Effects of Endocrine Disruptors/Modulators on Fishes

Vicki S. Blazer and Luke Iwanowicz
National Fish Health Research Laboratory
Leetown Science Center
Kearneysville, WV

USGS
science for a changing world

Fish and Endocrine Disruptors/Modulators

- Fish are good indicators for the aquatic environment
- Fish health and fish populations
  - Commercially and recreationally-important species
  - Ecologically important species
- Indicators of effects on aquatic ecosystem and ultimately human health

Endocrine System of Vertebrates

- Complex system primarily involved in chemical communication
- Hormones are released from glands and affect target tissues
  - Travel through the bloodstream
  - Act locally

Endocrine Modulators

- Synthetic or naturally-occurring chemicals that modify or disrupt normal hormonal functions
  - Alterations in synthesis and metabolism
    - Improper balance or quantity of circulating hormones
  - Directly or indirectly interact with natural hormones
    - Change hormone message and alter cell activity
  - Induce creation of extra receptor sites
    - Amplify the hormone effect on cell activity
  - Block binding sites
    - Impair normal cell activity
  - Bind to receptors (hormone mimics)
    - Abnormal, unpredictable cell activity

Endocrine Disruption

- Reproductive
- Thyroid
- Adrenal (Interrenal, chromaffin)
- Neuroendocrine

- Immune system/disease resistance
  - Cortisol
  - Estrogens/androgens

2002 Sampling With WV DNR South Branch Potomac

- randomly examined a variety of fish species focusing on the external lesions
  - Variety of causes – bacterial, parasitic, noninfectious
  - Suggested some environmental stressors that may cause immunosuppression and increased disease susceptibility

Sampling In South Branch Potomac (WV DNR)

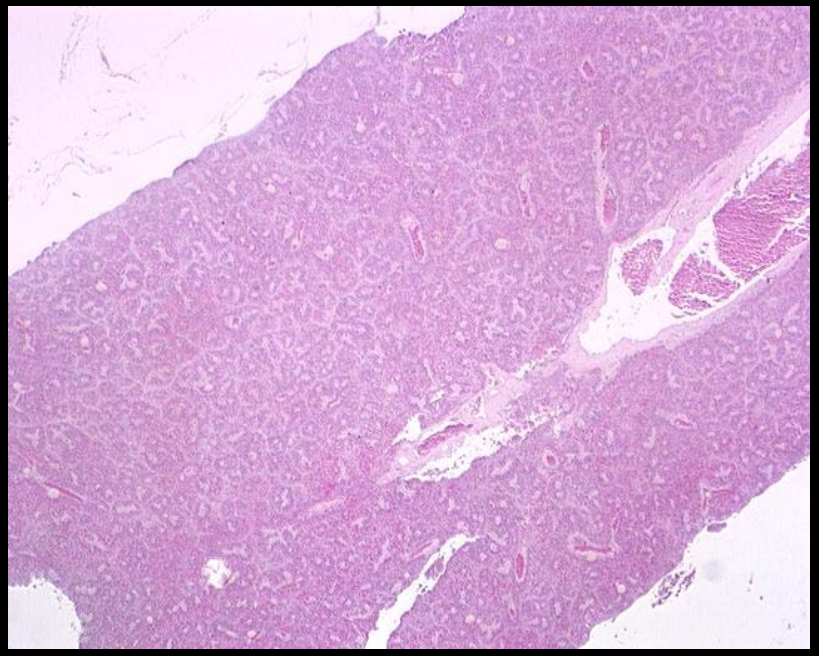
- 2003 – more intensive sampling – 7 sites in the South Branch, 1 in Cacapon, 1 in Lost River, 1 in North River drainages
  - Necropsy-based assessment, pieces of liver, kidney, spleen, gonad and skin lesions
  - Histologic evaluation of all the organs
  - Finding of intersex

Fish Lesions/Fish Kills
Smallmouth Bass and Other Species

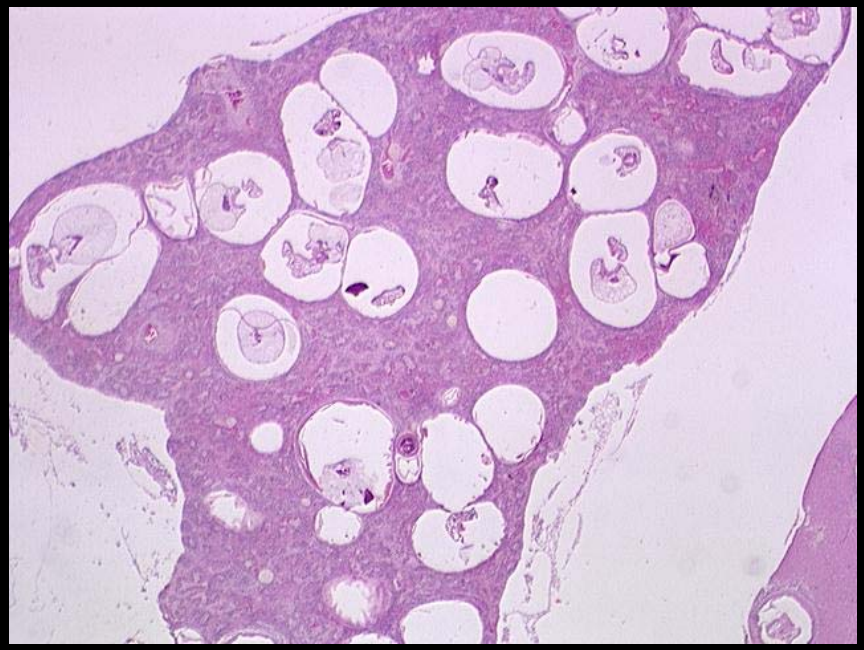
- Potomac River – WV, MD

- Shenandoah River – VA, WV
- Susquehanna River – PA
  - Skin lesions are associated with a number of opportunistic bacterial pathogens

