A Comprehensive Survey of Endocrine Active Compounds and Fish Effects in the Chicago Area Waterway System

A presentation at the

Metropolitan Water Reclamation District
Of Greater Chicago

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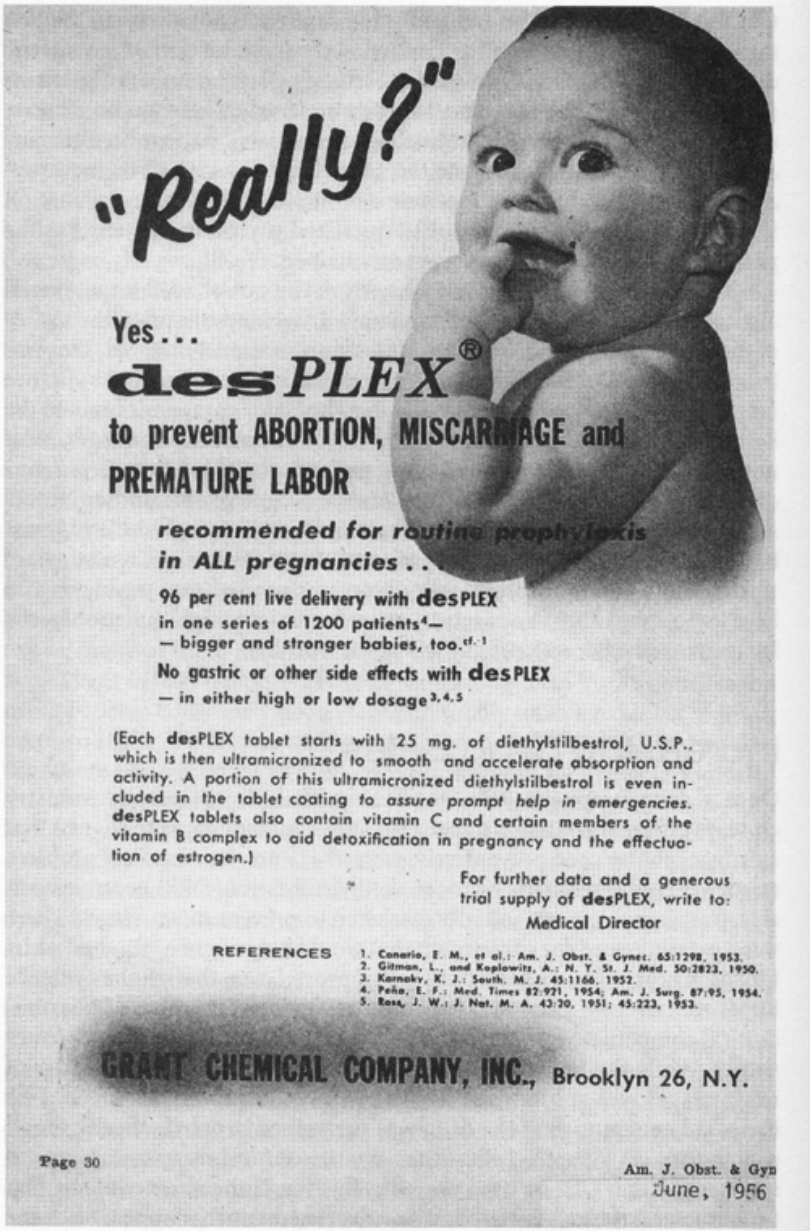


Figure 6-1 An ad promoting diethylstilbestrol addressed to obstetricians.

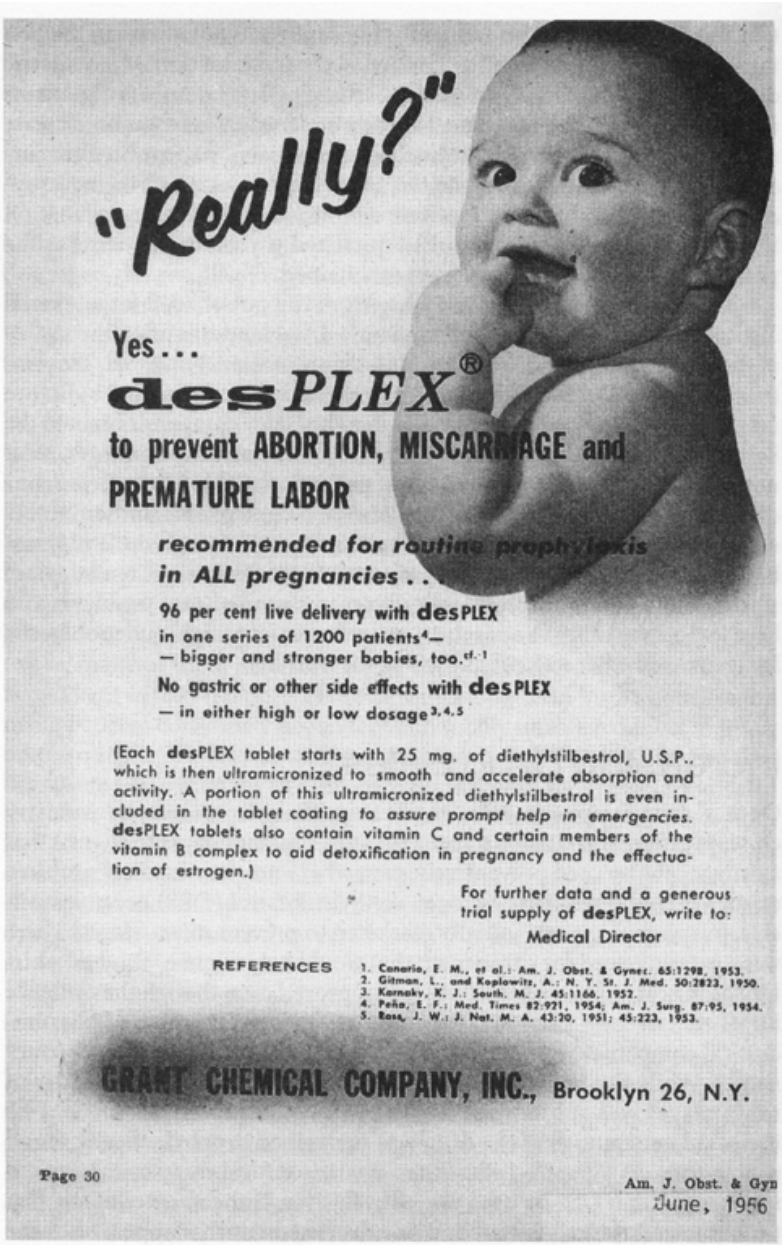


Figure 6-1 An ad promoting diethylstilbestrol addressed to obstetricians.

Diethylstilbestrol (DES)

- **1940-1970 prescribed to prevent miscarriage.**
- **1953 found to be ineffective.**
- **Continued prescribed during pregnancies until 1971.**

Figure 6-1 An ad promoting diethylstilbestrol addressed to obstetricians.

DES – "Women"

- Increased risk of breast cancer.

DES – Daughters

- Increased risk of a rare cancer.

DES – Sons

- Possible association with gender dysphoria and genital abnormalities.

