Effects of Endocrine Disrupting Compounds on Fish - What Do We Know and What Don't We Know:

Part A: Overview

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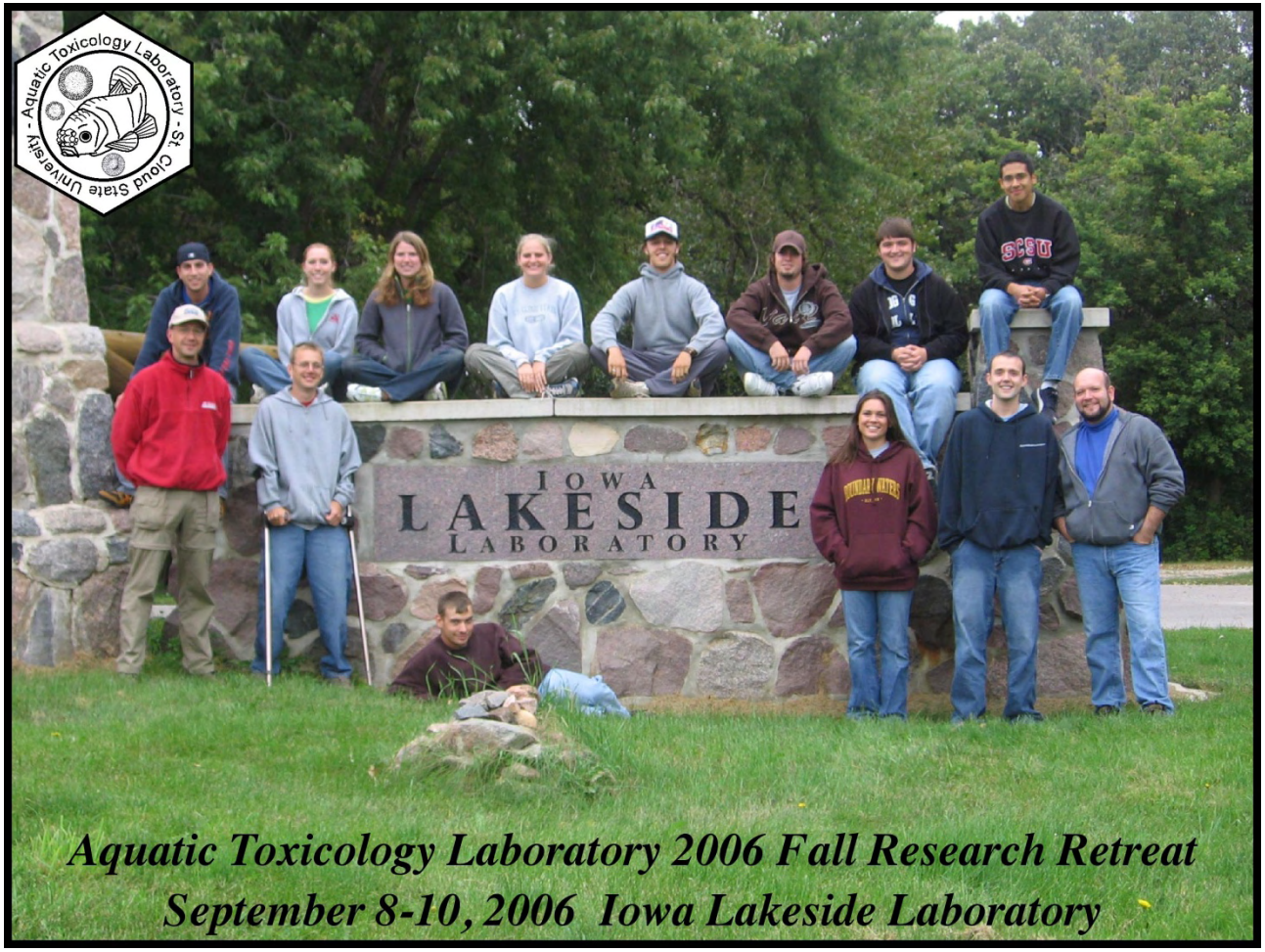

Aquatic Toxicology Laboratory - St. Cloud State University

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Minnesota Pollution Control Agency

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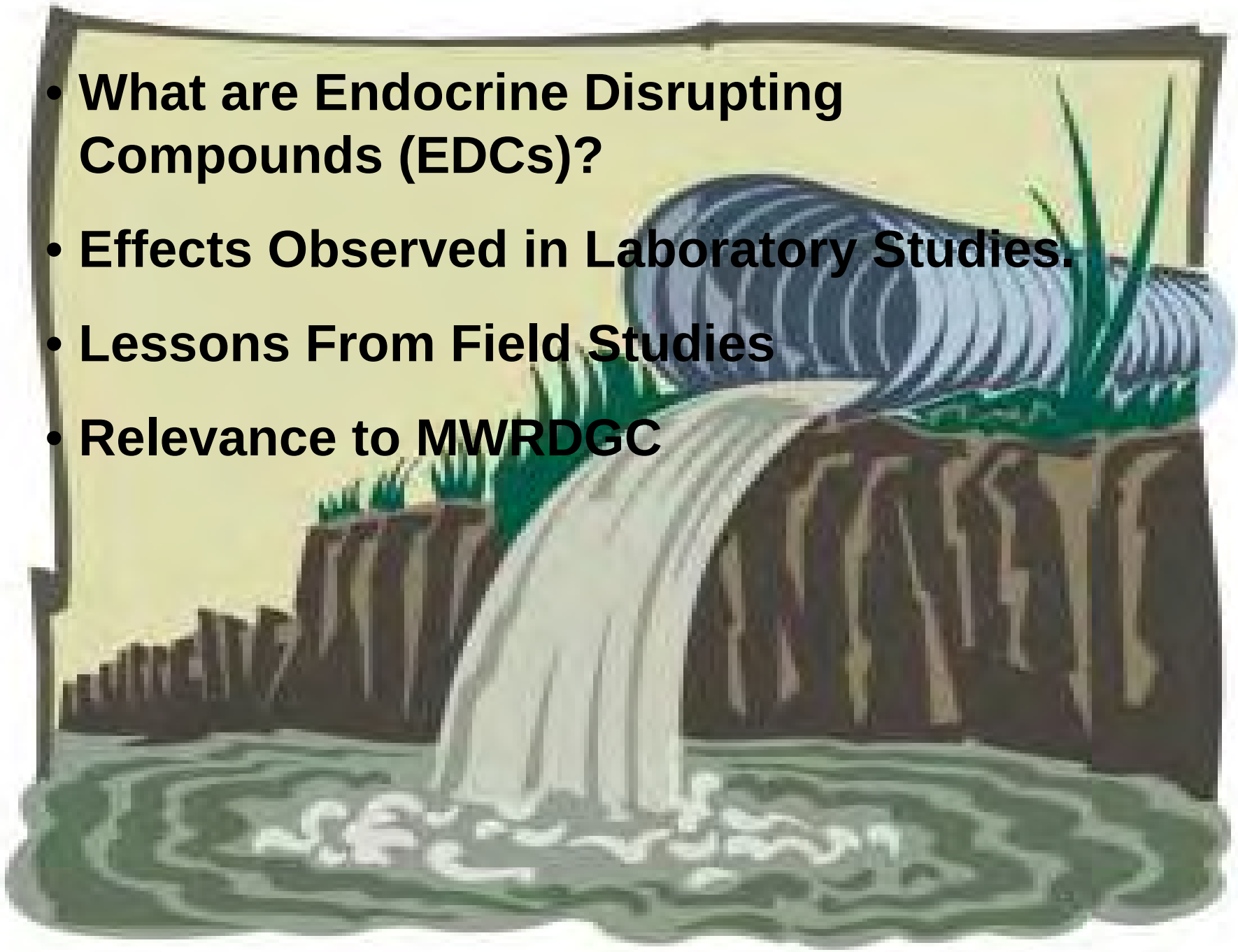


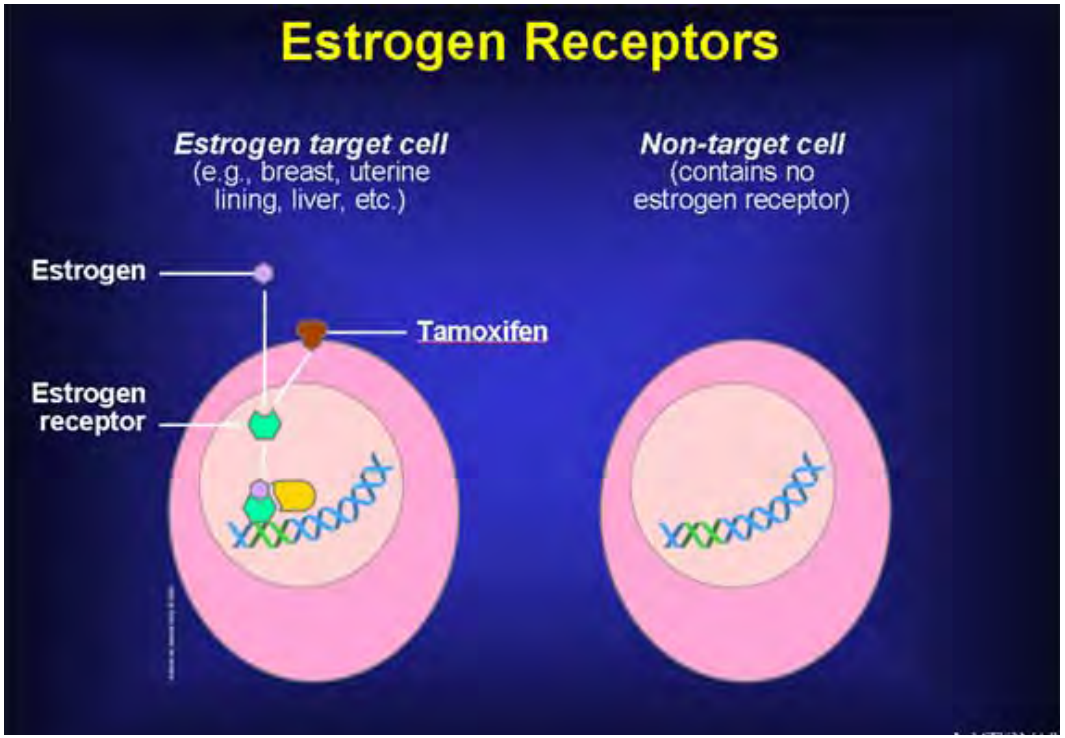
- **What are Endocrine Disrupting Compounds (EDCs)?**
- **Effects Observed in Laboratory Studies.**
- **Lessons From Field Studies**
- **Relevance to MWRDGC**