Internal Lesions - Parasites

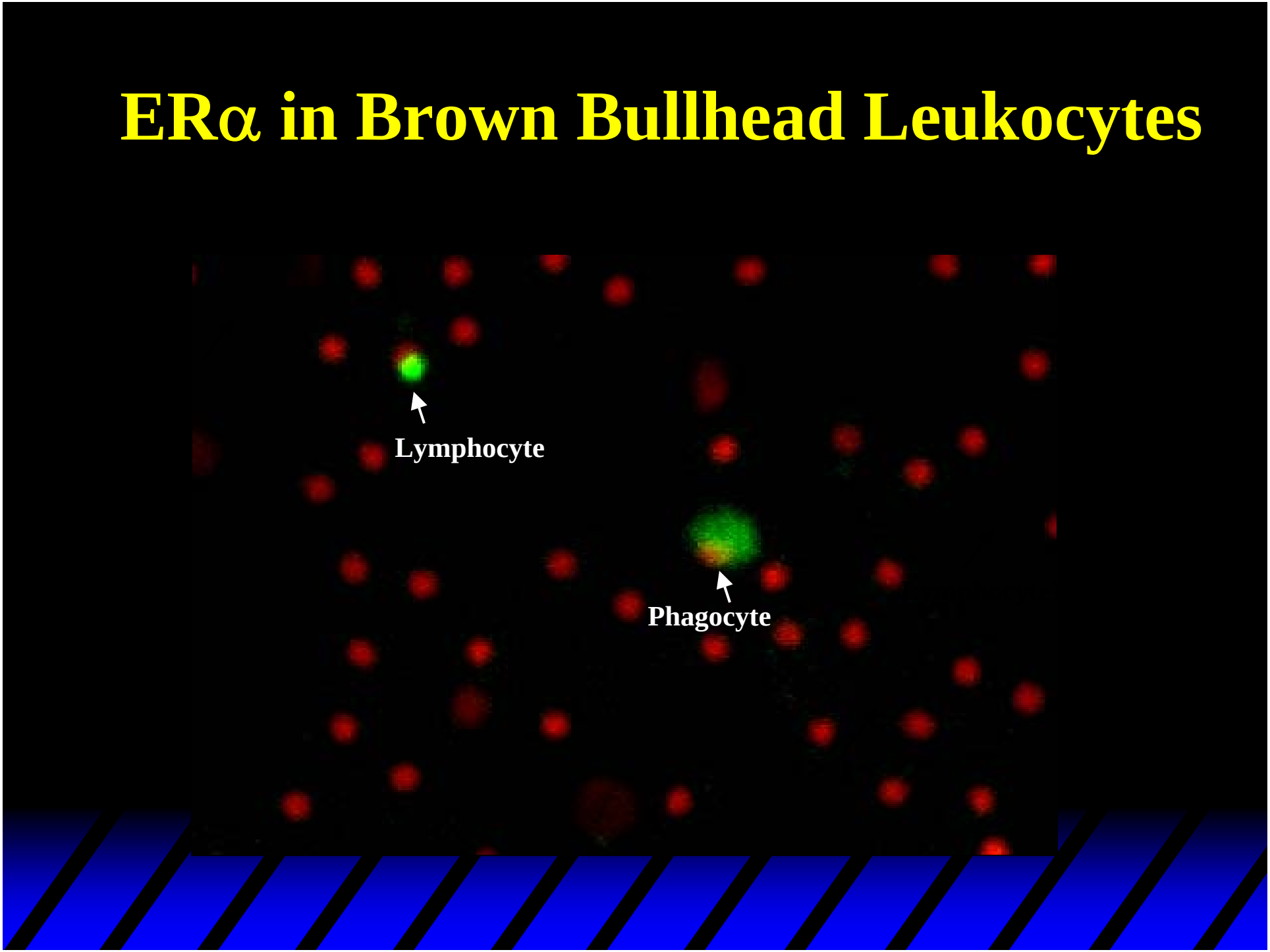


Spleen from bass in North River



Spleen from bass at Indian Rock

ERα in Brown Bullhead Leukocytes



Effects of Estrogen on Brown Bullhead Leukocytes

- Estrogen exposure reduces phagocytic index
- Estrogen influences bactericidal activity
- Estrogen influences nitric oxide production
- Estrogen influences respiratory burst activity

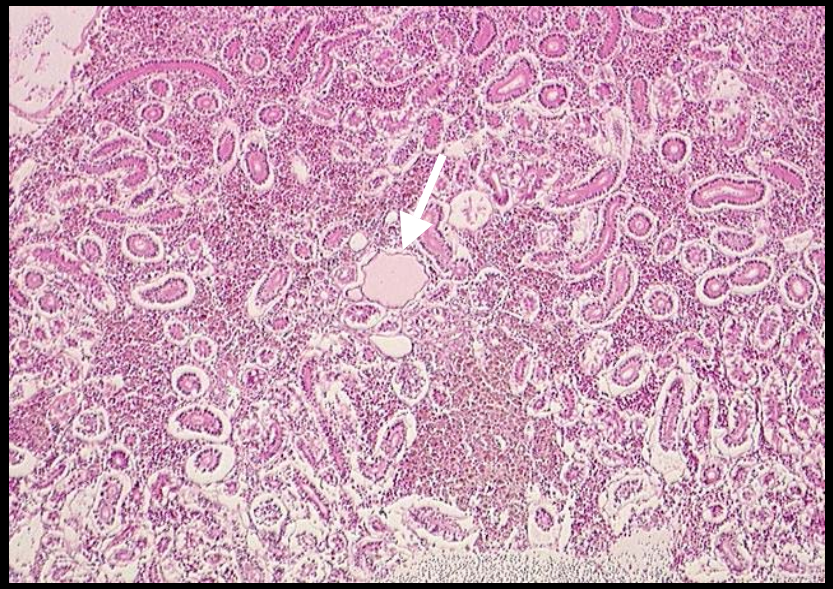
Indicators of Endocrine Modulation in Fishes