DES – Third Generation

- Higher incident rate of irregular menstrual periods

→ **prescribed as <u>estrogen</u>-replacement until 1997.**

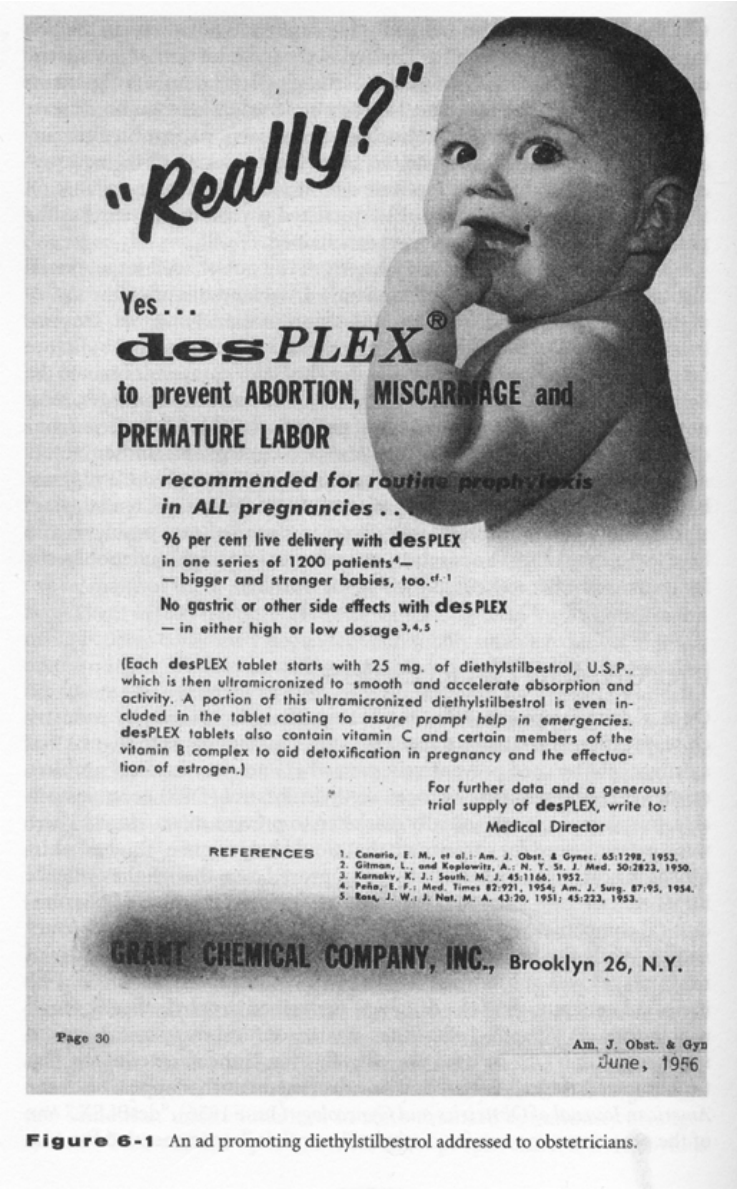
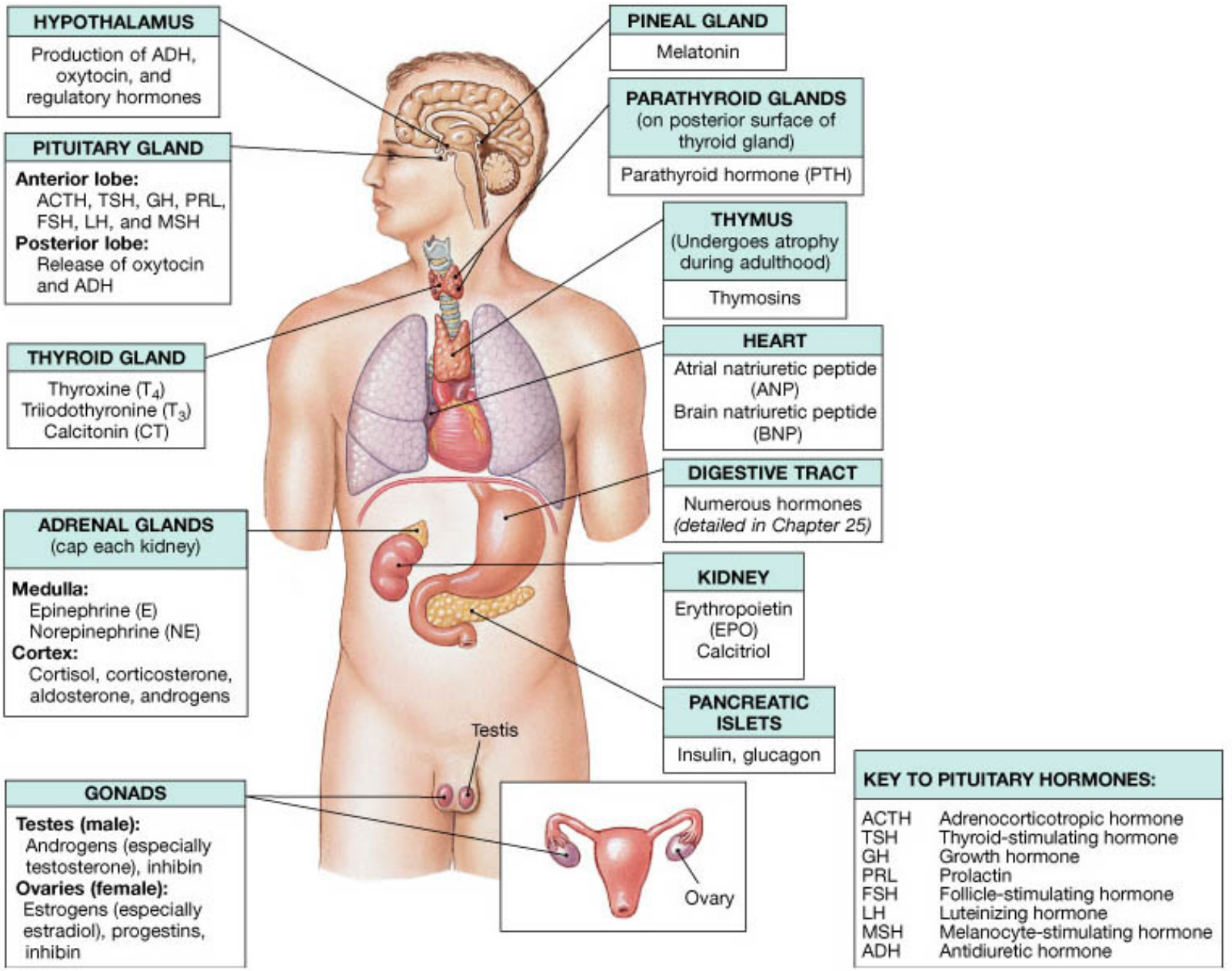


Figure 6-1 An ad promoting diethylstilbestrol addressed to obstetricians.