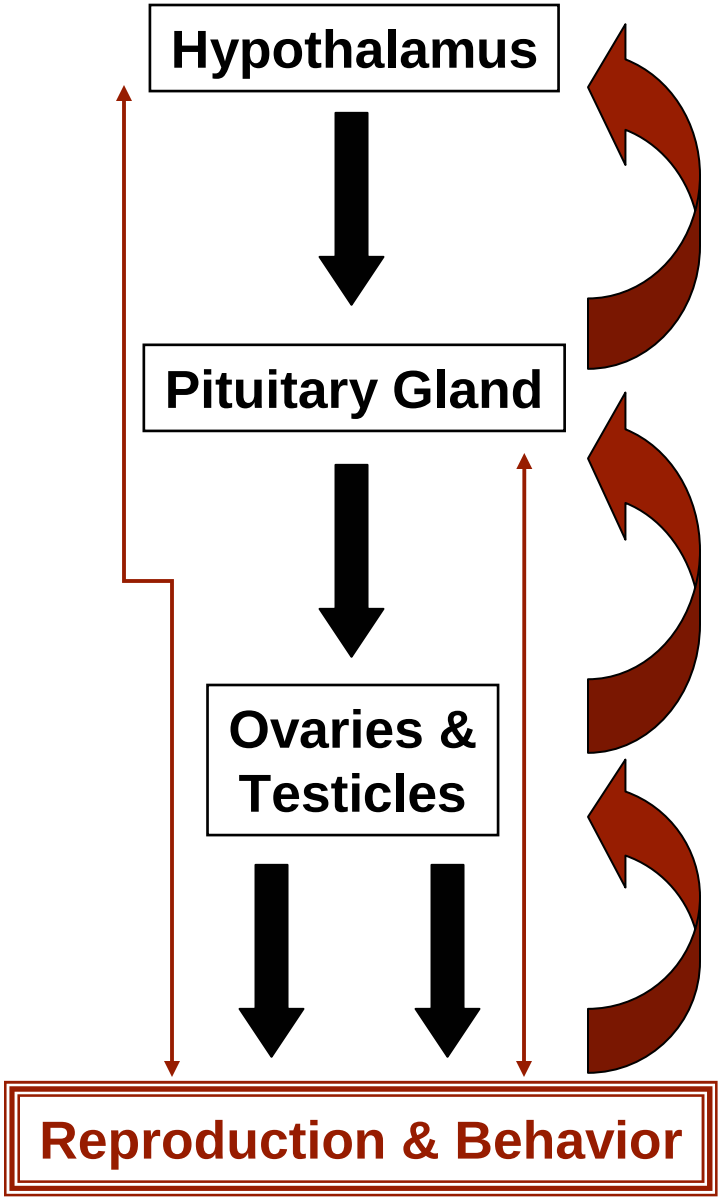
Endocrine Disrupting Compound - One Definition

"An Endocrine Disrupting Compound is an Anthropogenic Compound that may have an Adverse Effect Mediated Directly through the Endocrine System of Fish, Wildlife, or Humans."

Endocrine Disrupting Compound - Mode of Action

- *Interact with hormone receptors.*
- *Little change in the past 500 million years.*
- *Hormones in fish and humans are remarkably similar.*





Endocrine Disrupting Compound - Diversity

Pharmaceuticals & Personal Care Products (PPCPs)

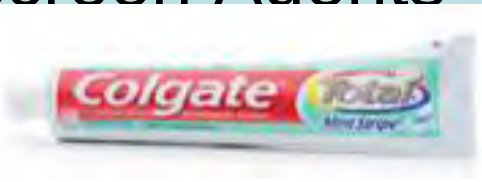
Bath additives, shampoos, skin care products, hair sprays, oral hygiene, soaps, detergents

Fragrances

Preservatives

Disinfectants/Antiseptic

Sunscreen Agents



Daughton and Ternes

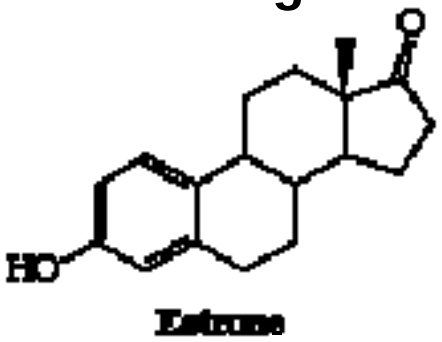
Alkylphenol Ethoxylates (APEs)

- Nonyl & Octyl Phenol Ethoxylate
- High Production Volume Chemical
    - ~ 391.5 million lbs/year NPEs and 77 million lbs/yr OPEs used in North America (U.S. & Canada) in 2003
- Uses - Detergents, wetting agents, dispersants, emulsifiers, solubilizers and foaming agents
- Industrial applications - Pulp and paper, textiles, coatings, agricultural pesticides, lube oils and fuels, metals and plastics
- Chief concern is NP based compounds.
  – OP is also toxic, and more potent ED but only = 10 – 15% of APEs used

Endocrine Disrupting Compound - Abundance

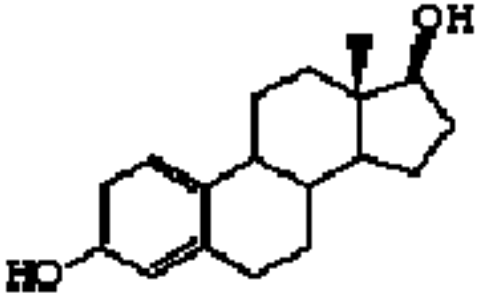
- A snapshot from effluents flowing into the Mississippi River:

Natural Estrogens:



Estrone

nd - 50 ng/L



17 β-Estradiol

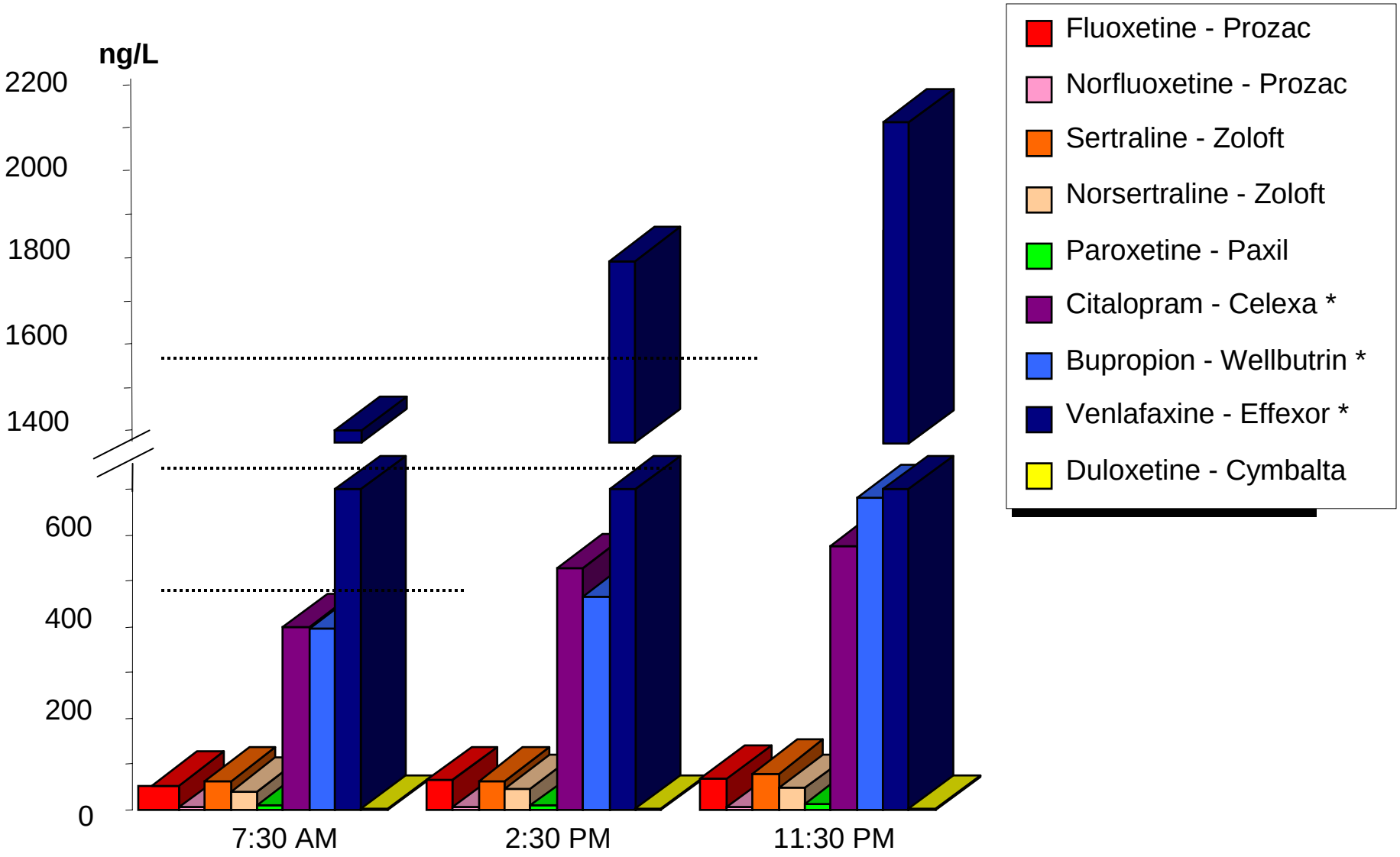
nd - 7.3 ng/L

Synthetic Emerging Contaminants:

- **Ethynylestradiol (birth control): nd - 3 ng/L**
- **Total alkylphenols (detergents): > 50,000 ng/L**
- **Bisphenol A (plasticizer) : 35.5 - 9,026 ng/L**
- **Carbamazepine (anti-epileptic): 823 - 1,360 ng/L**
- **Triclosan (anti-microbial): 82 - 318 ng/L**
- **Perfluorochemicals (non-stick Teflon): 3.6 - 28 ng/g in fish tissue**

nd = non-detectable; ng/L = parts per trillion

Endocrine Disrupting Compound - Pharmaceuticals

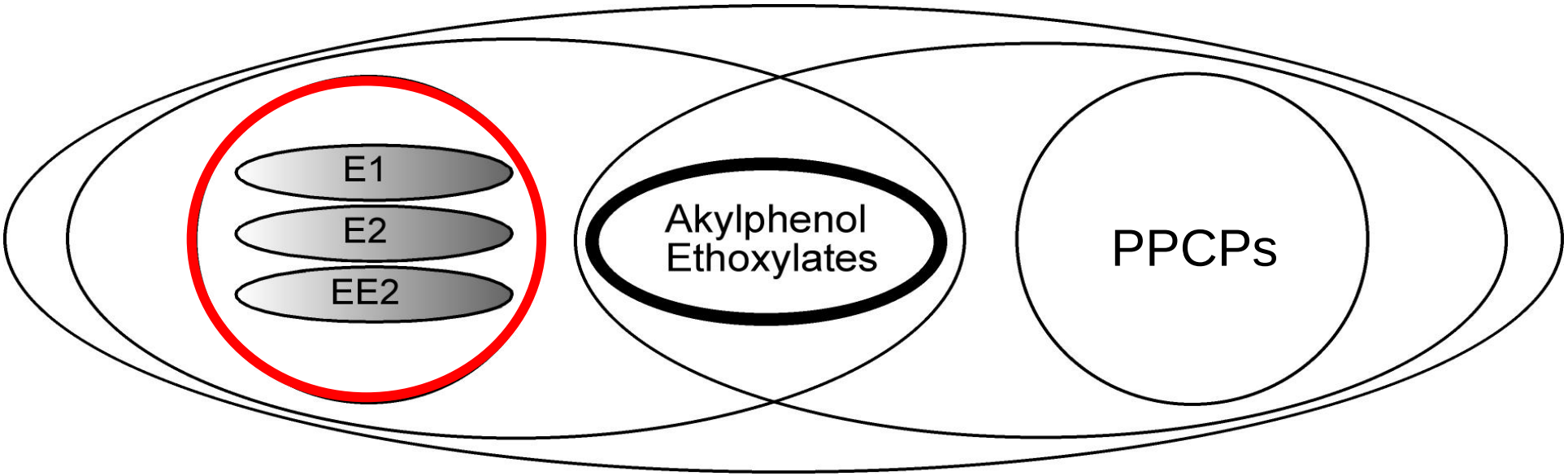


Total SSRI concentration: > 5,000 ng/L !

Laboratory Studies - Questions

Emerging contaminants enter the aquatic environment as mixtures:

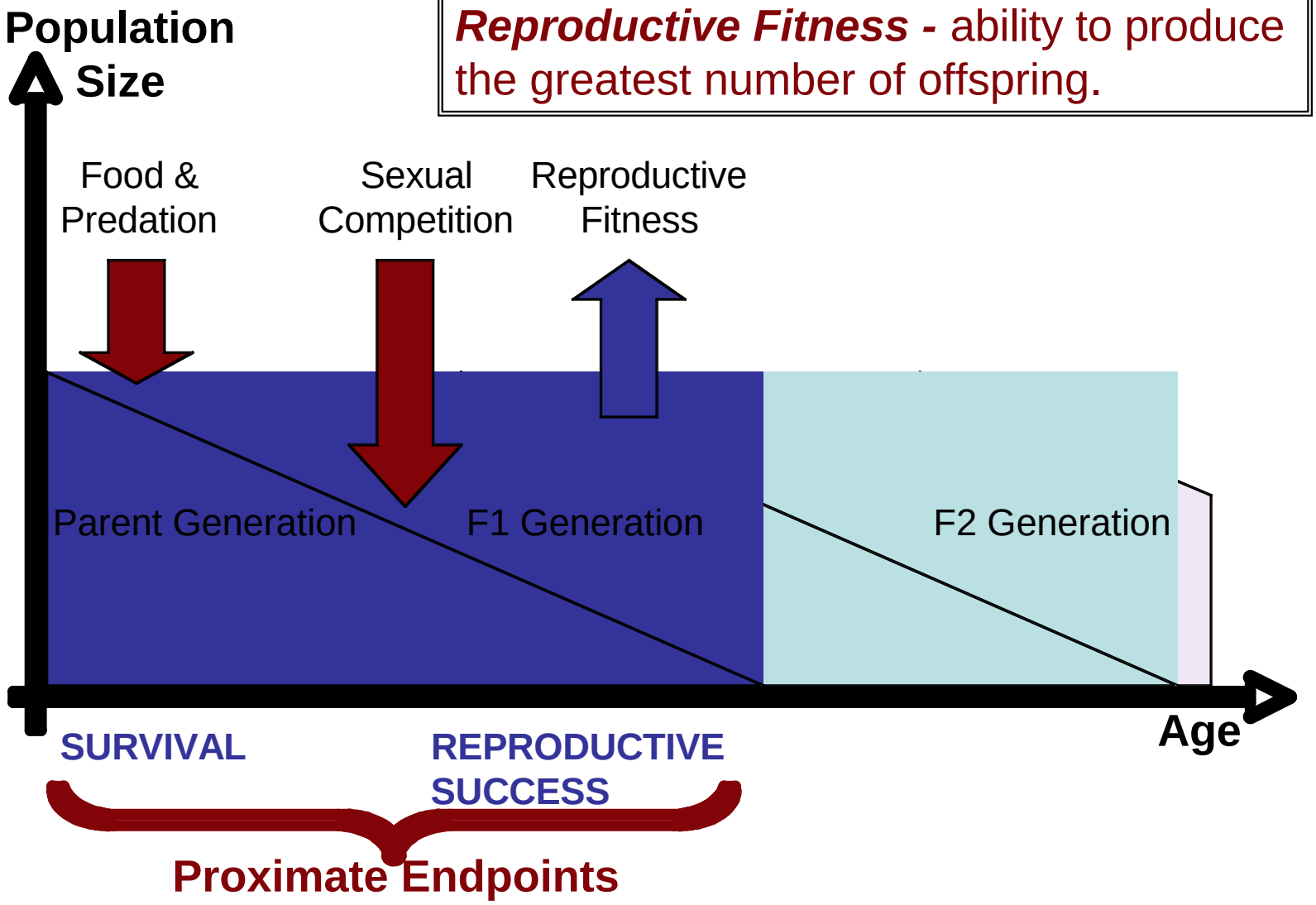
1. What are the effects of individual compounds?
2. What are the effects of mixtures?
3. Can the sum of effects of individual compounds account for the effect of whole effluent?



Laboratory Studies - Concept

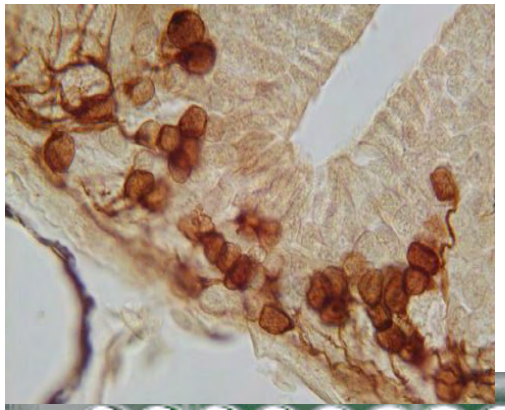
The Ultimate Endpoint

Reproductive Fitness - ability to produce the greatest number of offspring.