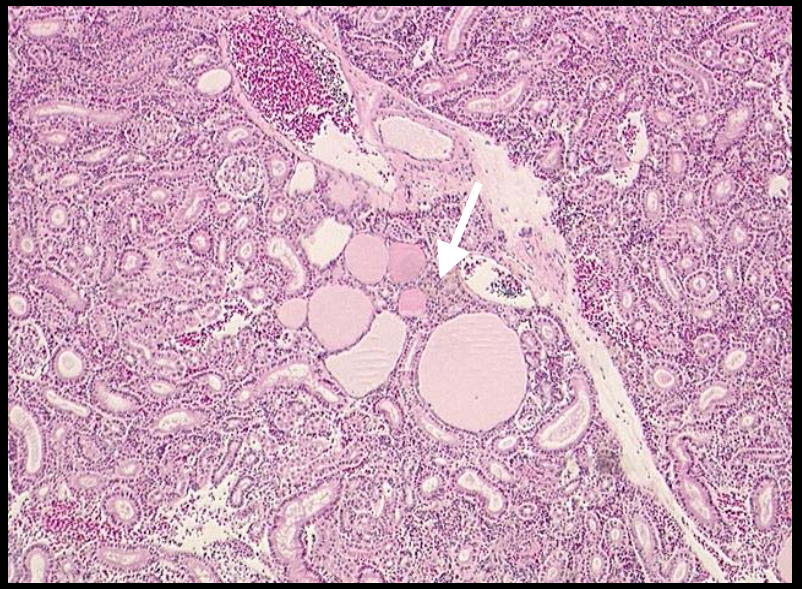
- **Morphological changes**
  - Secondary sex characteristics, altered growth, altered organosomatic indices
- **Circulating hormone levels**
  - Testosterone, estrogen, thyroxine (T_3, T_4,), cortisol
- **Molecular changes – mRNA, estrogen receptors**
- **Microscopic (histologic) changes**

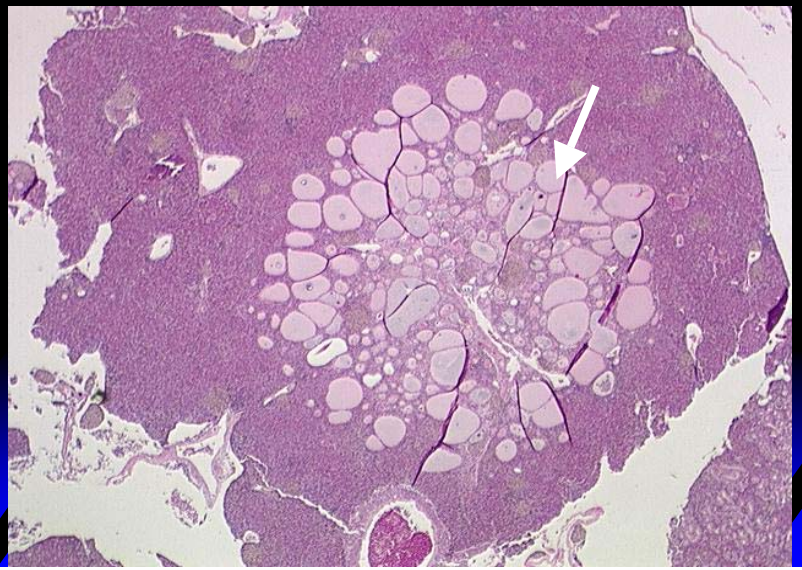
Difficulties in Assessing Endocrine Disruption in Fish

- Endocrine organs tend to be diffuse tissues rather than discrete organs
  - Interrenal (adrenal cortex) and chromaffin (adrenal medulla) dispersed throughout anterior kidney
  - Thyroid follicles around vessels gill arch

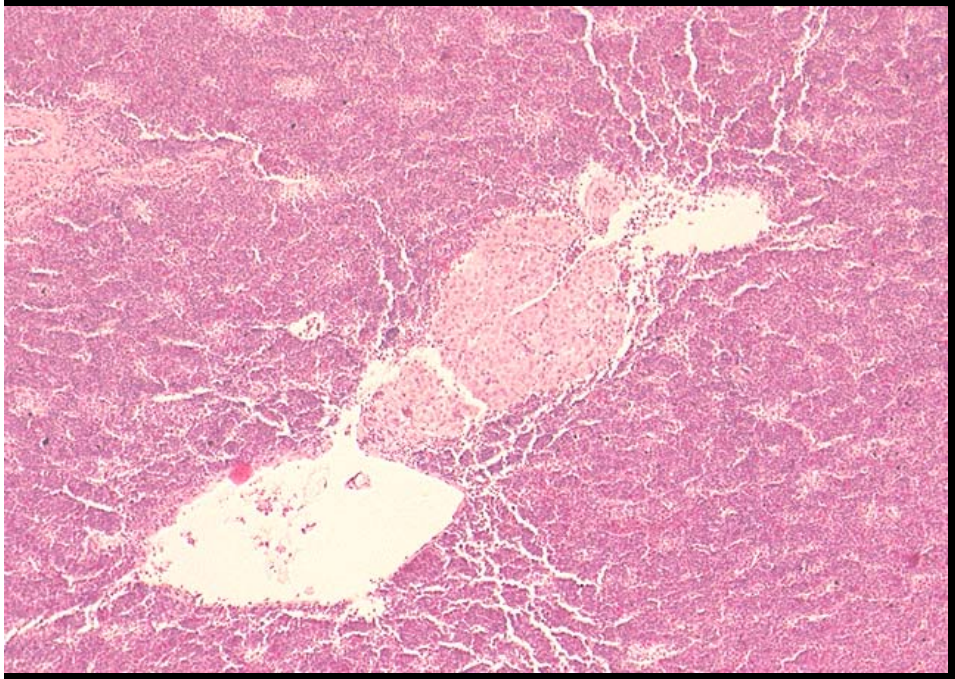
Ectopic Thyroid in Kidney of Carp
Rio Grande BEST Project



