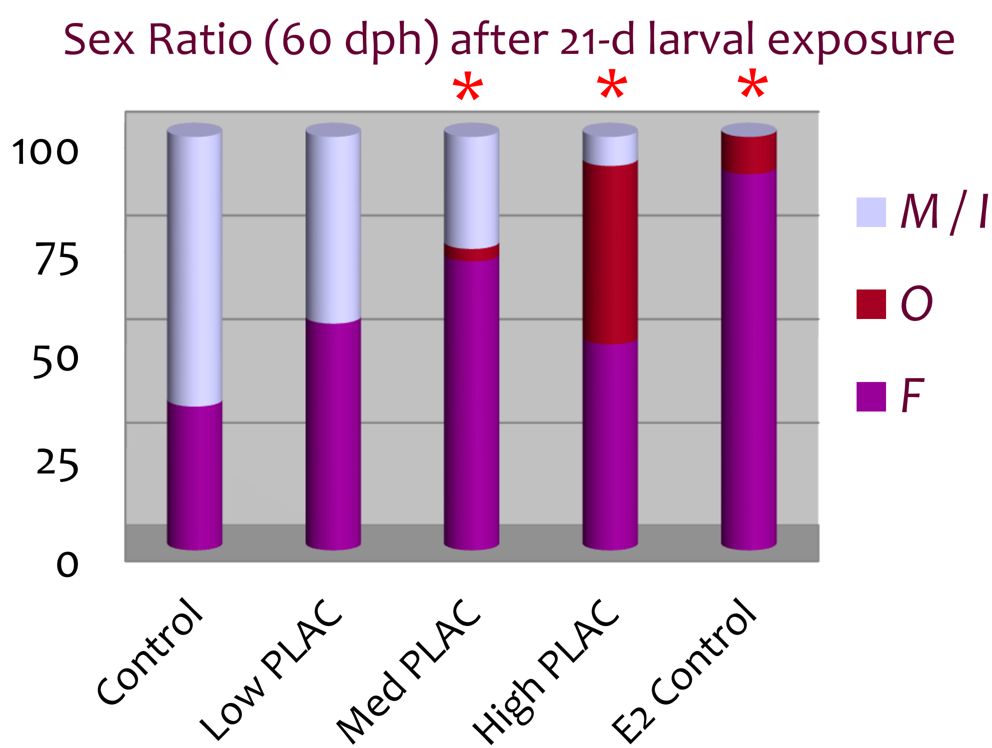
Control: 55% Spermatozoa

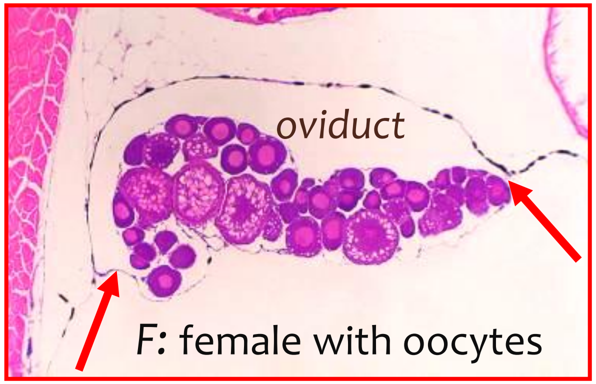


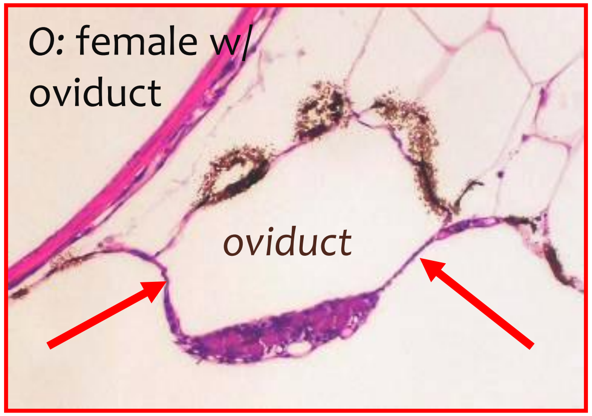
E2 Control: 18% Spermatozoa

BIOLOGICAL INDICATORS

- Gonadal development and gender differentiation