Endocrine Active Compounds (EACs)

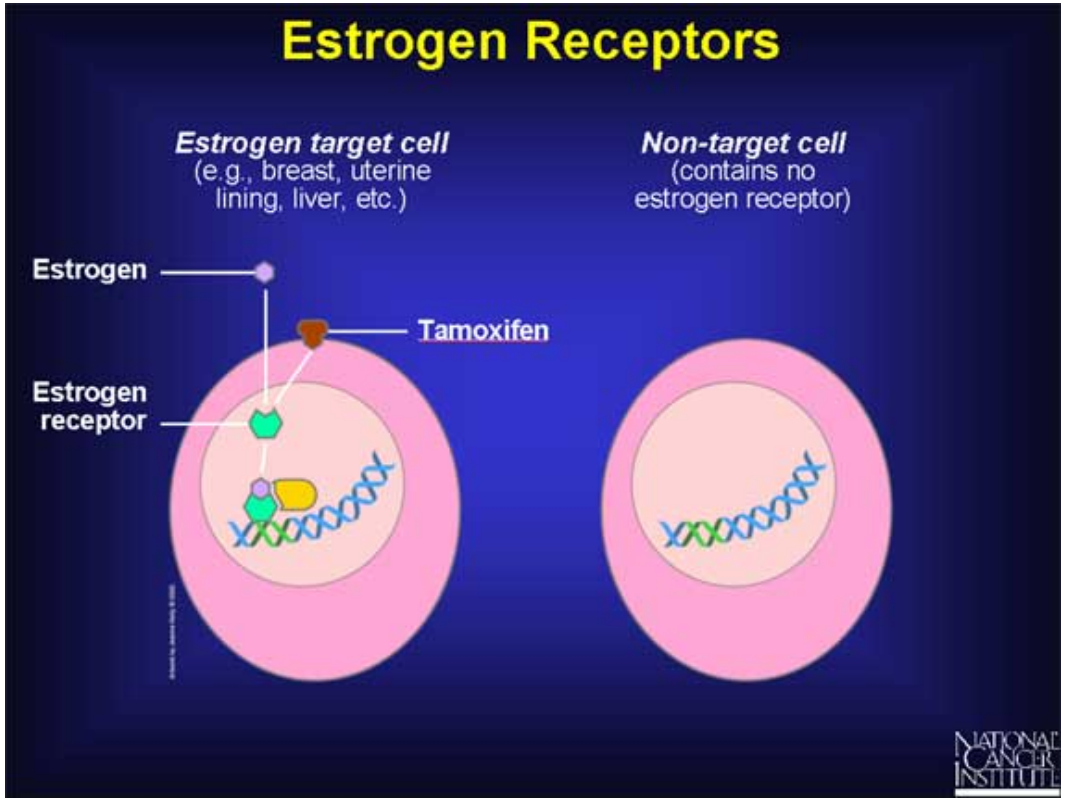
An exogenous chemical that causes adverse health effects in an organism, or its progeny, consequent to changes in the endocrine function.

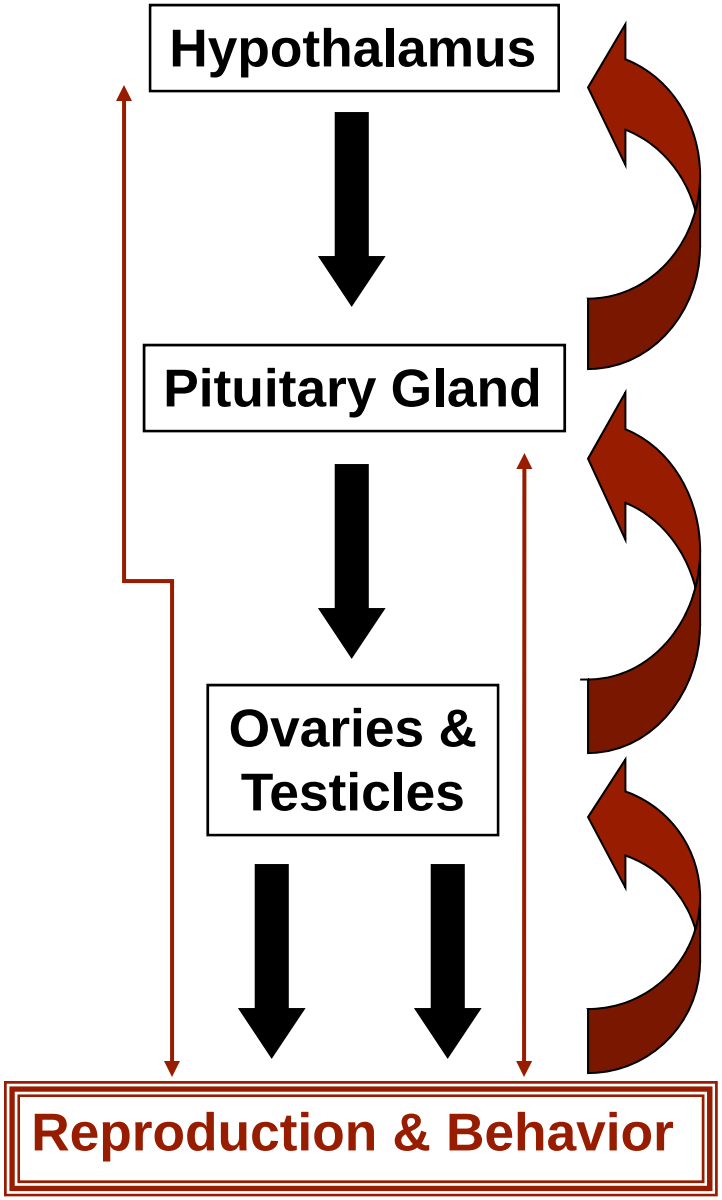
Complexity of the Endocrine System



Endocrine Active Compounds (EACs)

- *Little change in the past 300+ million years.*
- *Hormones in fish and humans are remarkably similar.*





Endocrine Active Compounds (EACs)

This USGS Survey Found:

- **22** Antibiotics
- **14** Prescription Drugs
- **5** Nonprescription Drugs
- **15** Hormones and Steroids
- **39** Household and Industrial

•Kolpin, Furlong, Meyer, Thurman, Zaugg, Barber, and Buxton, 2002, Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. Streams, 1999-2000: A national Reconnaissance: ES&T, v. 36, p. 1202.

Drugs in the drinking water

Tests have detected minute concentrations of pharmaceuticals in the drinking water supplies of at least 46 million people in two dozen major American metropolitan areas, an Associated Press investigation has found. The federal government does not regulate prescription drugs in water.

ST. CLOUD Times

Partly sunny not as cold
High 36 | Low 21
Complete forecast, 12A

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MEDICINES FOUND IN U.S. WATER SUPPLIES

STILL OPTIMISTIC
HUSKIES LOOK AHEAD AFTER DISAPPOINTING FINISHES SPORTS

More health news on msnbc.com

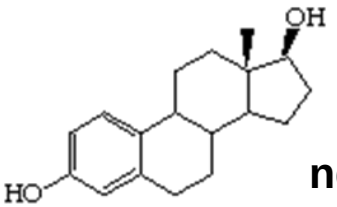
Pharmaceuticals lurking in U.S. drinking water

AP probe found traces of meds in water supplies of 41 million Americans

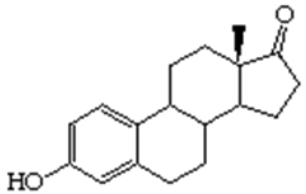
Endocrine Active Compounds (EACs)

➢ A snapshot from <u>effluents</u> flowing into the Mississippi River:

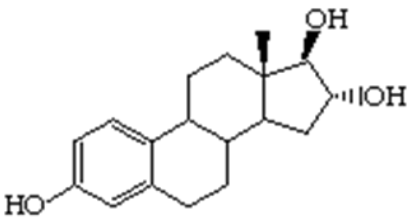
Natural Estrogens:



17 β-Estradiol **nd - 7.3 ng/L**



Estrone **nd - 50 ng/L**



Estriol **nd - 40 ng/L**

Synthetic Endocrine Active Compounds:

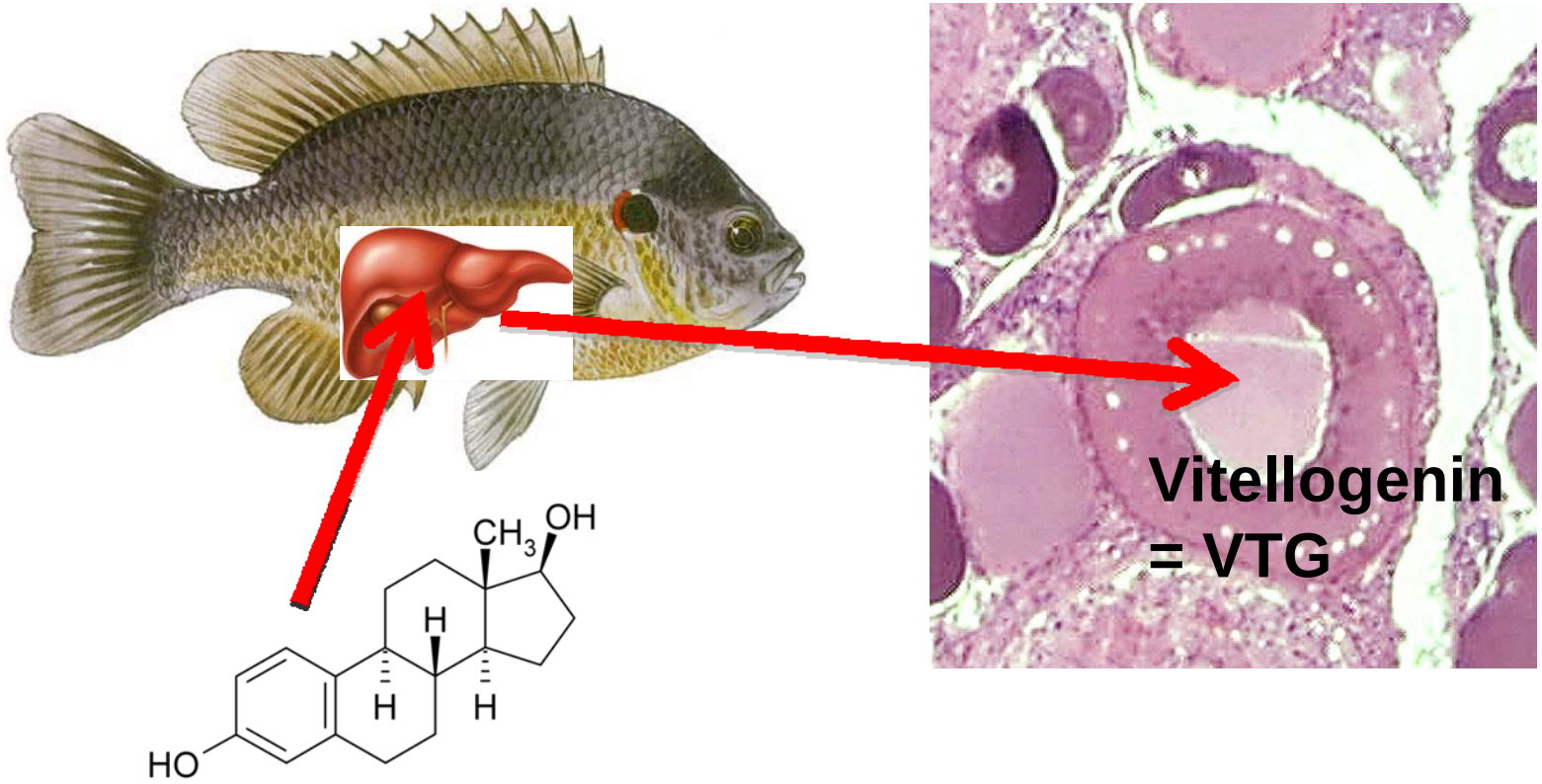
- **Ethynylestradiol** (birth control):
  - up to 3 ng/L
- **Nonylphenol** (detergent):
  - up to 13,000 ng/L
- Total alkylphenols: > 50,000 ng/L
- Combined **Mood-altering drugs**:
  - ~ 10,000 ng/L
- **Triclosan** (anti-microbial): up to 318 ng/L
- **Bisphenol A** (BPA): ~100-1,000 ng/L

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

Endocrine Active Compounds (EACs)

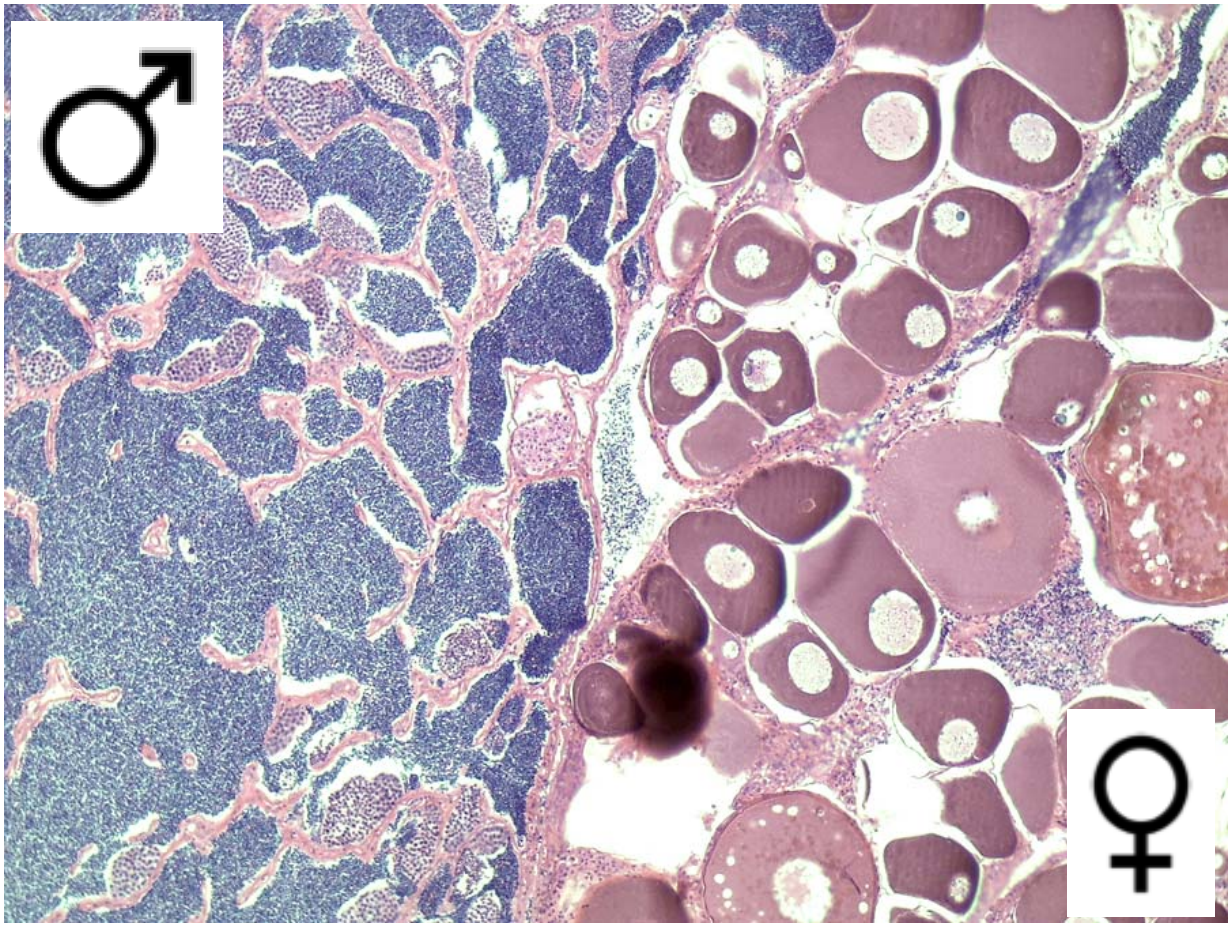
Common Endpoints

1. **Vitellogenin** – an egg yolk protein usually found in female fish, but also produced in males when exposed to estrogens.