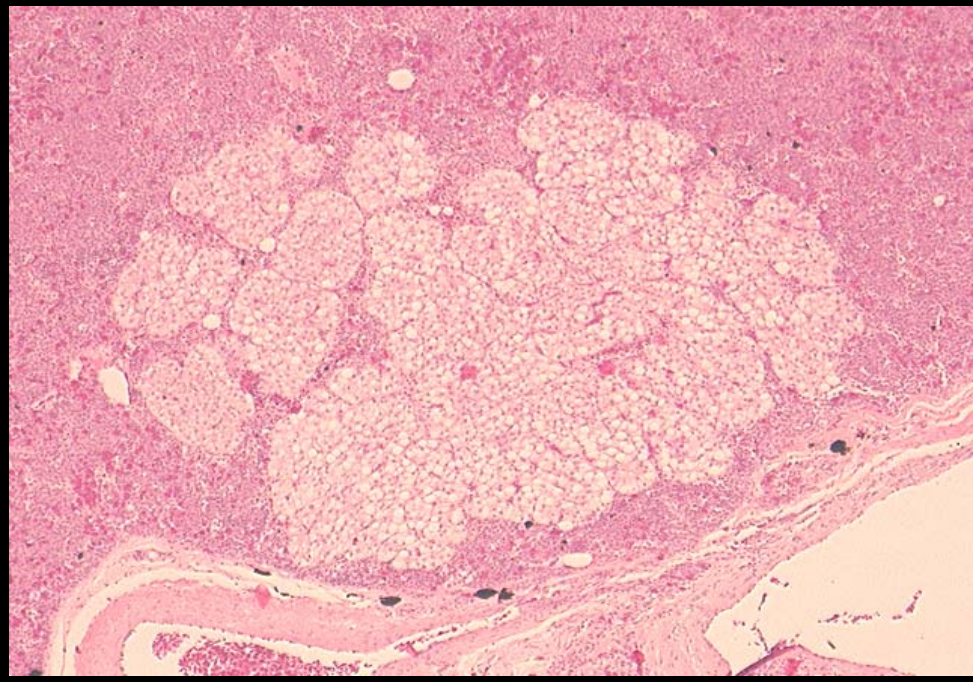




Endocrine Changes - Interrenal Tissue Paddlefish Study

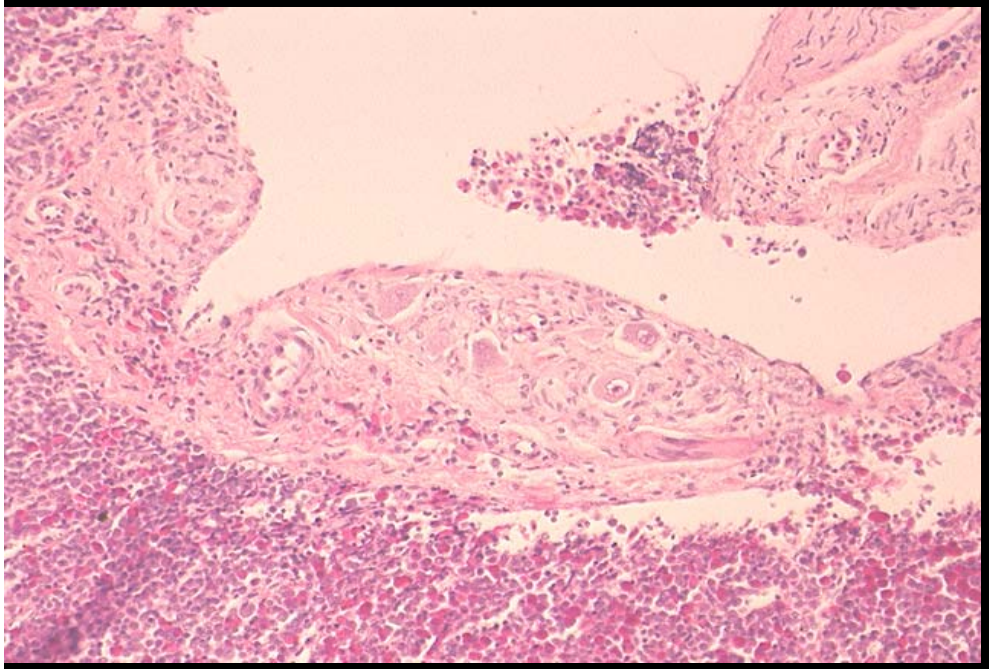


Control site

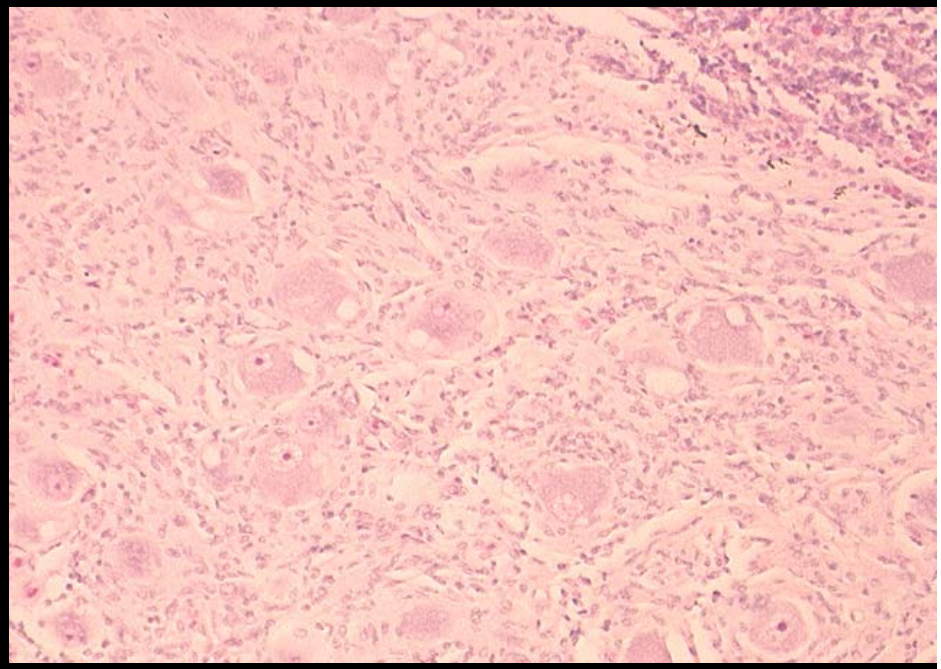


Contaminated site

Endocrine Changes - Chromaffin Tissue Paddlefish Study



Control site



Contaminated site

Bioindicators of Reproductive Endocrine Disruption

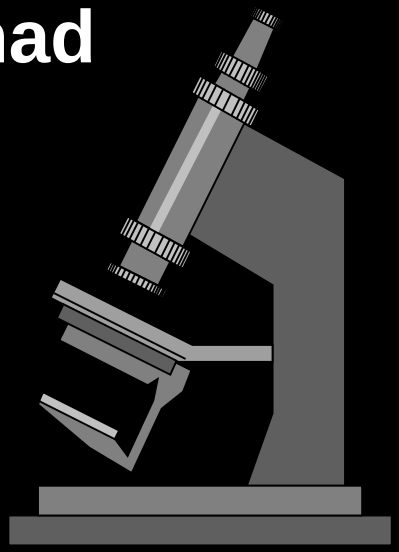
- Morphological Changes
  - Secondary sexual characteristics
  - Gonad size (gonadal somatic index)
- Circulating Hormone Levels
  - Estrogen
  - Testosterone
  - E/T ratios
- Circulating Vitellogenin