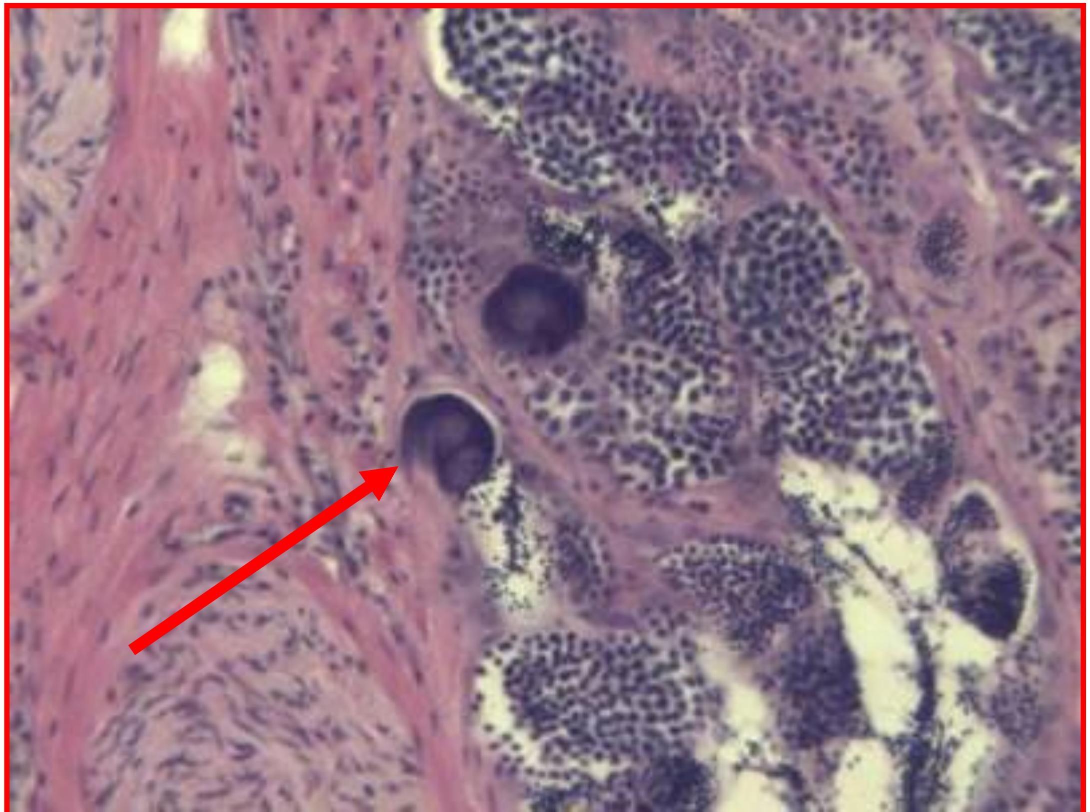






Biological indicators

- Male (♂) Gonadal abnormalities (intersex) - testicular oocytes - (eggs) within males testis

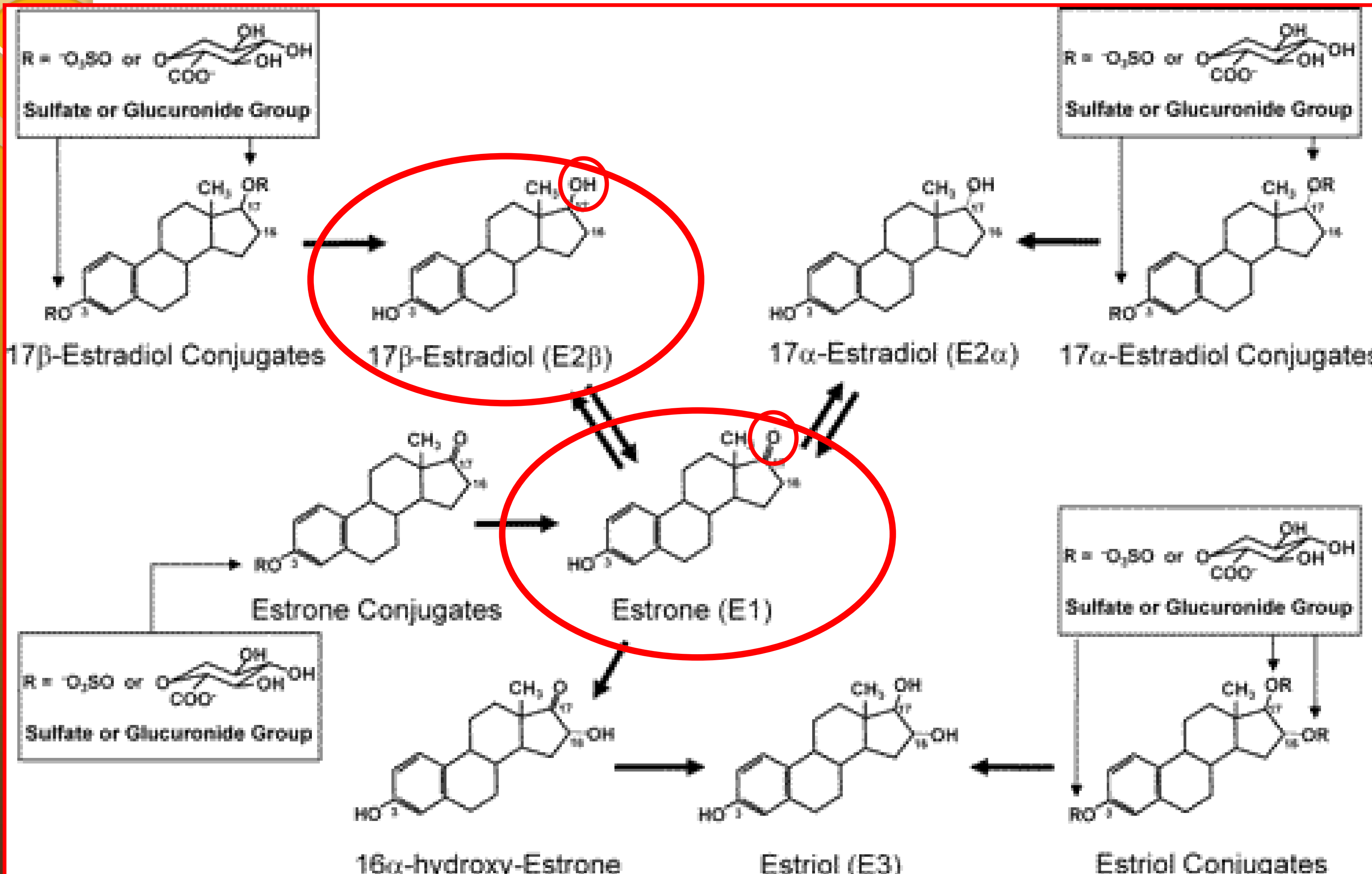


Summary of Fathead Minnow Laboratory Results

PLACs are capable of:

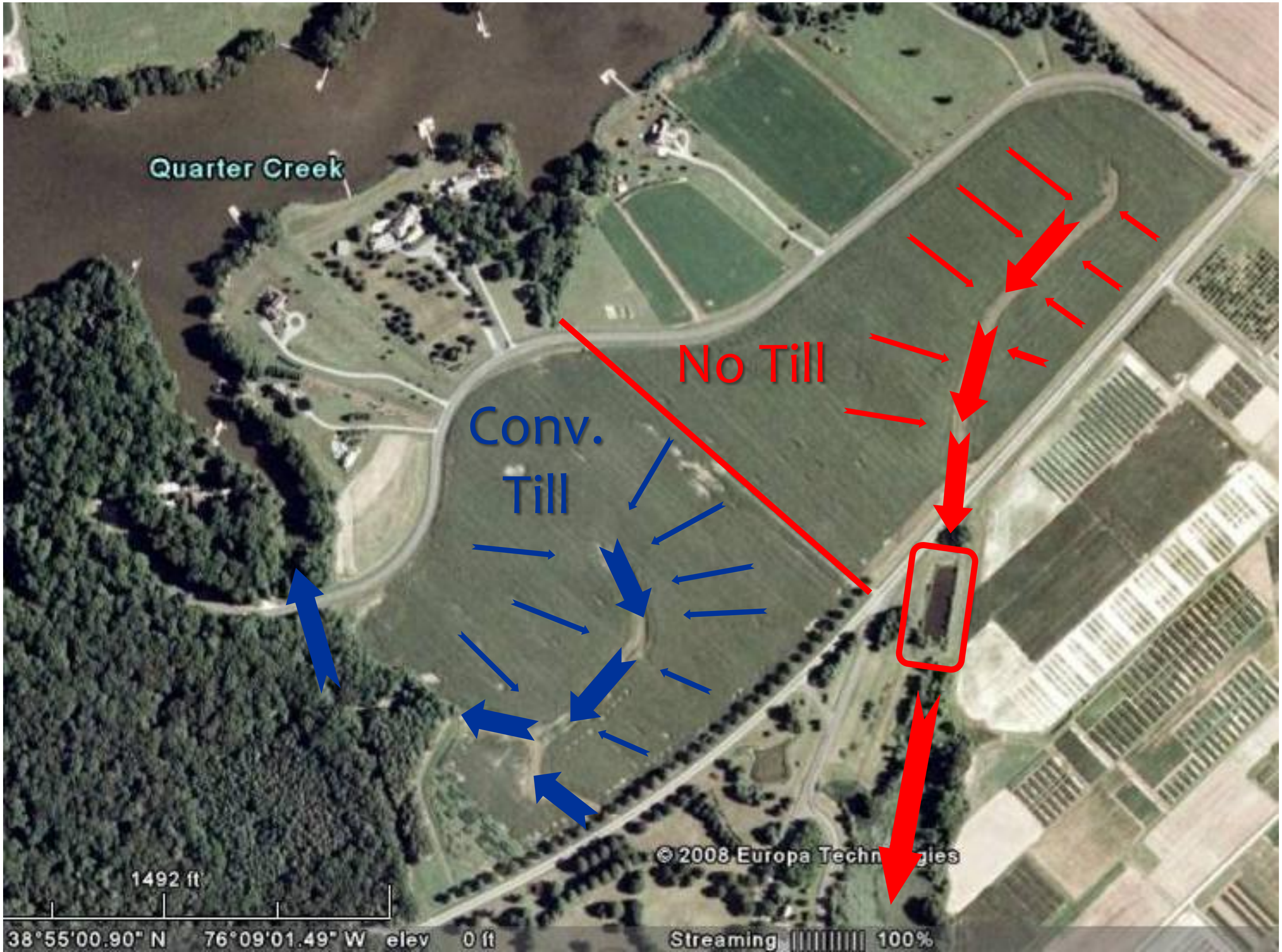
- Inducing VTG in mature male fish
- Inhibiting gonadal maturation of male fish
- Feminizing male gonadal development following larval exposures

A good time to discuss estrogen chemistry…



Wye Research and Education Center (WREC)

Research Fields



Poultry Litter

- Whole-house clean-out from a standard broiler operation
- Accumulation over 2 years (10 – 12 flocks)
- >200 tons (100,000 kg)
- 3,000 kg/acre