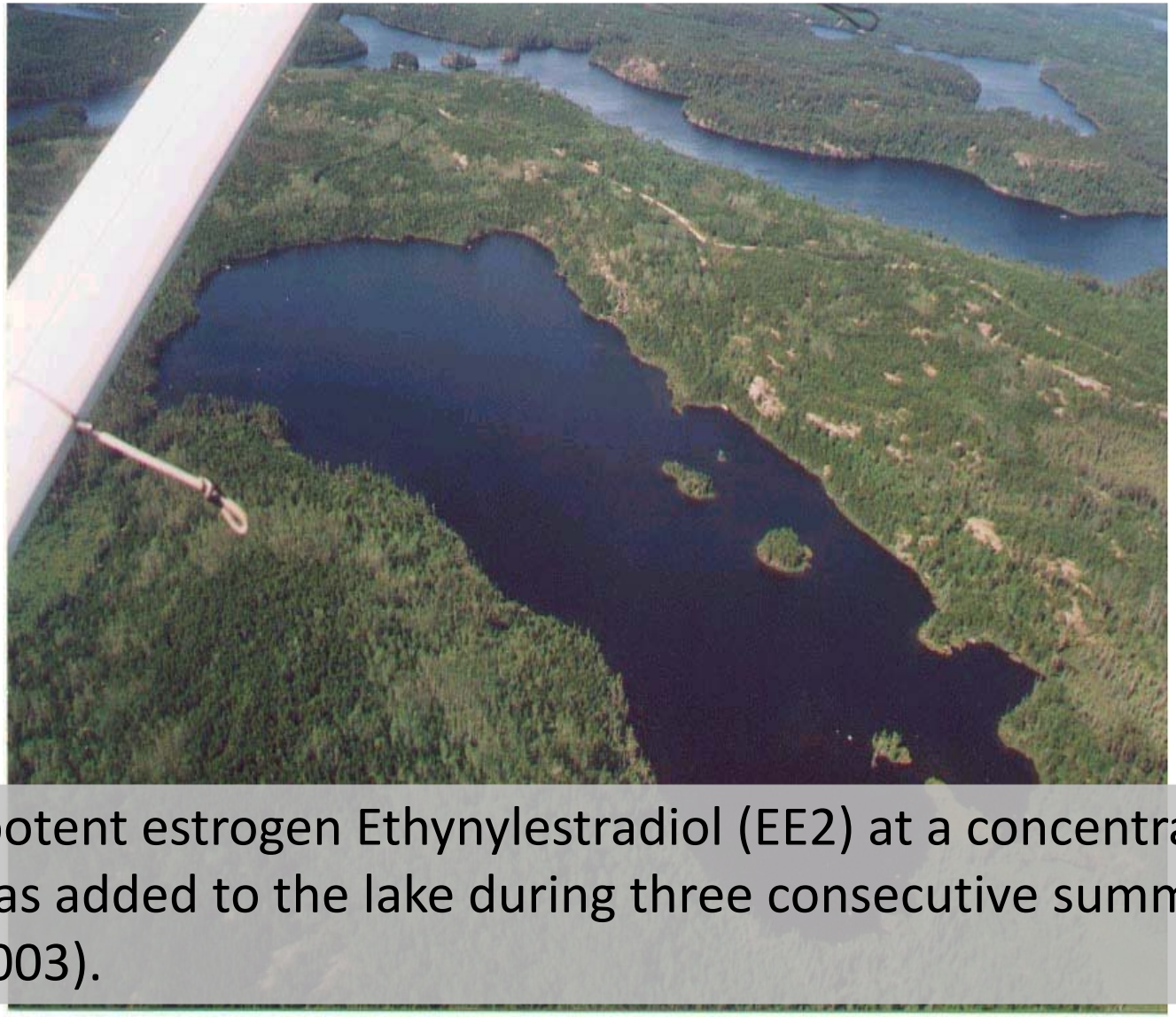


Endocrine Active Compounds (EACs) – Common Endpoints

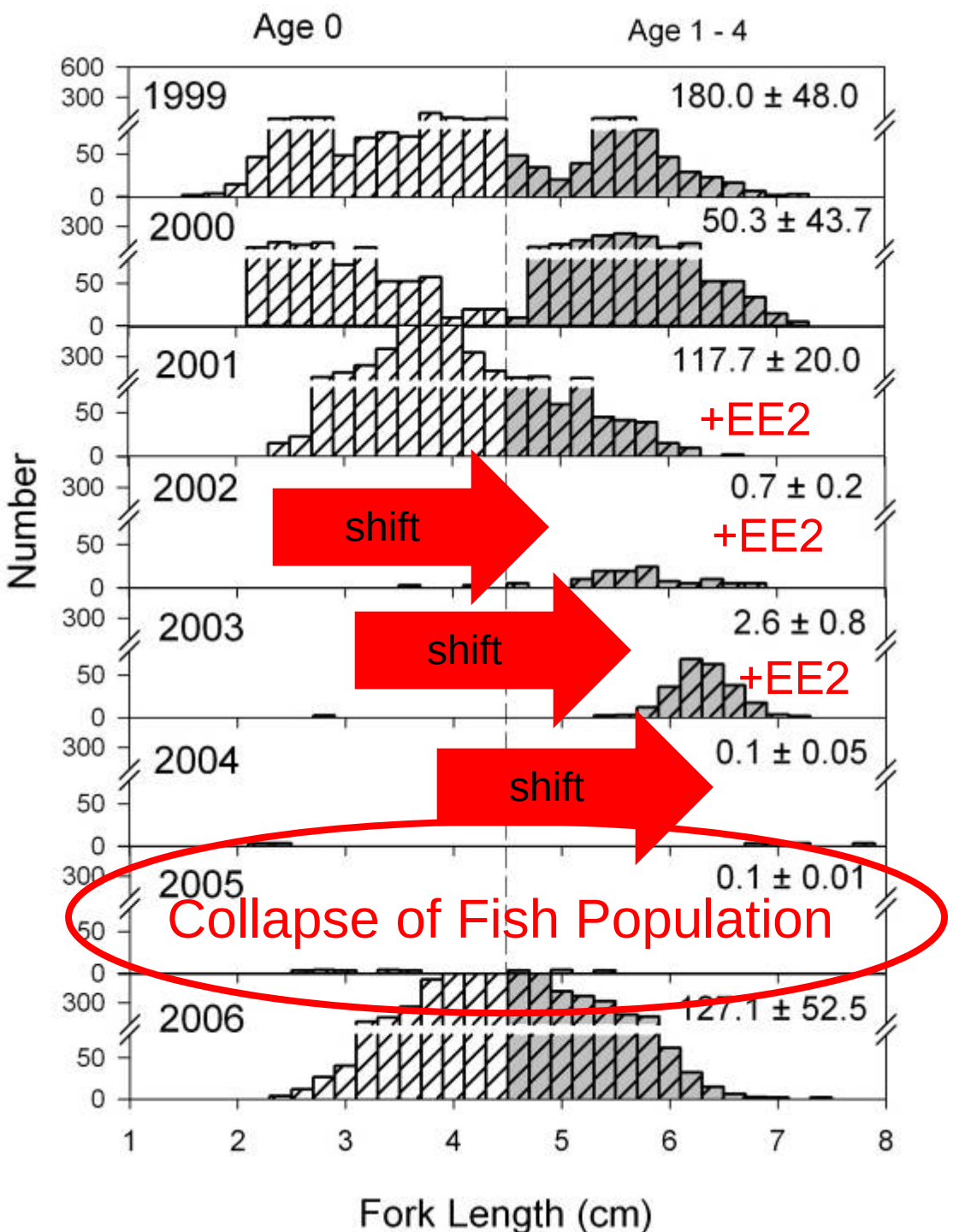
2. Histology of the reproductive tissues