- Histologic Indicators

Difficulties in Assessing Endocrine Disruption in Fishes

- Many factors influence hormone levels
  - Sexual maturity, age
  - Climatic/Environmental factors – water temperature, photoperiod
  - Diet
  - Environmental factors - water quality
  - Time of year/day of collection

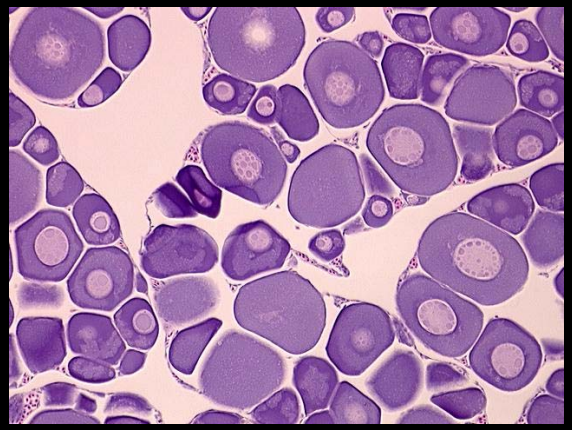
Histopathologic Endpoints Reproductive Studies

- Developmental stage of the gonad
  - Females stages 0 to 5
  - Males stages 1 to 4
- Percent atresia
- Sertoli cell proliferation
- Gonadal tumors
- Ceroid/lipofuscin (pigmented cell accumulations)
- Intersex