Tillage Practices

No-Till

Conventional Till

Turbo Till

Sub-surface Injection

Controlled field investigation

No-Till Runoff from 2008 Litter Application

Controlled field investigation

- NT runoff exposures
- Pond exposures
- Caged in pond

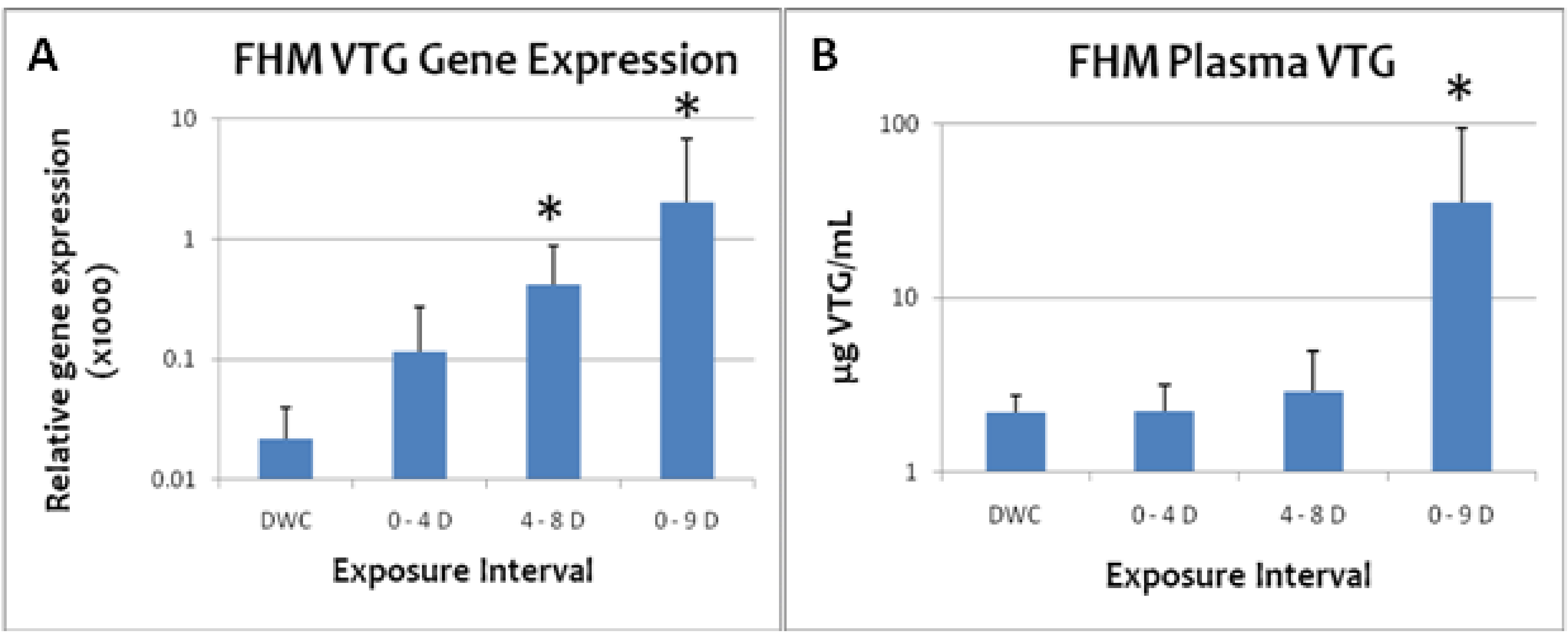
Controlled Field Investigation

Exposures of adult male fathead minnows in microcosm

Water from NT retention pond after poultry litter application

Measured 17β-estradiol = 3 ng/L

Measured estrone = 8 ng/L



CONTROLLED FIELD INVESTIGATION

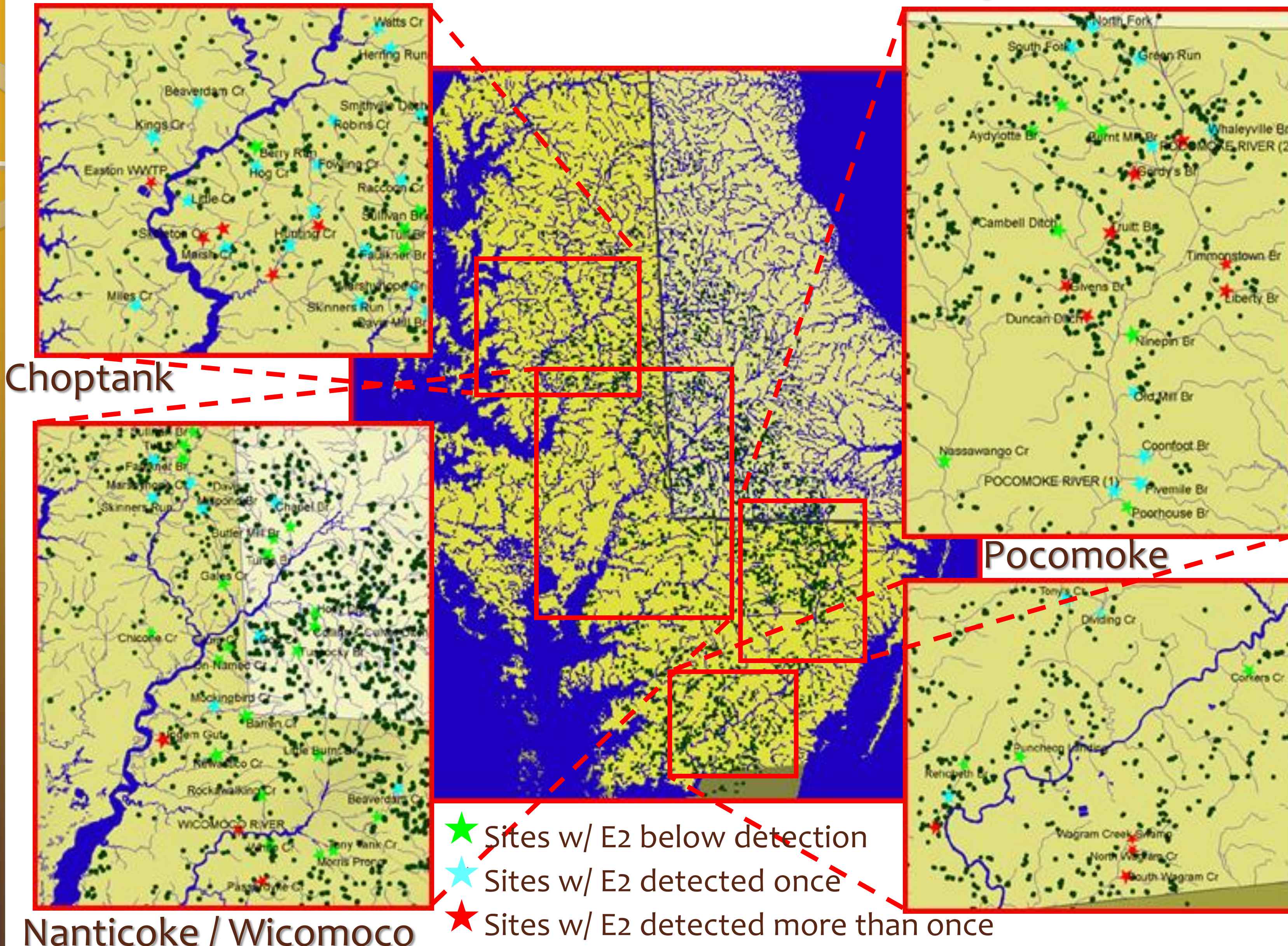
Relative abundance of fecal estrogens in runoff following poultry litter application

Year	Tillage Practice		Reduction
2002	No Till	Conv Till	40%
2008	No Till	Turbo Till	58%
2009	No Till	Turbo Till	52%
2010	No Till	Turbo Till	38%
2011	No Till	Sub-Surface	82%