Documented Environmental Effects - Experimental Lakes Area – Ontario, Canada

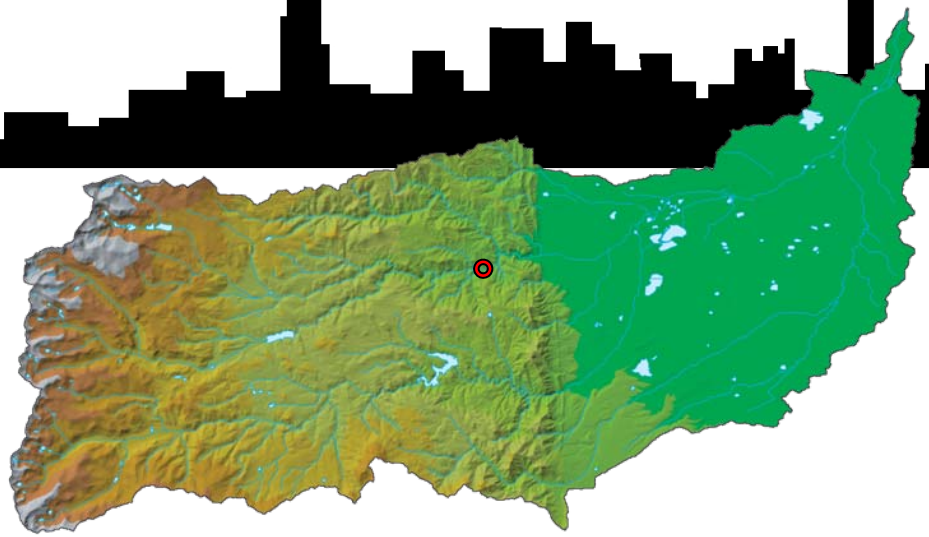


→ The potent estrogen Ethynylestradiol (EE2) at a concentration of 5ng/L was added to the lake during three consecutive summers (2001-2003).



Kidd et al., 2007. PNAS

Documented Environmental Effects



Boulder Creek, CO

(Vajda et al. 2008)

Total Estrogenicity: 11 to 31 ng/L E2

Vitellogenin in Males: 500x control conc.

Fish Sex Ratio: < 25% males

Intersex Frequency: 18 – 22% of males

Documented Environmental Effects

North Shore Channel, IL

(Barber et al. In review)

Total Estrogenicity: 9 to 19 ng/L E2

Vitellogenin in Males: 1,000x control conc.

Fish Sex Ratio: ~ 50% males

Intersex Frequency: 0% of males

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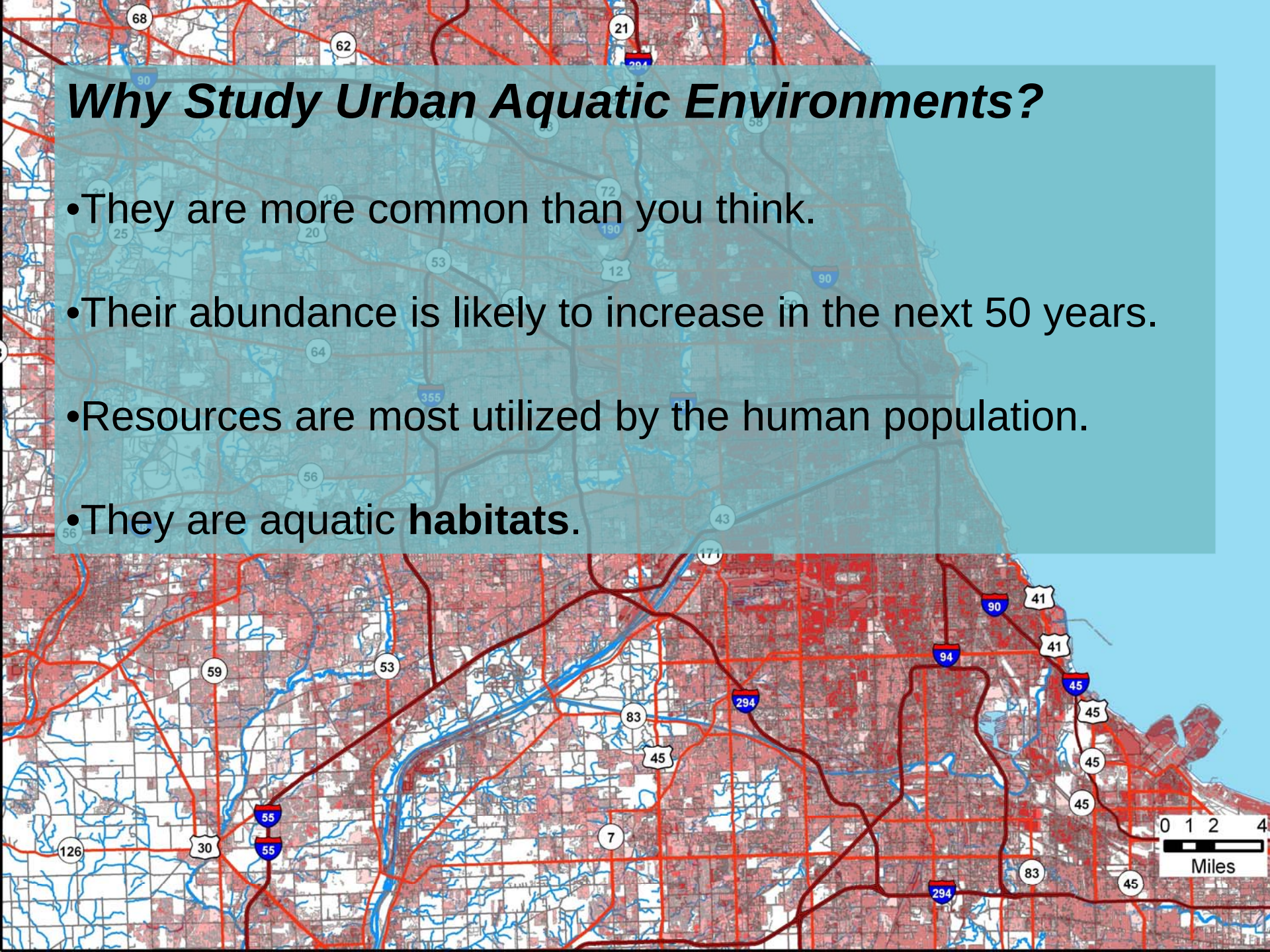
Intersex Frequency: 18 – 22% of males