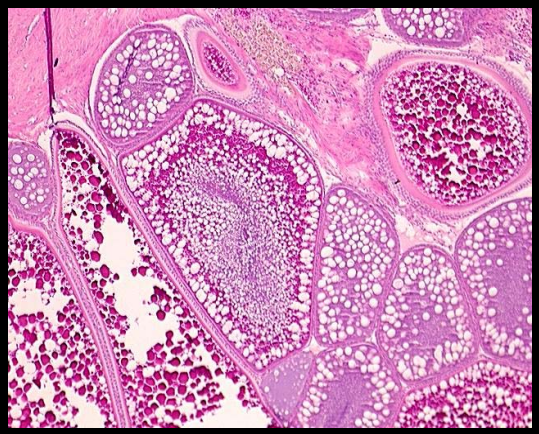
Gonadal Stages - Female

Stage 0



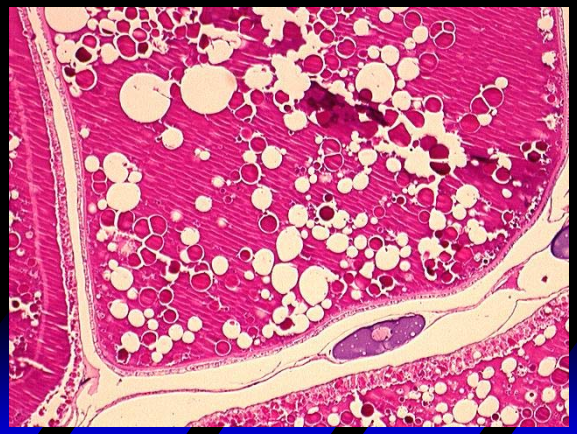
Stage 1

Stage 2

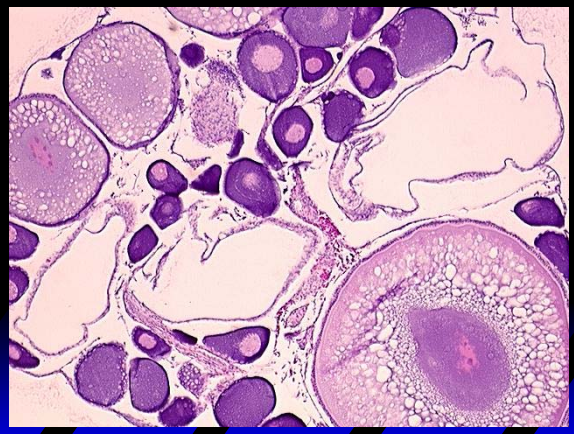


Stage 3

Stage 4



Stage 5

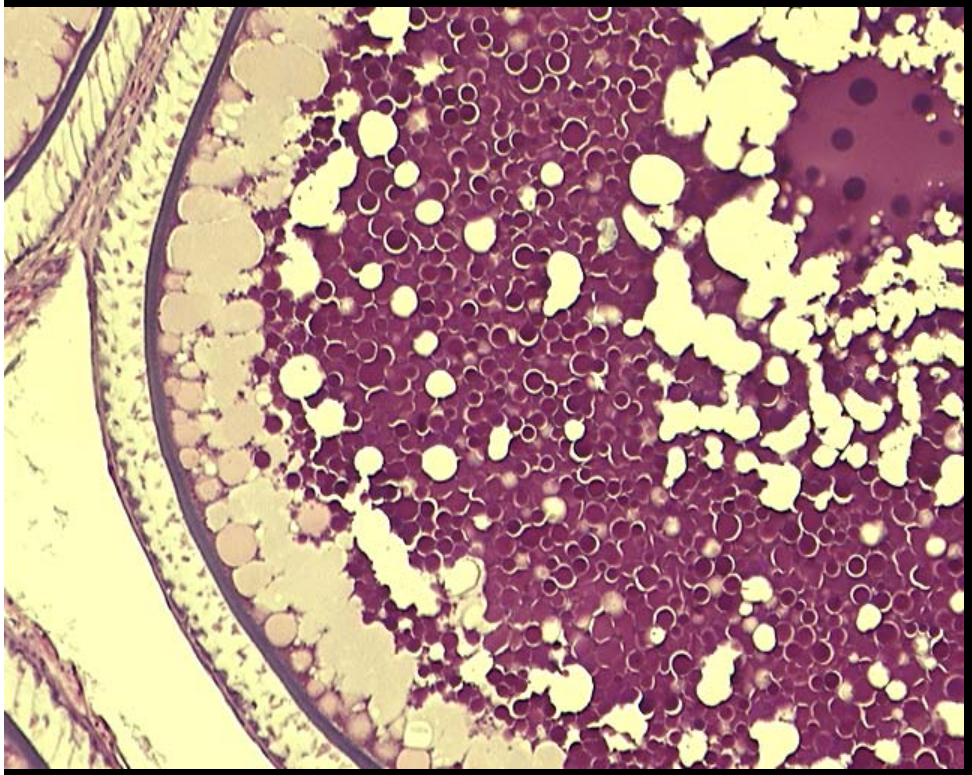


Lake Michigan Yellow Perch

- Overall decline of the yellow perch population noted since 1994
- Series of weak year classes since 1991
  - limited recruitment to the adult population
- Shift in age-class structure
  - lack of young fish