Summary of Controlled Field Investigations

- Fecal steroids readily transport from litter-amended fields to surface waters via runoff
- Amount transported is a function of precipitation frequency/intensity and of tillage practice employed
- Transport under NT practices generally greater than other practices
  - Conventional and Turbo-Till similar w/ 40% to 60% reduction
  - Sub-Surface Injection >80% reduction
- Runoff from litter-amended fields capable of inducing ED in fish

IN SITU FIELD INVESTIGATION: 2004



Sites w/ E2 below detection

Sites w/ E2 detected once

Sites w/ E2 detected more than once

Poultry houses (within Chesapeake watershed)

IN SITU FIELD INVESTIGATION: 2004

Of 90+ sites visited…
~66% had detectible E2

Field collections: 2005/2006

Field collections: 2005/2006

Fish and frogs were collected from:

- 20 sites in Pocomoke & Choptank watersheds
- All had detectible E2 in 2004 survey
  - 12 common fish species
  - 3 common frog species
  - 480 total specimens
- Gonads (♂ & ♀) examined histologically for ED-related pathology

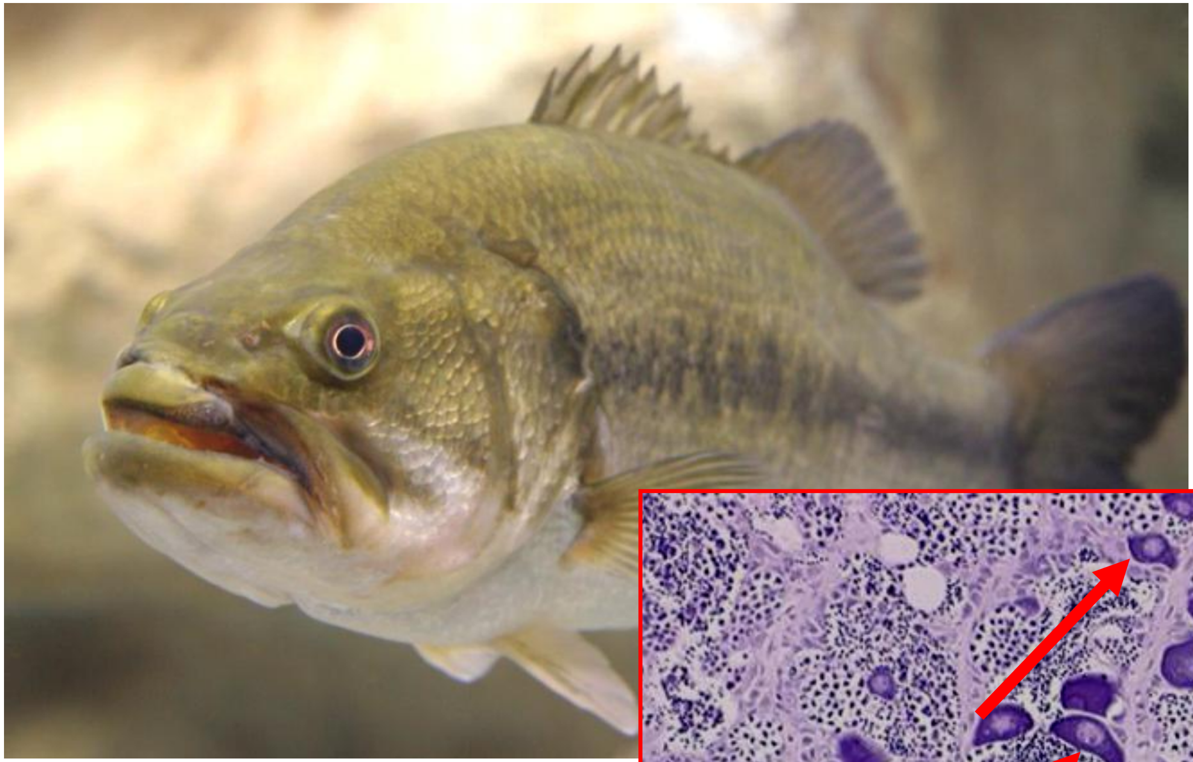
Fish

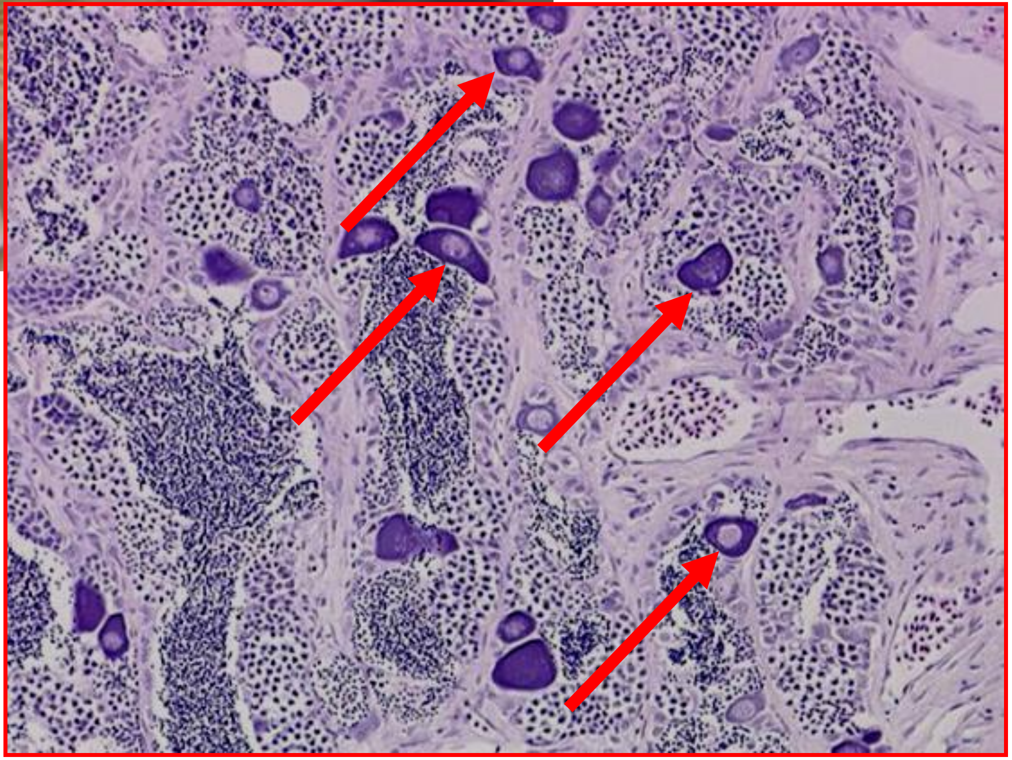
Creek chubsucker	*Erimyzon oblongus*
Golden shiner	*Notemigonus crysoleucas*
Redfin pickerel	*Esox americanus*
Chain pickerel	*Esox niger*
Largemouth bass	*Micropterus salmoides*
Fallfish	*Semotilus corporalis*
Redbreast Sunfish	*Lepomis auritus*
Bluegill	*Lepomis macrochirus*
Pumpkinseed	*Lepomis gibbosus*
White perch	*Morone americana*
Yellow perch	*Perca flavescens*
Gizzard shad	*Dorosoma cepedianum*