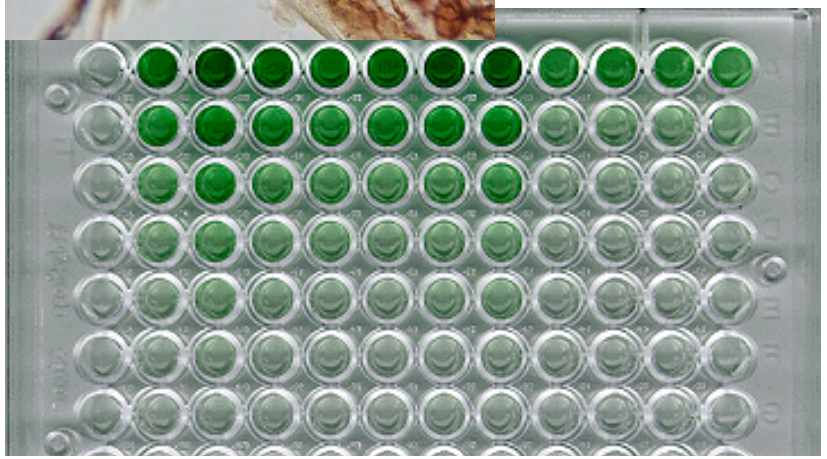


Laboratory Studies - Endpoints

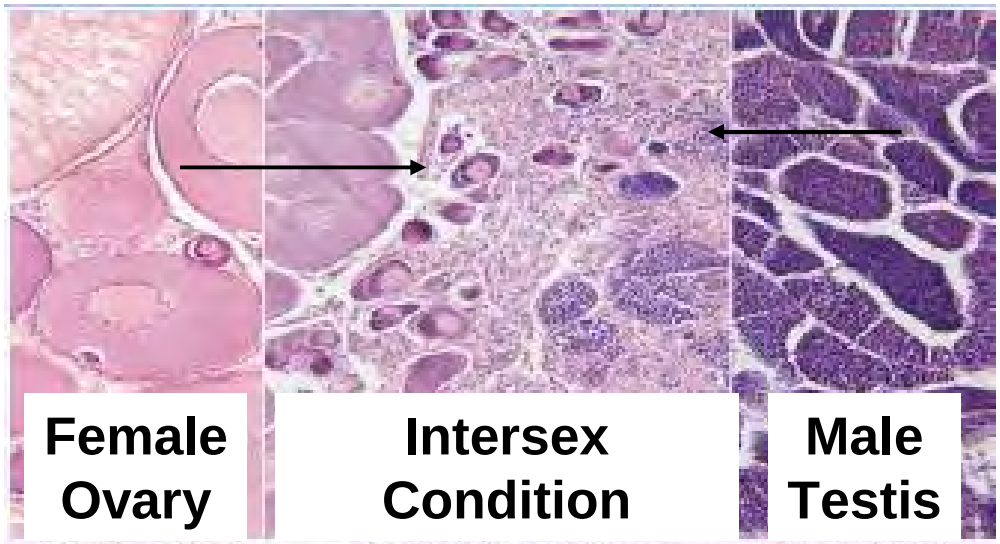
Tubercles & Dorsal Pad

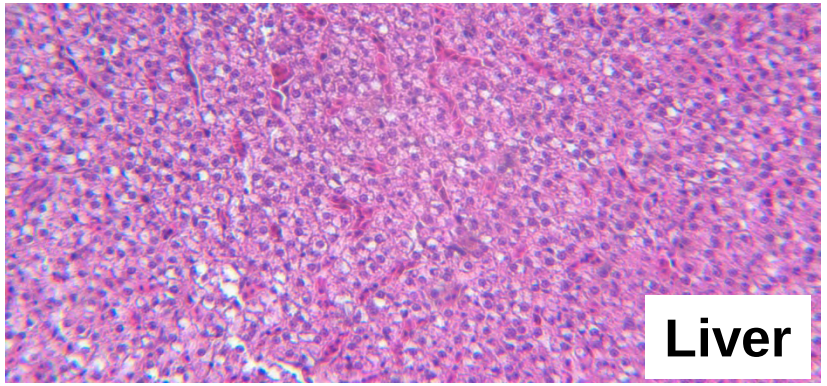


Brain Activity



Vitellogenin - Egg Yolk Protein





Laboratory Studies - Male exposures

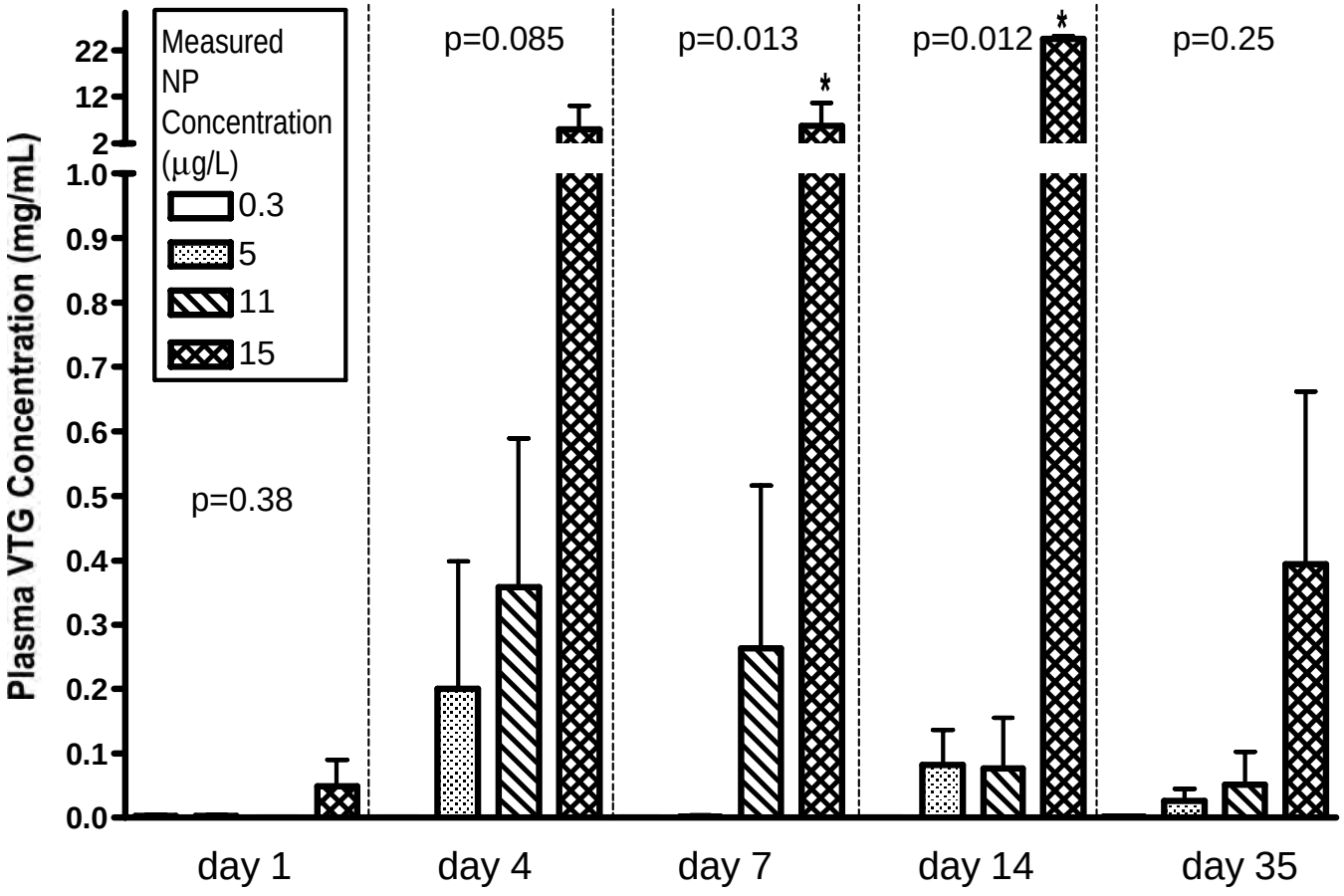
Pimephales promelas

Experimental Design - Mature Male Fathead Minnow Exposure

Expose mature male fathead minnows for 21 days to graded series of nonylphenol.

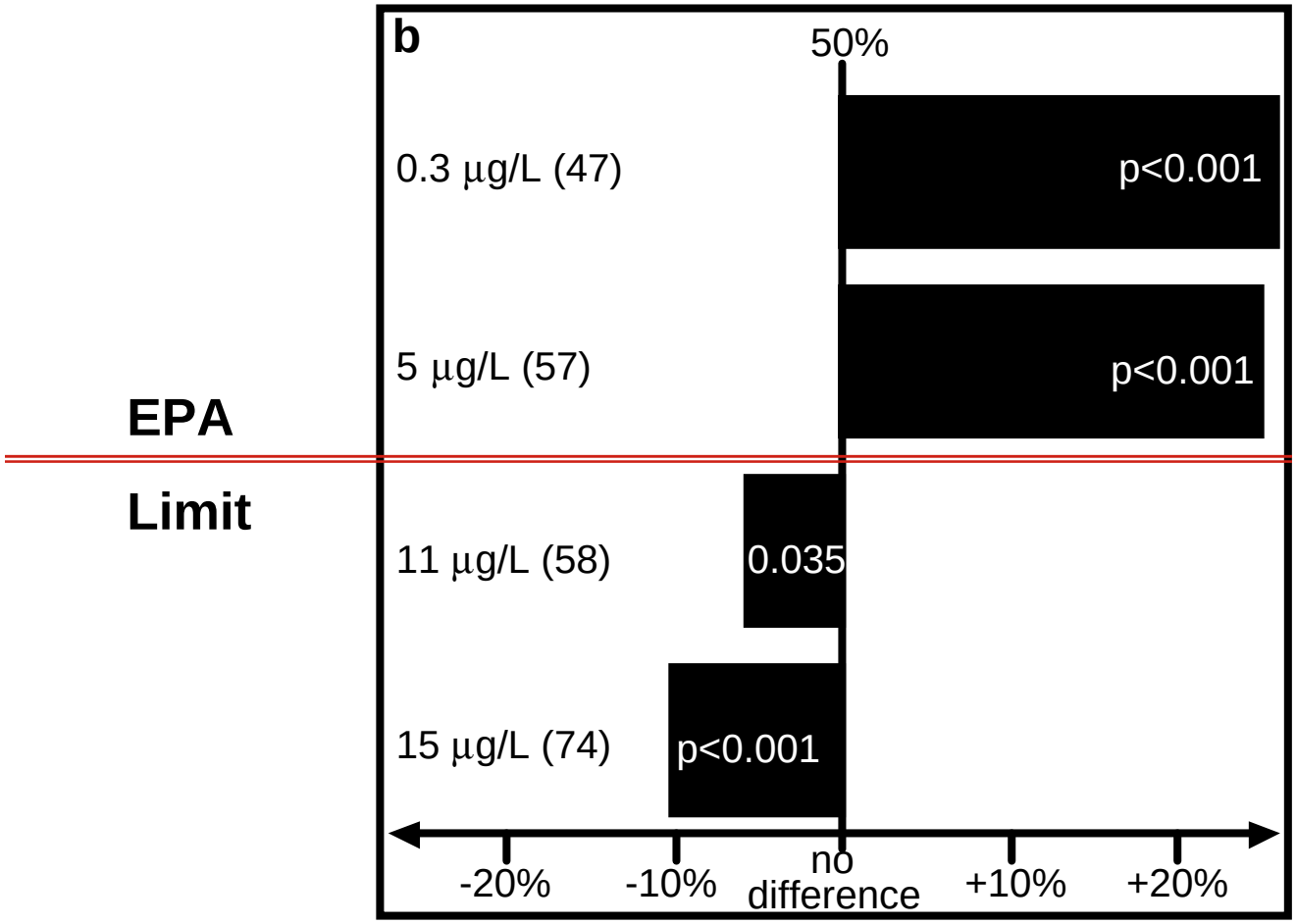
- Competitive spawning
- Secondary Sex Characters
- Vitellogenin
- Histology

Laboratory Studies - Male exposures



Vitellogenin Concentrations (mg/mL)

Laboratory Studies - Male exposures



Competitive Spawning

Schoenfuss et al. 2008. Aquatic Toxicology.