Comparison of Yellow Perch Females

Year Class 90 Winter 1996 Collection



Lake Mendota

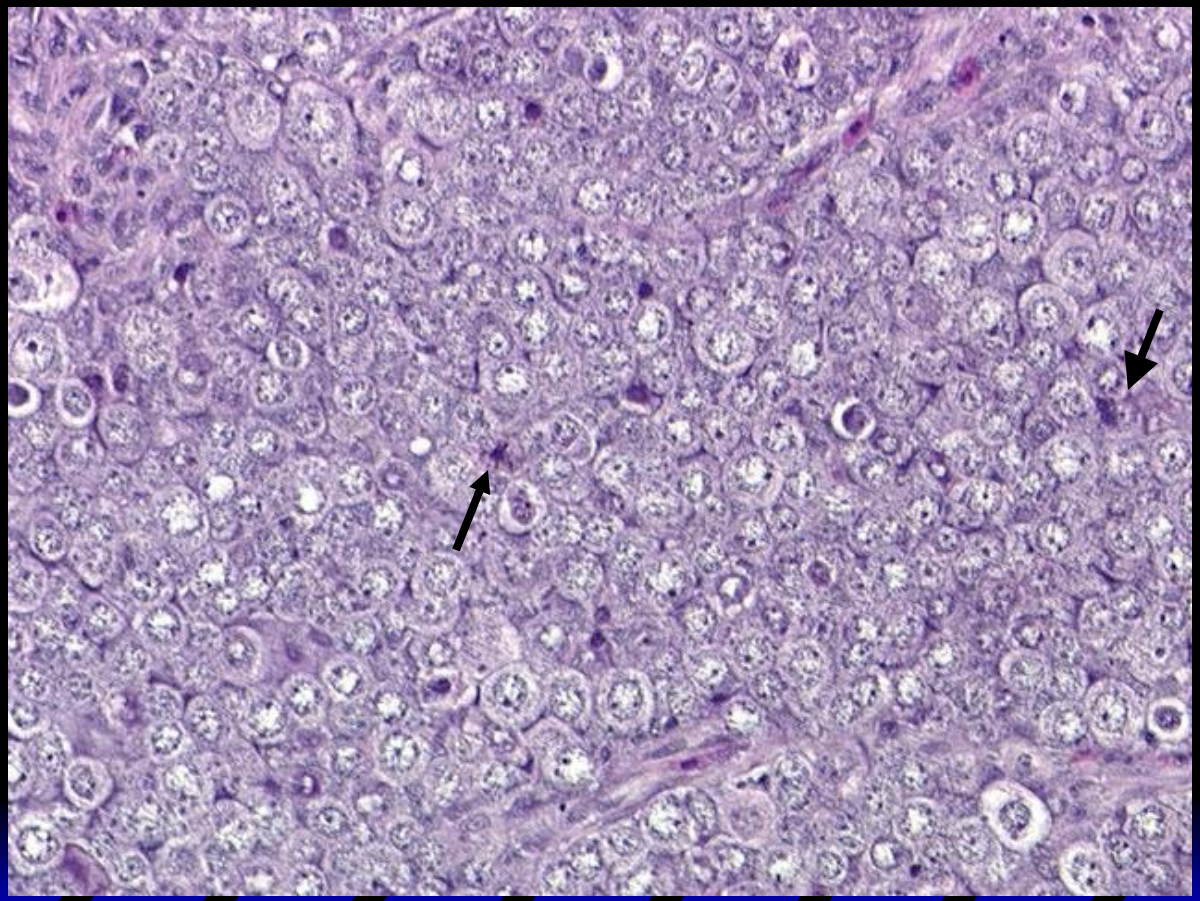
Lake Michigan

Stage 3

Testicular Tumors (Seminoma)
Lake Michigan Yellow Perch

Sample Date	Prevalence
Winter 96	31%
Spring 97	27%
Fall 97	25%

Perch Testicular Neoplasia
Seminoma



Diffuse sheets of large cells with abundant clear cytoplasm, distinct cell membranes, and round nuclei with 1 or 2 nucleoli, intersected by thin bands of fibrovascular connective tissue. Mitotic figures are common.

Human Testicular Cancer

- Rates of testicular cancer have increased sharply in the past three decades in many countries (US, England, Denmarck, Canada)
- Particularly those affecting younger men- the seminomas

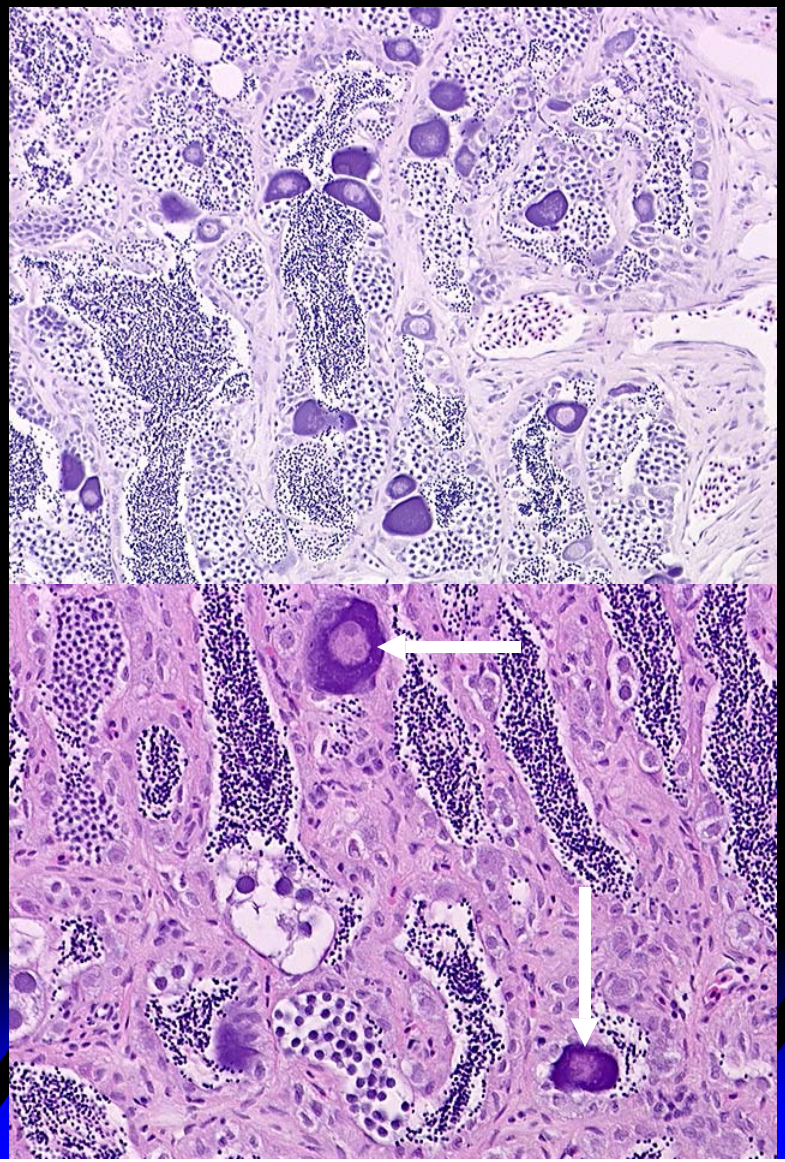
- In Ontario the cancer rate is up nearly 60% from 1964
  - Rising 2.4% a yr for 15-29 year olds
  - Rising 1.5% a yr for 30-44 year olds

Canadian Medical Association Journal

Intersex in Fish

- Increasingly there are reports of individual wild fish with intersex or ovotestis
  - Most involve female germ cells or immature oocytes within a predominantly male gonad
  - Other reports include malformed/intersex reproductive ducts; large areas of ovarian tissue