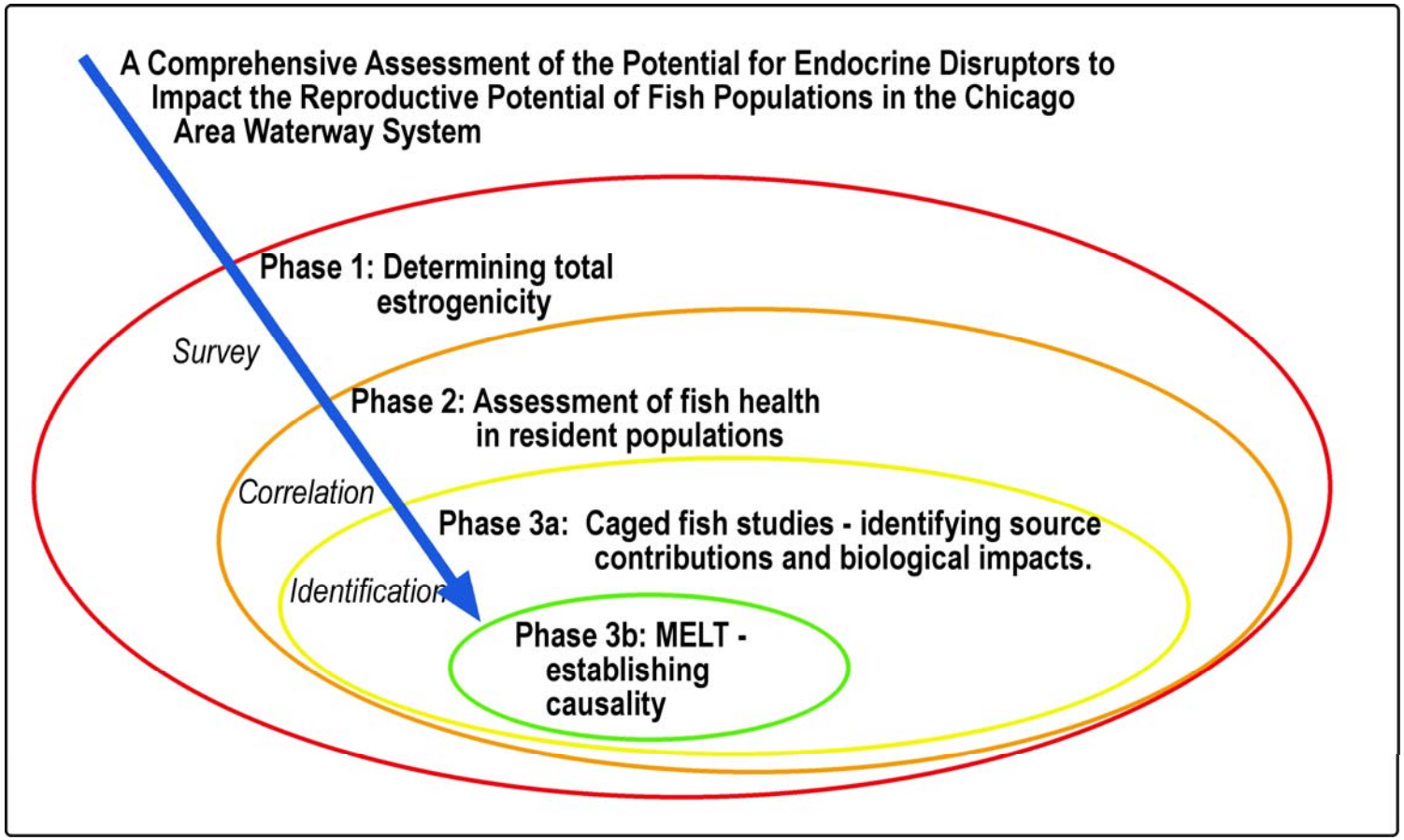
Why Study Urban Aquatic Environments?

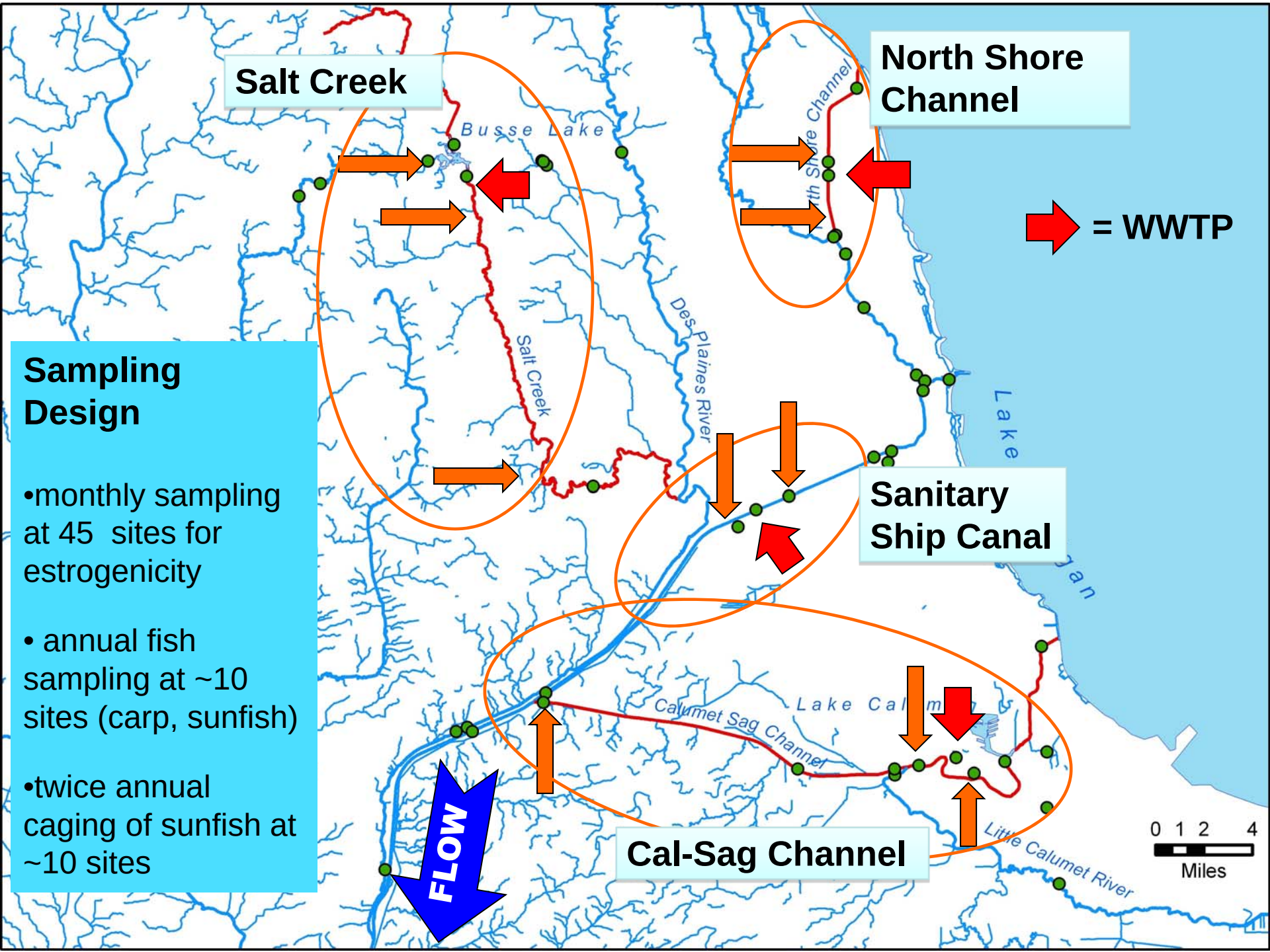
- They are more common than you think.
- Their abundance is likely to increase in the next 50 years.
- Resources are most utilized by the human population.
- They are aquatic **habitats**.