Laboratory Studies - Larval exposures

Pimephales promelas

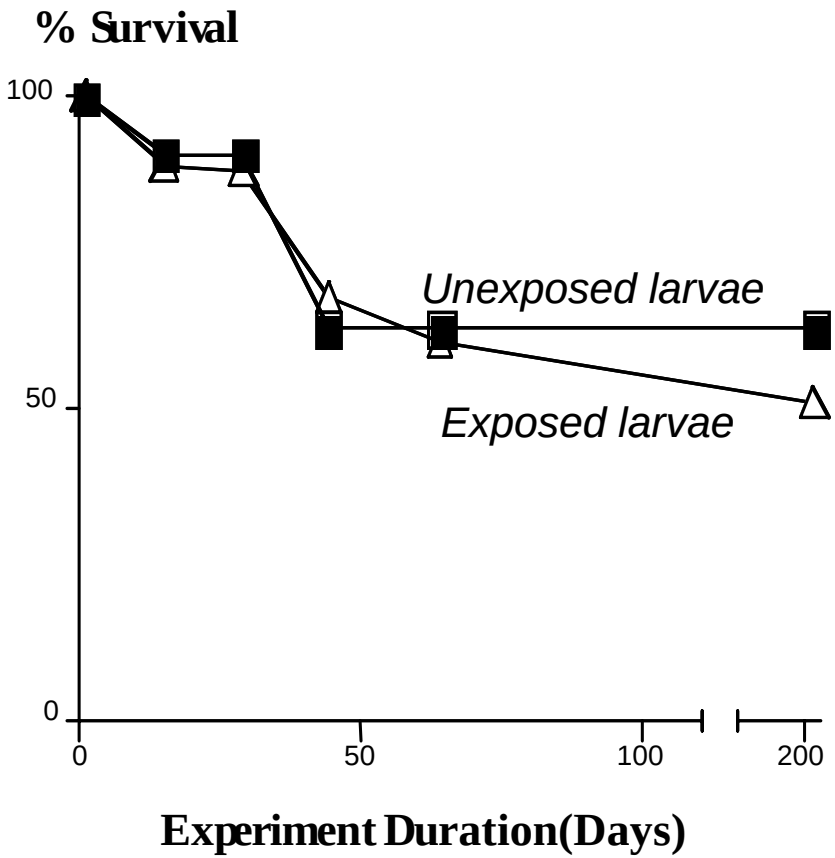
Experimental Design - Larval Fathead Minnow Exposure

Expose newly hatched fathead minnows for 63 days to nonylphenol or alkylphenol mixture based on effluent.

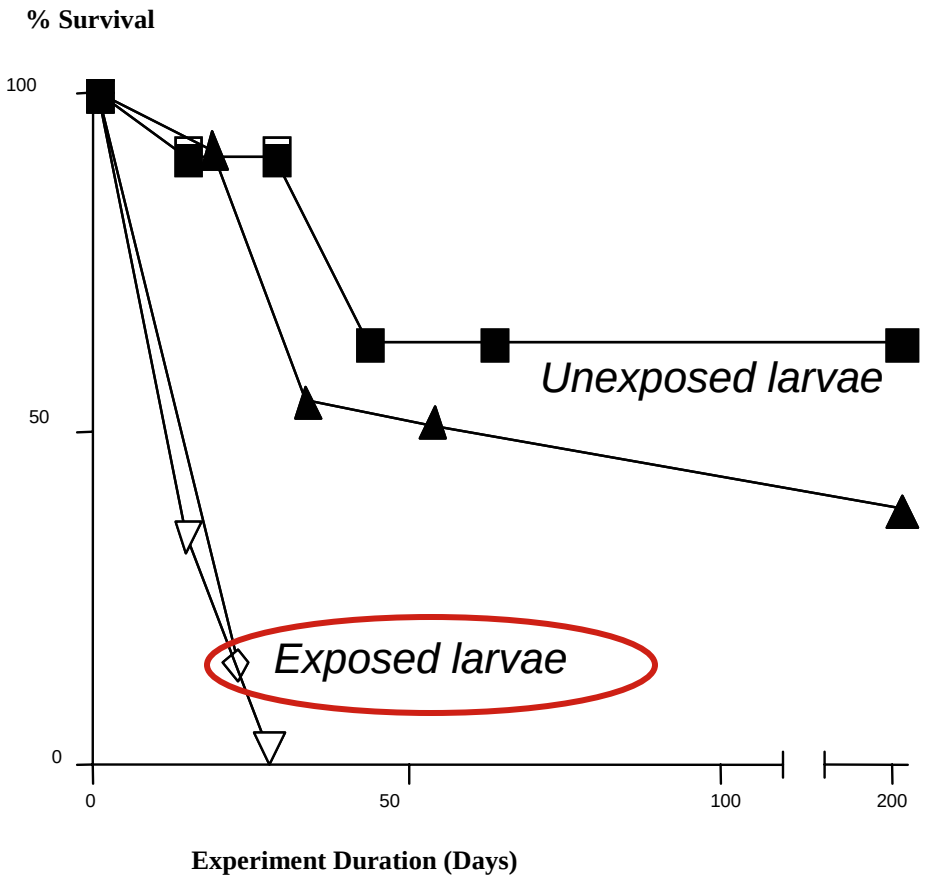
- Record survival and rear in clean water to maturity.
- Allow exposed mature males to compete with control males for access to spawning opportunities.