Frogs

American Bullfrog	*Rana catesbeiana*
Northern Green	*Rana clamitans* melanota
Southern Leopard	*Rana sphenocephala* utricularia

FIELD COLLECTIONS: 2005/2006





Sampling coincided with reports of intersex (TO) in smallmouth & largemouth bass in the Potomac River collected in 2004

(Blazer et al., 2007)

Field collections: 2005/2006

Fish and frogs were collected from:

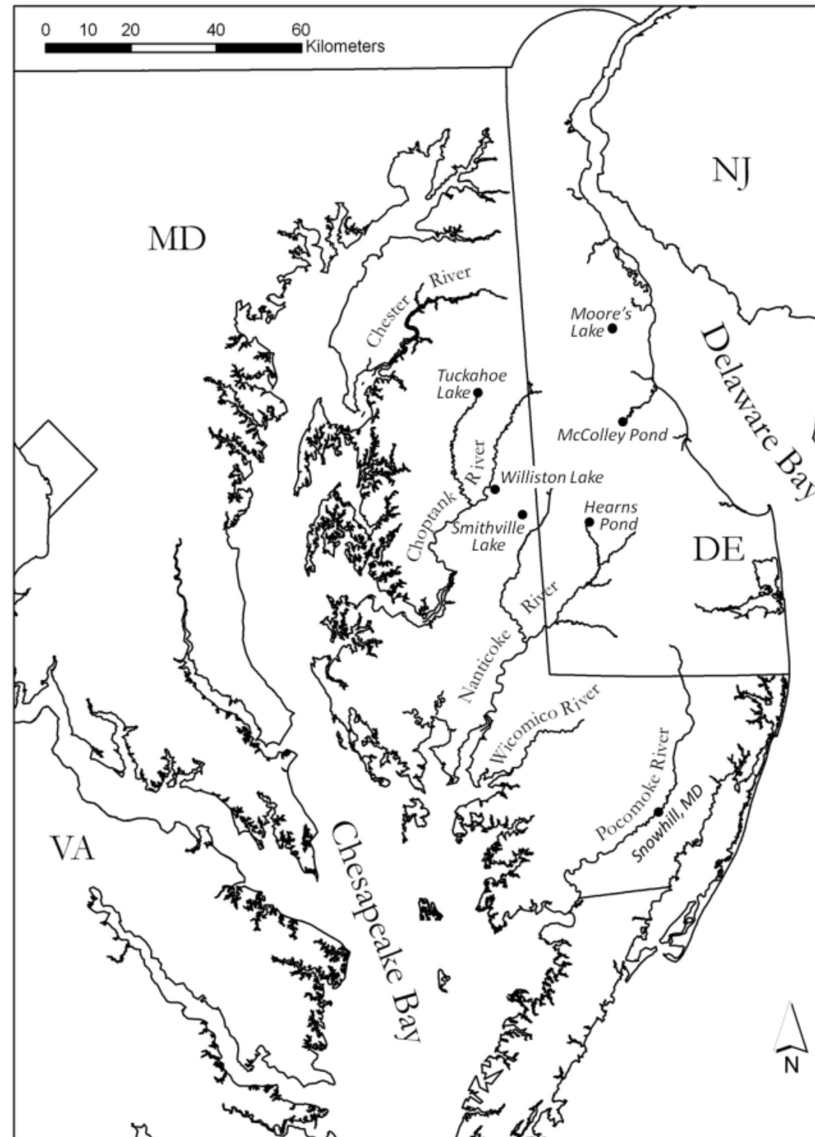
- 20 sites in Pocomoke & Choptank watersheds
- All had detectible E2 in 2004 survey
  - 12 common fish species
  - 3 common frog species
  - 480 total specimens

- Only 25 mature largemouth bass
- Of those – only 13 males
- Of those – 2 possessed TO (15%)

- Gonads (♂ & ♀) examined histologically for ED-related pathology

FIELD COLLECTIONS: 2008/2009
DELMARVA LAKE SAMPLING

- Abundant fish population – better chance to collect sufficient male LMB
- Receive run-off from large ag-influenced watersheds
- Water has longer "residence time" in system