Intersex in Normally Gonochorist Fishes

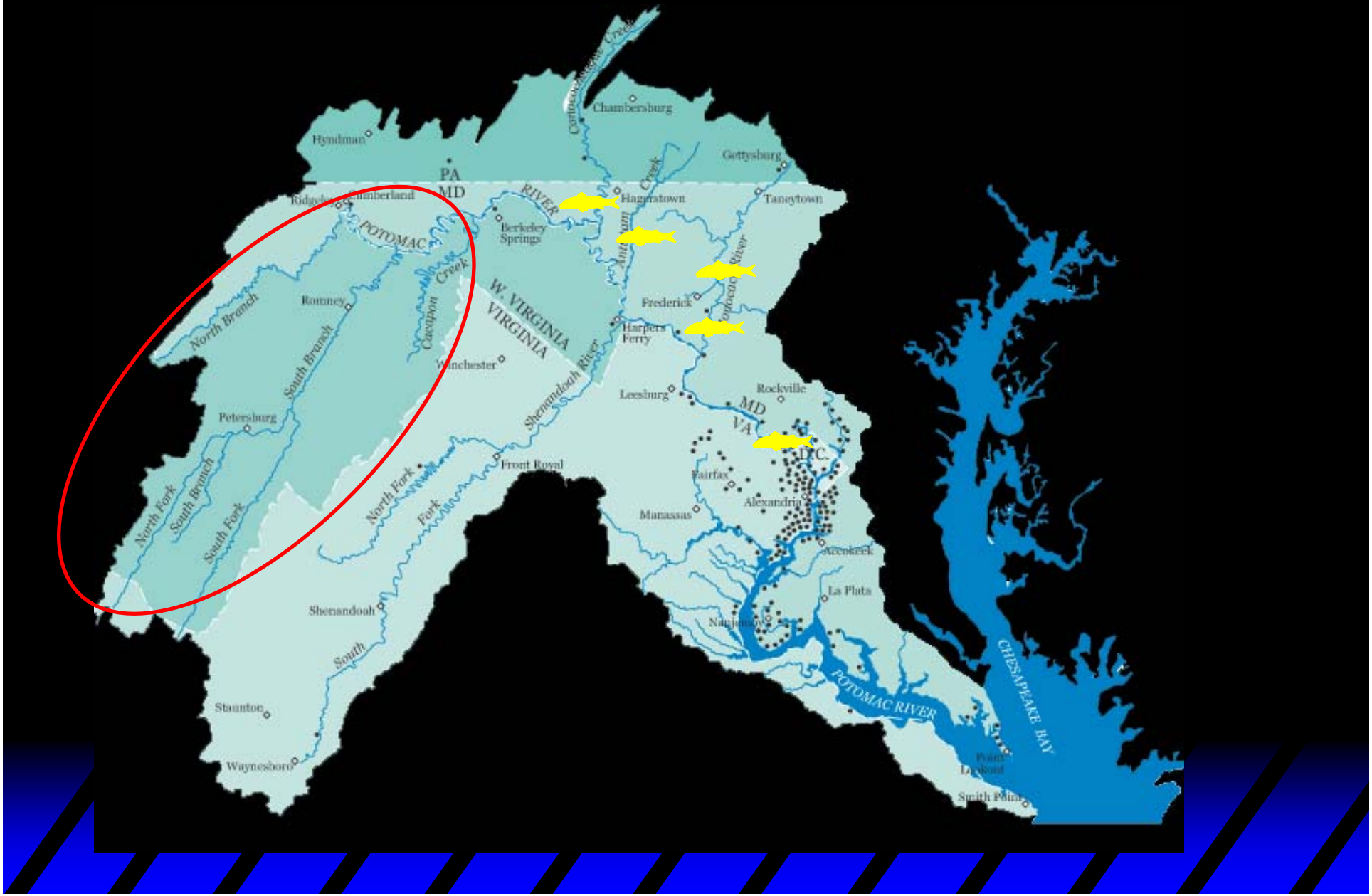


- Immature oocytes within testes
- Suggested as a marker of endocrine disruption
- Used as an indicator of exposure to estrogenic compounds

Intersex
South Branch Potomac

Site #	Summer 2003 % Intersex (# of males)	Spring 2004 % Intersex (# of males)	Summer 2004 % Intersex (# of males)
1	33.3% (6)	100% (7)	50% (4)
2	ND	75% (12)	25% (8)
3	0% (4)	84.6% (13)	40% (10)
4	ND	69.2 (13)	36.4% (11)
5	60% (5)	90% (10)	66.7% (12)
8	80% (10)	72.2% (11)	33.3% (6)

Potomac Watershed



Effects of Intersex

- Studies with wild roach (*Rutilus rutilus*)
- Intersex males
  - Produced up to 50% less milt (per gram)
  - Reduced percent of motile sperm
  - Lowered ability of sperm to successfully fertilize eggs and produce viable offspring

Vitellogenin

- Vitellogenin is the serum/plasma phospholipoglycoprotein precursor to egg yolk
- Normally found in measurable amounts only in the blood of sexually mature egg-laying vertebrates
- Estrogen stimulates the liver to produce vitellogenin which travels to the ovaries via bloodstream and is sequestered by developing oocytes

Vitellogenin in Male Fish

- Males have the gene to produce vitellogenin - usually not turned on
- Exposure to natural estrogens (phytoestrogens), synthetic or natural estrogens from human and agricultural sources, and estrogen mimics such as toxaphene, dieldrin, endosulfan, PCBs, alkylphenols and other chemicals may stimulate vitellogenin production by males

Endocrine Disruption in Potomac River

- Male smallmouth from the some of the same sites as those with intersex also had circulating vitellogenin
- Largemouth bass also have intersex, although at a lower prevalence (0-30%)

Other Potential Effects of Exposure to Estrogenic Compounds

- Behavioral effects?
  - Courtship
  - Mate competition
  - Nest guarding

Using Biological Responses to Screen Water/Sediment Extracts for Estrogenic Potential

- **E-screen**
  - **Estrogen sensitive mammalian cell line – estrogenicity can be tested by assessing proliferation**
- **Primary fish hepatocyte cultures**
  - **Culture hepatocytes in culture and look for vitellogenin production in tissue culture supernatants after exposure**