Laboratory Studies - Larval exposures

- Survival



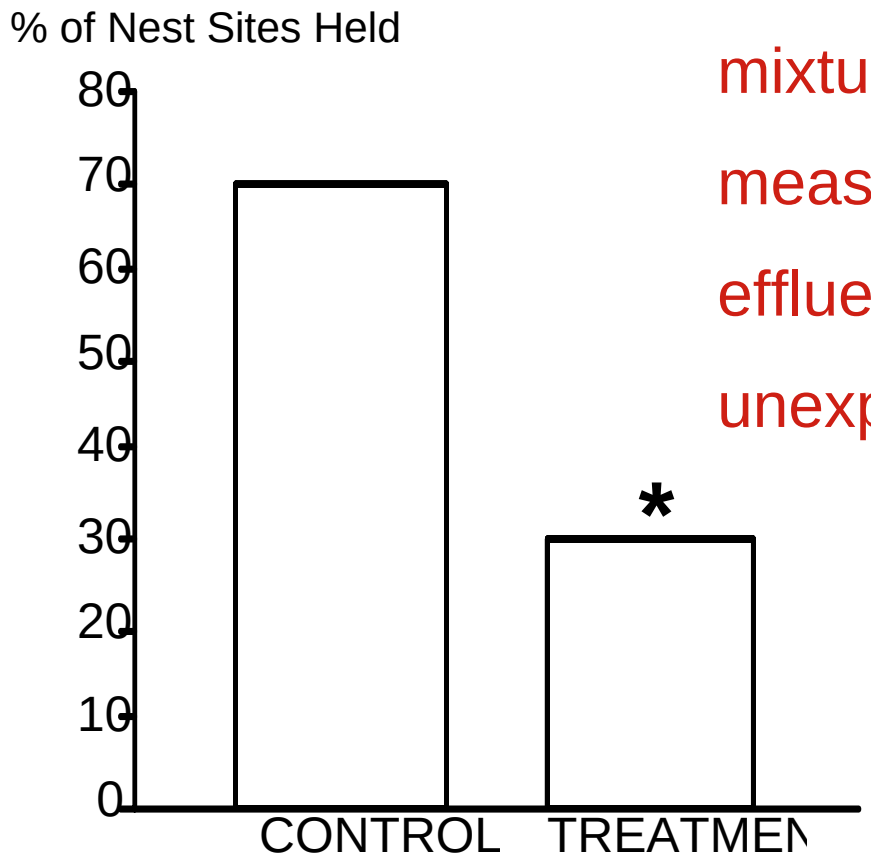
Larval Survival in Nonylphenol



Larval Survival in Alkylphenol Mixture

Laboratory Studies - Larval exposures

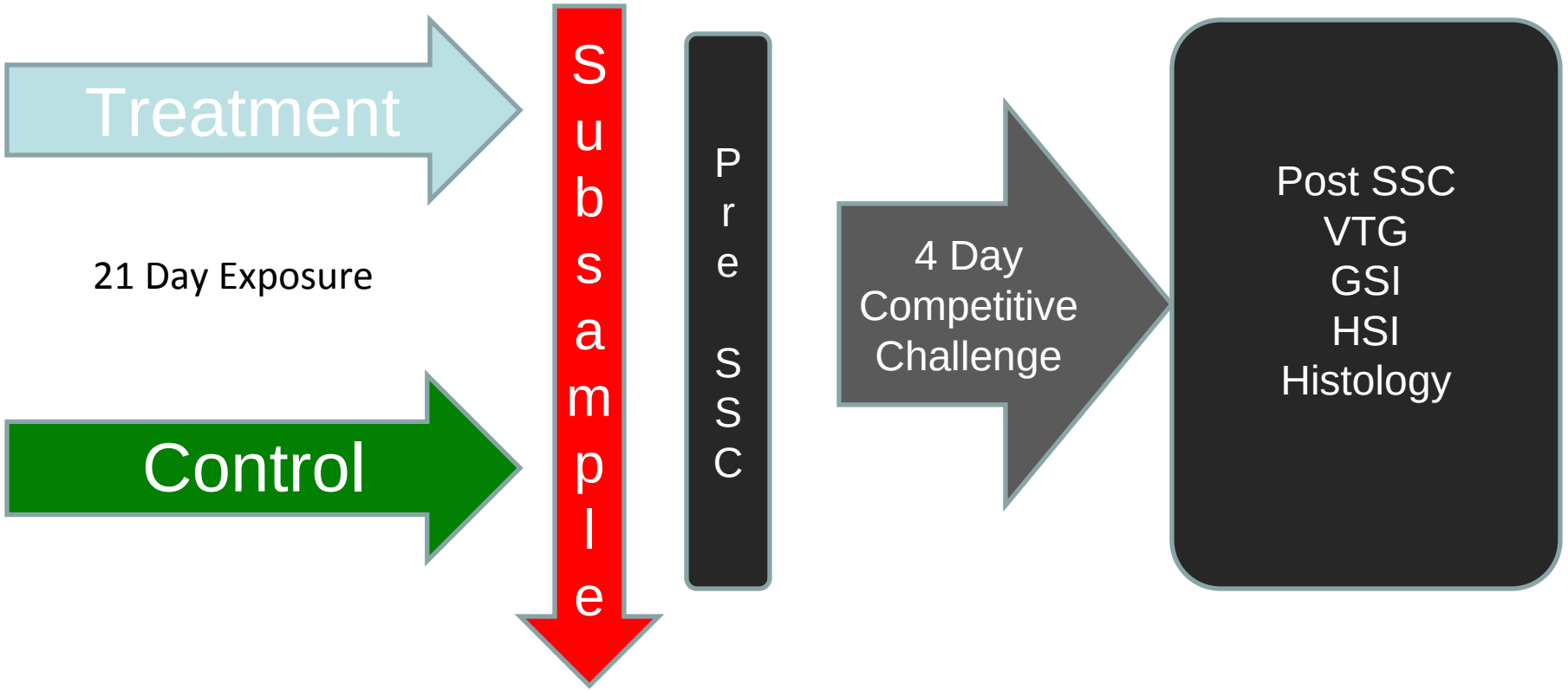
- Reproductive Ability



Larval fish exposed to an alkylphenol mixture at half the concentration measured in a treaded wastewater effluent cannot compete with unexposed fish once they are adults.

Bistodeau et al. 2006. Aquatic Toxicology.

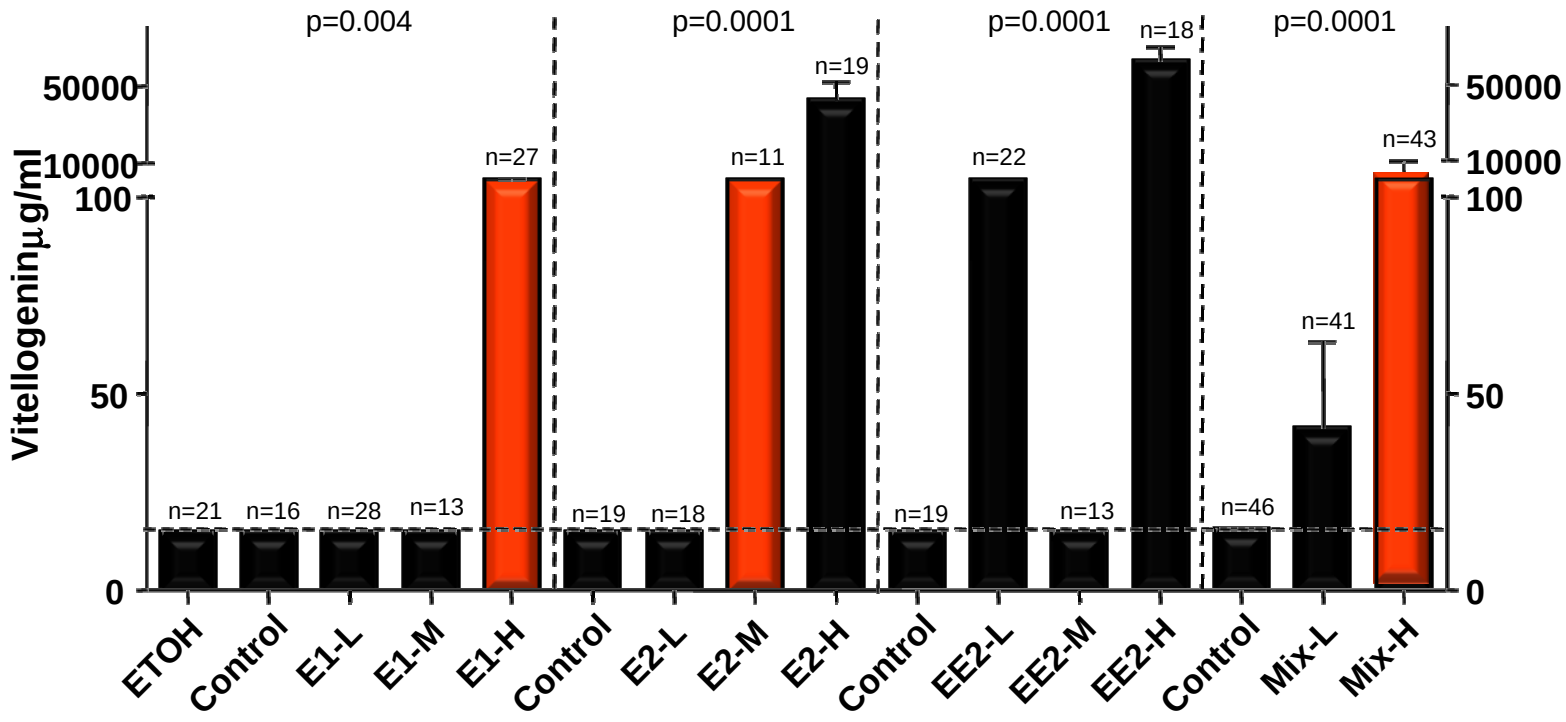
Experimental Analysis



Nominal Exposure Concentrations

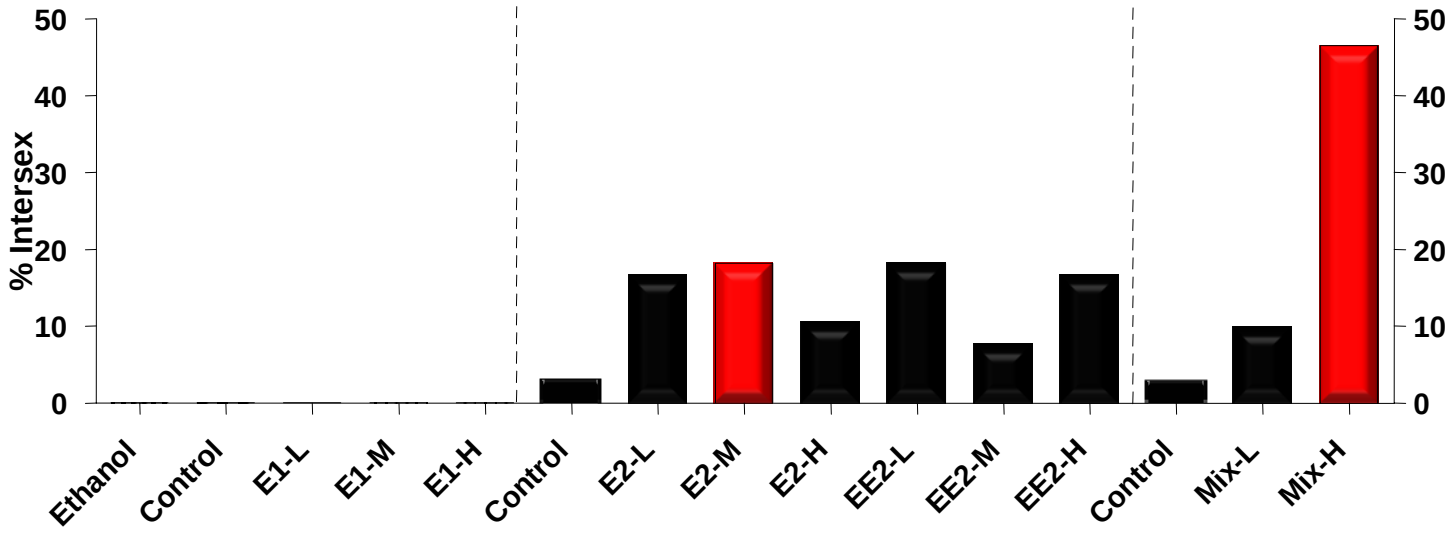
(ng/L)	Low		Med	High	
Estrone (E1)	5 (0.5)		50 (5)	100 (10)	
Estradiol (E2)	1		10	50	
Ethynylestradiol(EE2)	0.1 (1)		2.5 (25)	10 (100)	
Mixture	E1	10	N/A	E1	30
	E2	1		E2	3
	EE2	0.1		EE2	0.3
	(3)	() = Estradiol Equivalencies		(9)	

Vitellogenin Analysis



Comparing estradiol equivalencies, VTG analysis demonstrate E1-H, E2-M, Mix-H all had similar VTG induction indicating that the addition of estrogens (E1, E2,EE2) has an additive effect.

% Intersex In Males



Comparing estradiol equivalencies, data indicates a synergistic effect.