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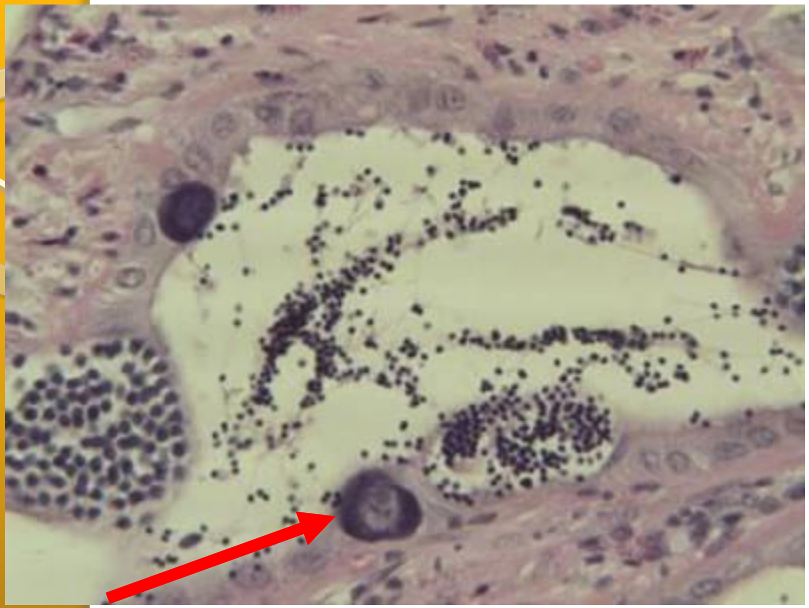
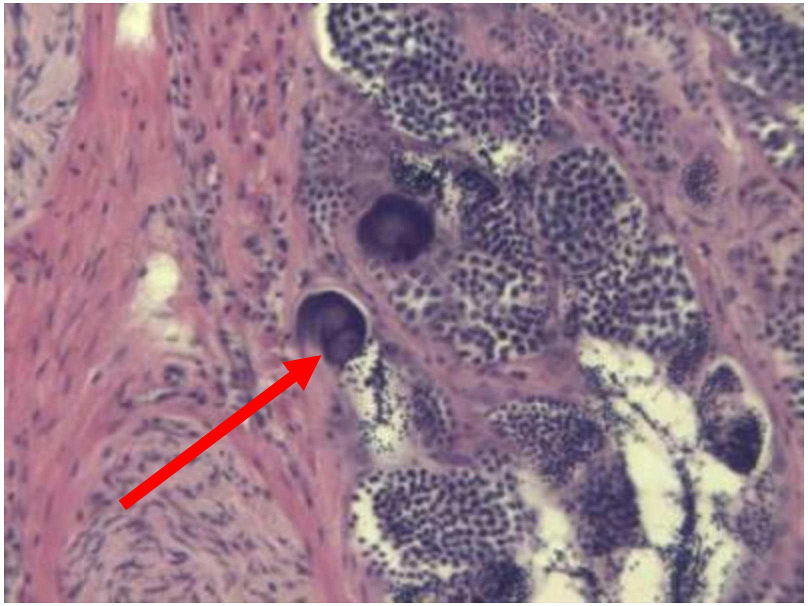
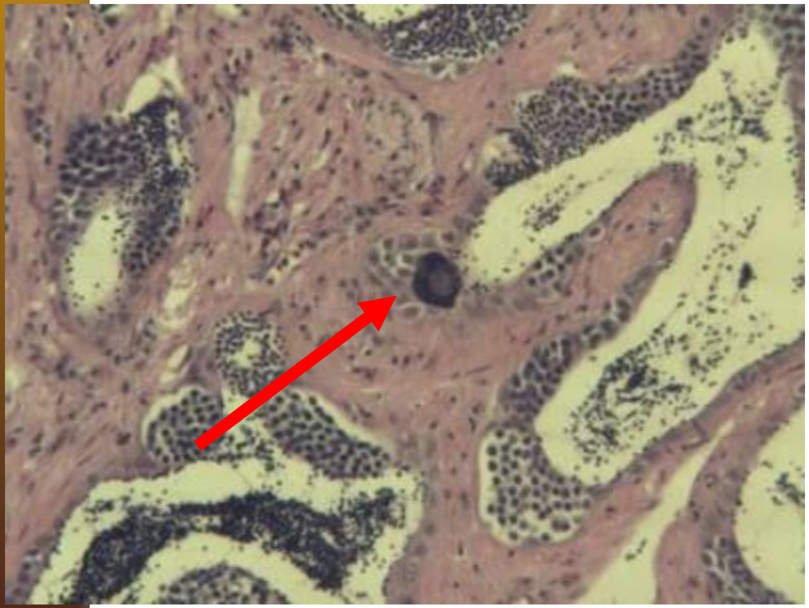
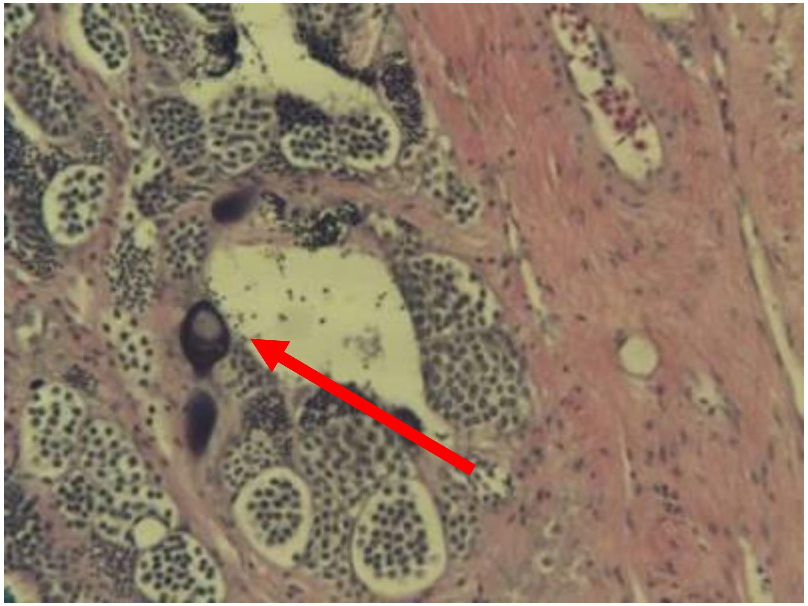
FIELD COLLECTIONS: 2008/2009