Field Studies - Questions

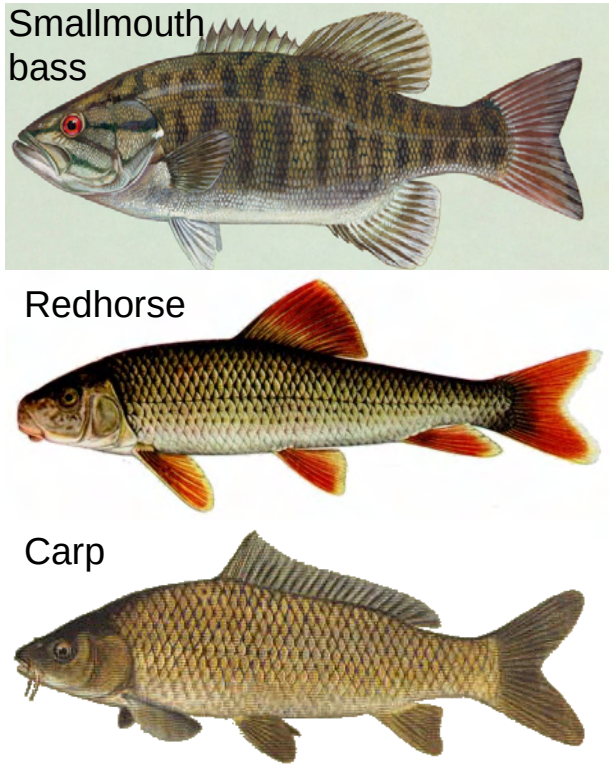
Emerging Contaminants have multi-tier effects on model organisms exposed in the laboratory.

1. Are these effects observable in wild fish?
2. Are these effects localized or wide-spread?
3. Are all fish affected equally?

2006 Mississippi River Survey

Field Studies - Mississippi River Survey

- Does repeated influx of contaminants result in cumulative increases in chemical load in the Mississippi River and concurrent increases in endocrine disruption?
- Does endocrine disruption correlate with the degree of dietary specialization?



Field Studies - Mississippi River Survey

Site	Sediment	Water
Bemidji	30	8
Grand Rapids	18	2
Brainerd	18	1
St. Cloud	18	1
Anoka	22	2
St. Paul WWTP	24	4
Hastings	21	2
Red Wing	7	1
Lake City	11	4
La Crescent	20	1

*Most commonly detected compounds:
Atrazin, Cholesterol, Alkylphenols, DEET

Field Studies - Mississippi River Survey

Mean vitellogenin concentrations

cies)

2. Location & Dilution & Sampling

Larg

Field Studies - Mississippi River Survey

Does repeated influx of contaminants result in cumulative increases in chemical load in the Mississippi River and concurrent increases in endocrine disruption?

?

- "Hotspots" rather then continuum on Upper River, continuous endocrine disruption on Lower River, noticeable effects of tributary dilution.

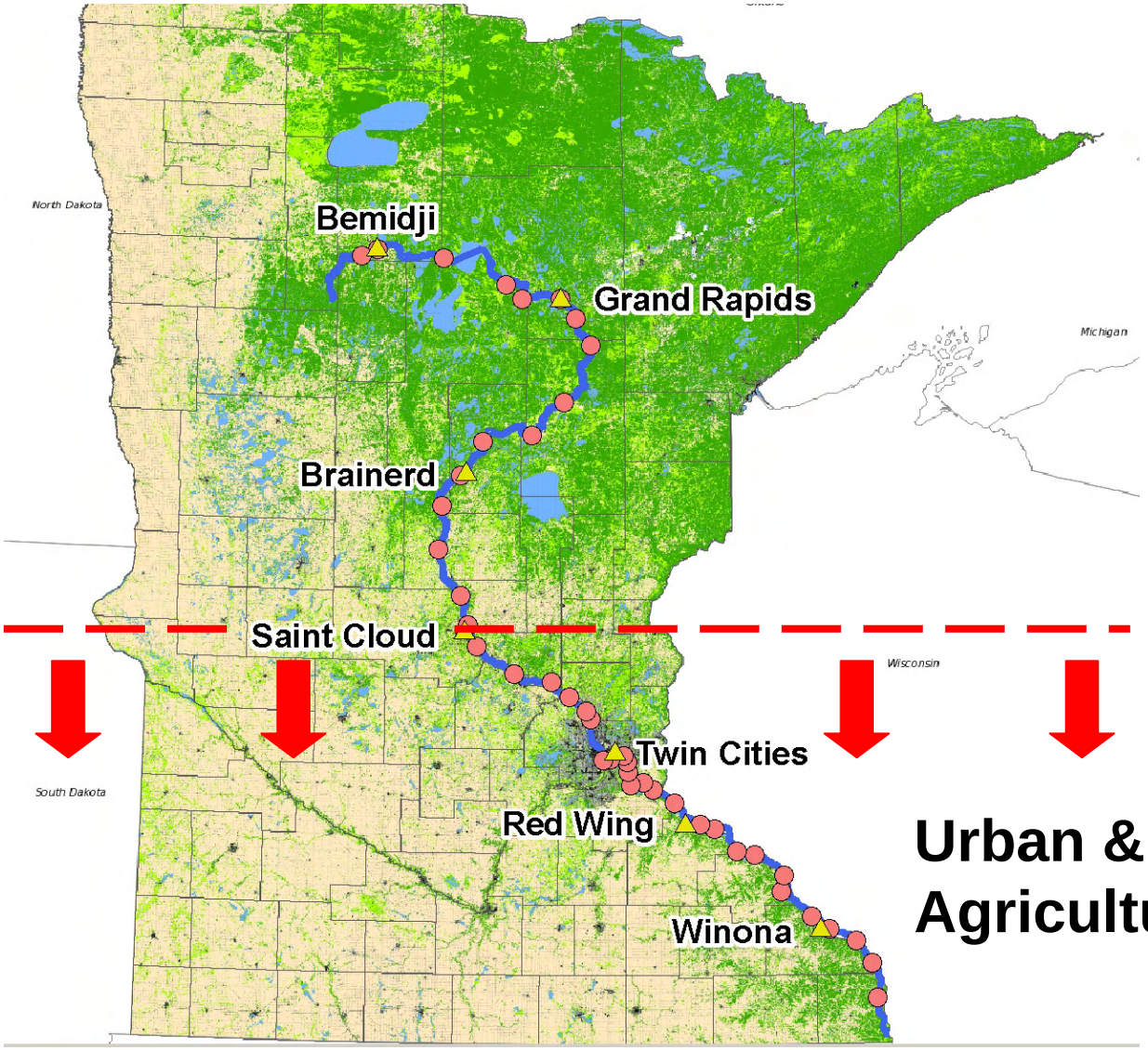
Does endocrine disruption correlate with degree of dietary specialization?

NO

- Carp and redhorse had less induction of vitellogenin despite being close to substrate with higher contaminant load.

Field Studies - Mississippi River Survey

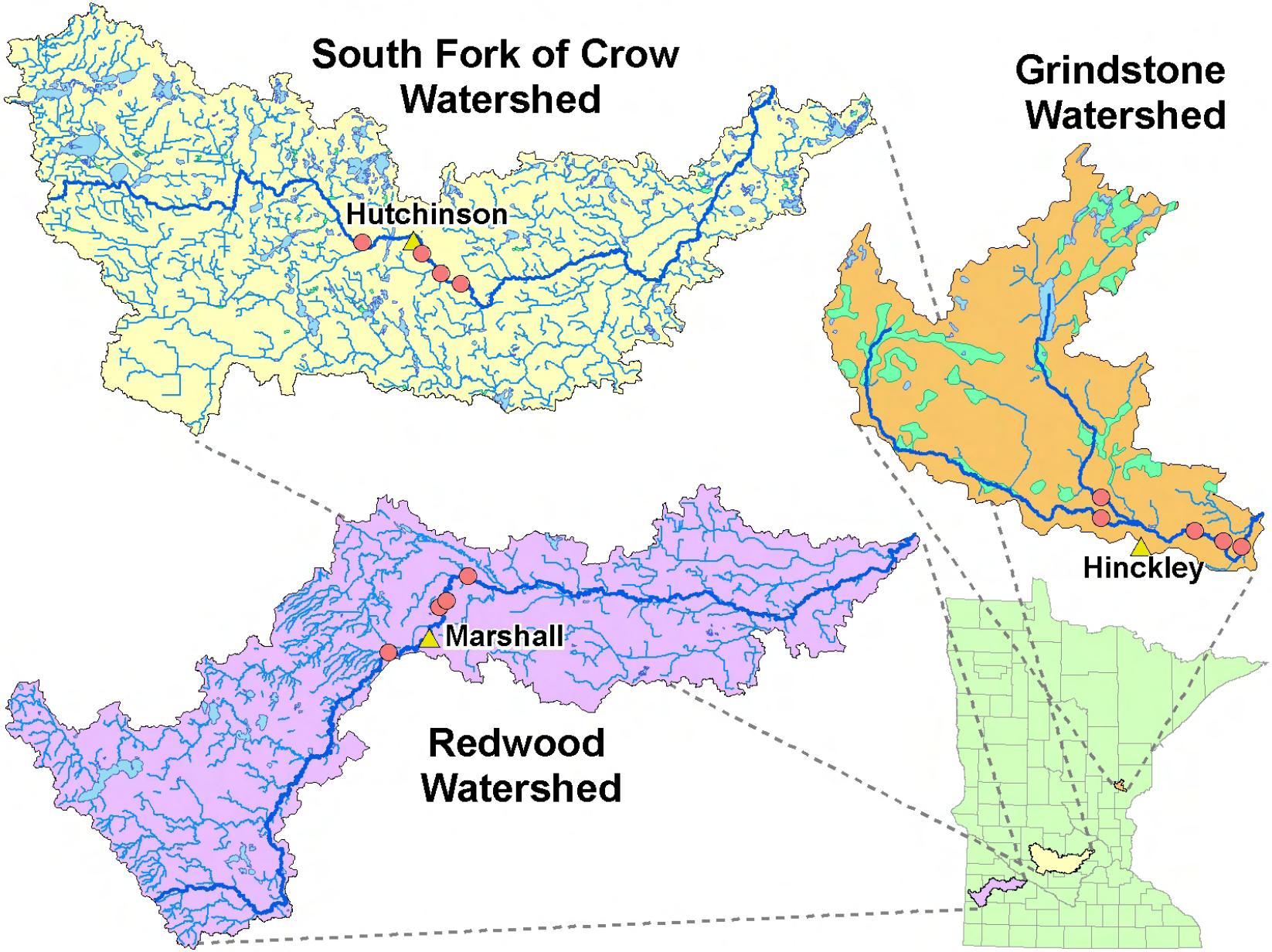
- **Land use appears to be an important factor in buffering the effects of EDCs!**



Field Studies - Mississippi River Survey

- **2007 Tributary Study**

Field Studies - Tributary study

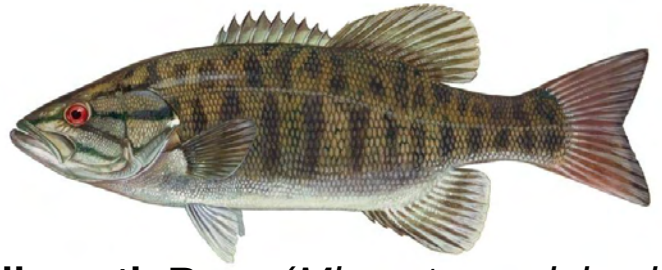


Field Studies - Tributary study

Common shiner (*Luxilus cornutus*)

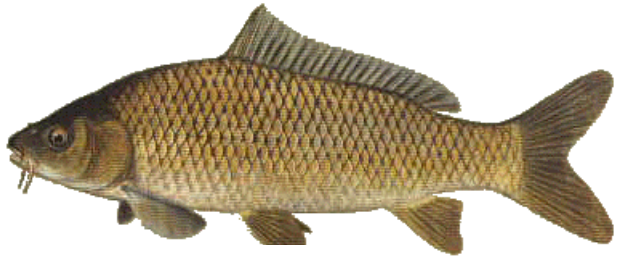
Fathead Minnow (*Pimephales promelas*)

Creek Chub (*Semotilus atromaculatus*)

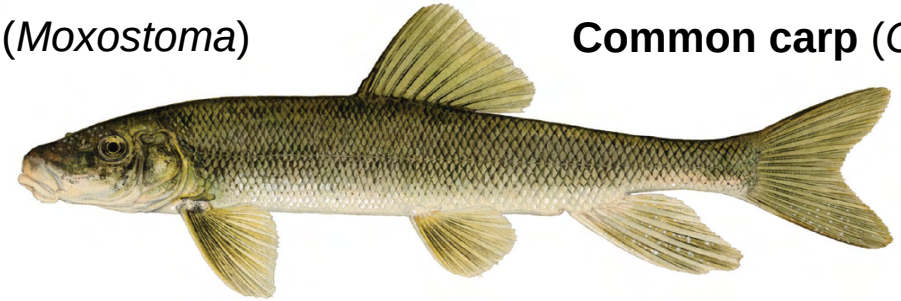


Smallmouth Bass (*Micropterus dolomieu*)

Redhorse (*Moxostoma*)

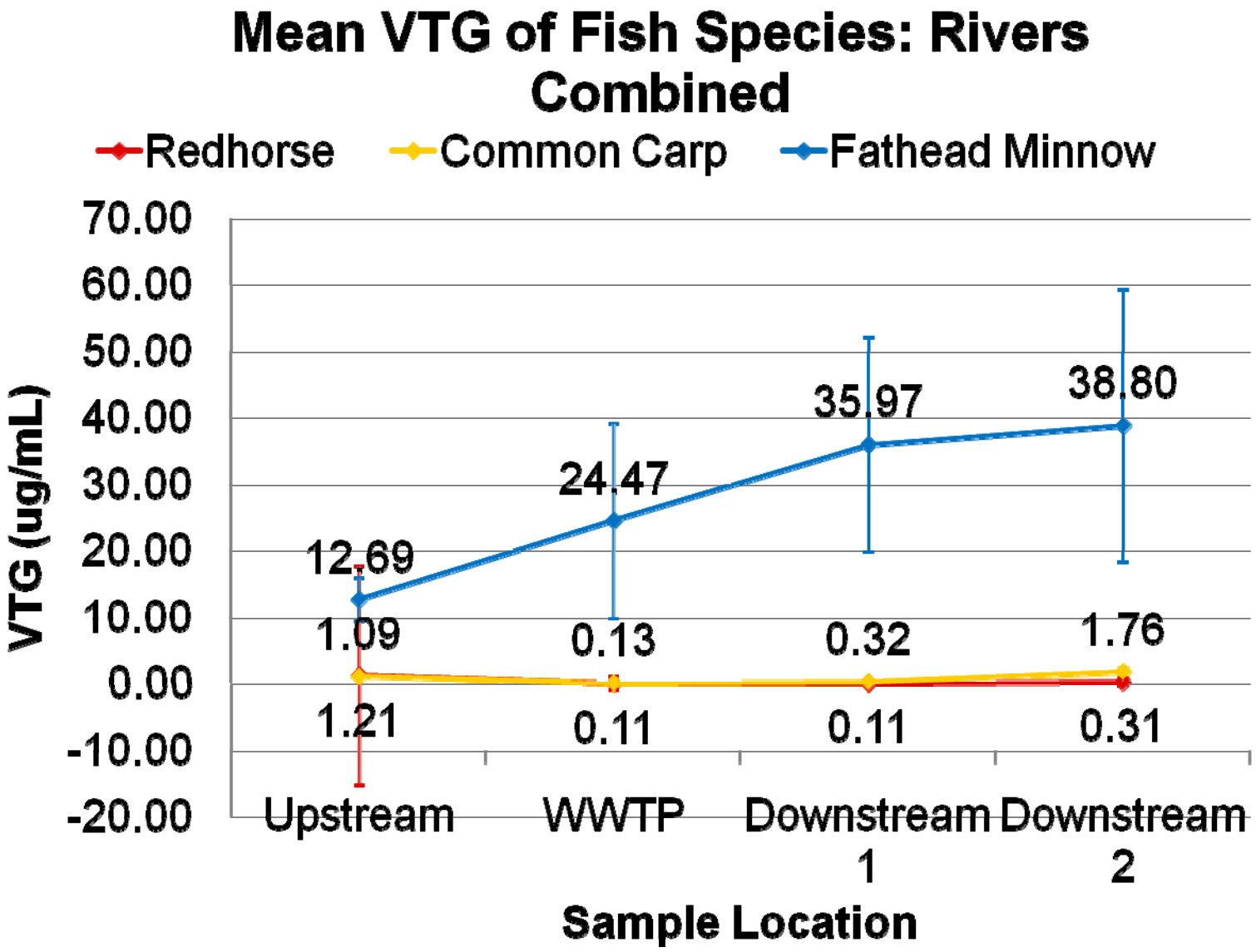


Common carp (*Cyprinus carpio*)



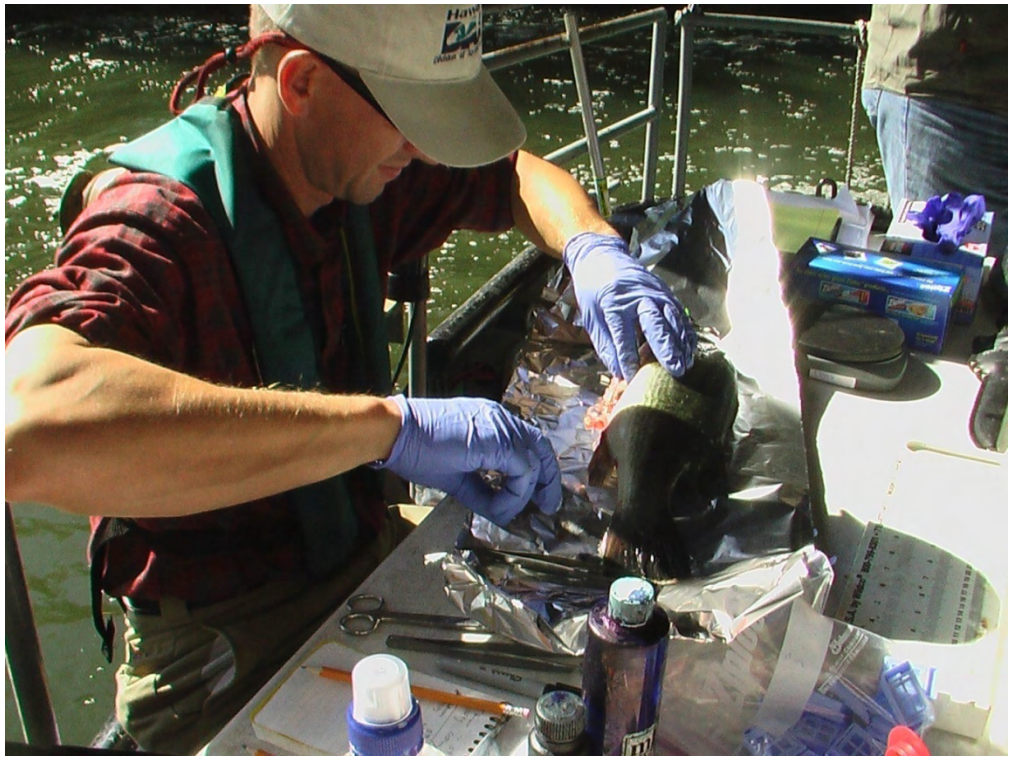
White Sucker (*Catostomus commersonii)*

Field Studies - Tributary study



- Fish response varies dramatically by species.

Relevance to MWRDGC



- Endocrine Disruption is a wide spread problem in the developed world.
- Any assessment of adverse effects requires a broad understanding of the biology of the aquatic ecosystem and of the chemistry of the EDCs.
- The “Tragedy of the Commons” scenario truly applies to EDCs, therefore education is key.

Thank You !