Intersex - testicular oocytes in LMB









Testicular oocytes in male largemouth bass on Delmarva – 2008/2009

Year	Lake/Pond	Occurrence	Severity
2008	Hearn's Pond, DE	88%	0.22 ± 0.16
	Moores Lake, DE	80%	0.37 ± 0.38
	McColley Pond, DE	67%	0.25 ± 0.26
	Williston Lake, MD	73%	0.24 ± 0.31
	Smithville Lake, MD	40%	0.11 ± 0.23
	Tuckahoe Lake, MD	42%	0.22 ± 0.48
2009	Tuckahoe Lake, MD - Spring	50%	0.16 ± 0.21
	Tuckahoe Lake, MD - Summer	33%	0.25 ± 0.42
	Pocomoke River, MD - Spring	33%	0.33 ± 0.56
	Pocomoke River, MD - Summer	80%	0.53 ± 0.40

Prevalence in Delmarva largemouth similar to reported levels from Western Shore (Potomac/Shenandoah) and nationally

Severity generally lower than reported in bass from Potomac River (≥ 0.6), but higher than bass from "minimally" impacted reference sites (≤ 0.1)

Full circle: Back to the lab - 2011/2012

- Reproduce FHM studies with LMB
  - Establish "proof-of-concept" that poultry litter-associated contaminants are able to induce ED
  - Identify developmental windows of sensitivity
  - Determine threshold concentrations
- Challenges…
  - Long-lived
  - Require ample space/water/food/etc
- Pilot study with assistance from AGNR
- Current Gemstone project
  - Expose larval / juvenile LMB to PLAC
  - Investigate occurrence of TO
  - Investigate Vtg induction

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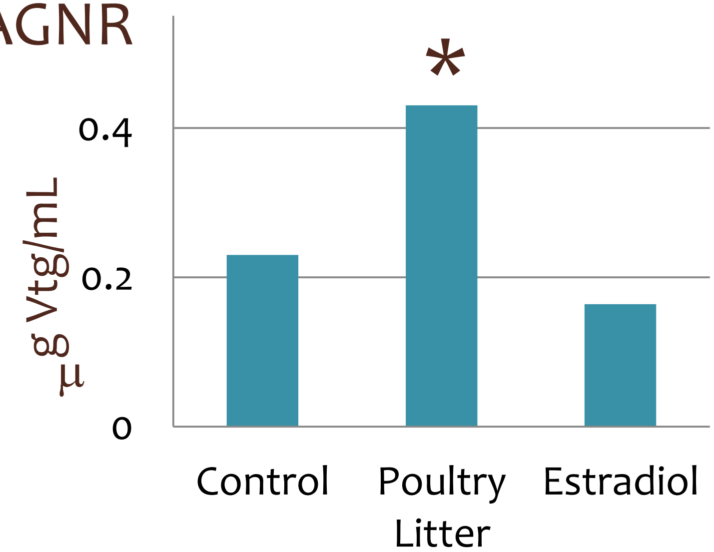
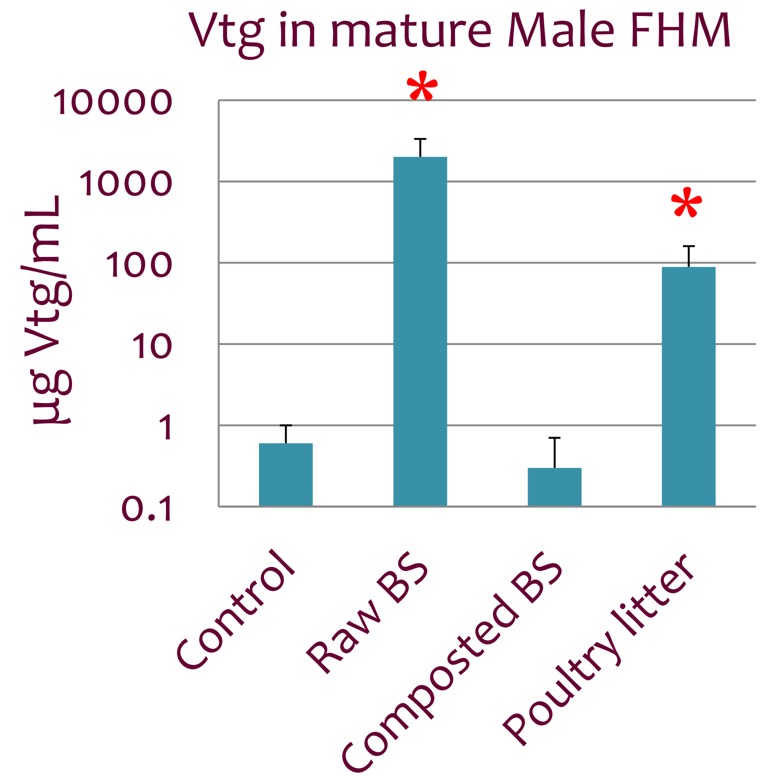


Where to next?

- Methods applicable to other complex media
  - Biosolids (BS)
- Develop non-lethal / minimally invasive sampling methods
  - Vtg in surface mucus/urine/plasma
  - Biopsies of testis for TO detection
- Immunotoxicity studies
  - Investigate immune competence of lab-reared bass
- Bio-informatics / Metabolomics
  - Quantify all low molecular weight molecules in biofluids / tissues
    - Identify target tissues
    - Investigate contaminant MOA



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- USDA
  - Nancy Shappell, PhD
- USGS
  - Vicki Blazer, PhD
  - Luke Iwanowicz, PhD
- USFWS
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  - Scott Stranko
  - Joseph Love, PhD
  - Don Pritchett
- CBF
  - Beth McGee, PhD
- Riverkeeper Alliance
  - Drew Koslow

THANK YOU!