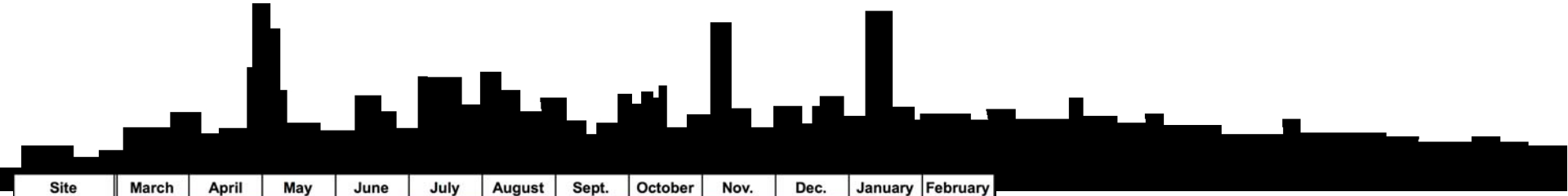

0 1 2 4
Miles

Study Overview




Salt Creek
North Shore Channel
= WWTP
Sampling Design
•monthly sampling at 45 sites for estrogenicity
• annual fish sampling at ~10 sites (carp, sunfish)
•twice annual caging of sunfish at ~10 sites
Sanitary Ship Canal
Cal-Sag Channel
FLOW
Busse Lake
Salt Creek
Des Plaines River
Calumet Sag Channel
Little Calumet River
0 1 2 4
Miles

Water Estrogenicity Sampling
24 Consecutive Months



Site	March	April	May	June	July	August	Sept.	October	Nov.	Dec.	January	February
RP 1												
RP 2												
RP 3												
RP 4												
RP 5												
RP 6												
RP 7												
WW 18												
WW 29												
WW 35												
WW 36												
WW 37												
WW 39												
WW 40												
WW 41												
WW 42												
WW 43												
WW 46												
WW 48												
WW 49												
WW 50												
WW 52												
WW 55												
WW 56												
WW 57												
WW 58												
WW 59												
WW 73												
WW 74												
WW 75												
WW 76												
WW 77												
WW 78												
WW 79												
WW 80												
WW 86												
WW 89												
WW 92												
WW 96												
WW 99												
WW 100												
WW 101												
WW 102												
WW 108												
WW 110												

sample collected & curated | sample not collected | estrogenicity analysis

Water Sampling

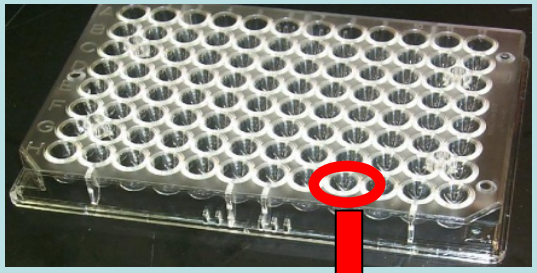
Collected water samples from 38 waterway locations and 7 Treatment Plants monthly.

Solid-phase extracted, eluded and curated > 1,000 samples

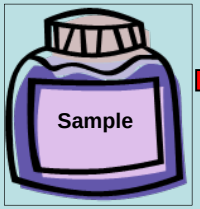
Analyzed quarterly samples for estrogenicity.

In Vitro Endpoint – Estrogenicity

Plate cells



Dose cells



Add Luciferin

Read luminescence

Estrogenicity Gradient
not measured
0
1
2
3
4
5
6

- 0, 1: Only background estrogenicity (natural sources)
- 2, 3: Elevated estrogenic activity (expected with human activity)
- 4, 5, 6: High estrogenic activity (likely to result in physiological response in fish)

Estrogenicity Gradient
not measured
0
1
2
3
4
5
6

Site ID	April 2009	July 2009	October 2009	May 2010	June 2010	September 2010
18	1	0	0	0	not measured	0
24	not measured	not measured	not measured	0	not measured	0
29	2	0	1	0	0	0
35	3	3	3	5	0	1
36	2	0	2	not measured	0	3
37	2	4	1	6	0	2
39	0	2	not measured	3	0	2
40	2	3	not measured	0	0	2
41	0	3	not measured	3	2	not measured
42	not measured	5	not measured	5	4	2
43	3	2	2	3	0	2
46	2	2	0	4	0	3
48	not measured	3	not measured	4	2	0
49	not measured	0	1	2	0	1
50	2	2	0	0	not measured	1
52	2	3	2	0	0	2
55	0	2	2	0	0	0
56	0	not measured	2	2	0	2
57	1	0	2	not measured	0	2
58	2	2	0	2	3	1
59	2	3	2	2	0	not measured
73	2	5	1	6	2	3
74	2	0	not measured	2	0	2
75	1	5	not measured	0	3	2
76	3	6	2	3	0	3
77	0	not measured	1	0	not measured	not measured
78	2	5	2	3	not measured	3
79	5	0	2	0	0	0
80	2	2	1	0	0	0
86	1	3	not measured	not measured	0	3
89	2	0	2	4	0	0
92	2	0	not measured	0	2	3
96	2	2	0	0	0	3
99	0	6	not measured	4	not measured	1
100	0	0	not measured	2	0	2
101	2	0	2	6	3	2
102	2	2	2	6	0	2
108	1	3	not measured	3	2	2
110	2	0	0	2	0	0
rp1	2	0	1	2	0	2
rp2	2	3	0	3	0	0
rp3	3	6	3	4	0	3
rp4	0	0	0	3	0	4
rp5	2	4	not measured	4	0	3
rp6	3	3	2	4	2	4
rp7	2	2	1	3	0	2

Estrogenicity Gradient
not measured
0
1
2
3
4
5
6

Site ID	April 2009	July 2009	October 2009	May 2010	June 2010	September 2010
18	1	0	0	0	not measured	0
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35	3	3	3	5	0	1
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39	0	2	not measured	3	0	2
40	2	3	not measured	0	0	2
41	0	3	not measured	3	2	not measured
42	not measured	5	not measured	5	4	2
43	3	2	2	3	0	2
46	2	2	0	4	0	3
48	not measured	3	not measured	4	2	0
49	not measured	0	1	2	0	1
50	2	2	0	0	not measured	1
52	2	3	2	0	0	2
55	0	2	2	0	0	0
56	0	not measured	2	2	0	2
57	1	0	2	not measured	0	2
58	2	2	0	2	3	1
59	2	3	2	2	0	not measured
73	2	5	1	6	2	3
74	2	0	not measured	2	0	2
75	1	5	not measured	0	3	2
76	3	6	2	3	0	3
77	0	not measured	1	0	not measured	not measured
78	2	5	2	3	not measured	3
79	5	0	2	0	0	0
80	2	2	1	0	0	0
86	1	3	not measured	not measured	0	3
89	2	0	2	4	0	0
92	2	0	not measured	0	2	3
96	2	2	0	0	0	3
99	0	6	not measured	4	not measured	1
100	0	0	not measured	2	0	2
101	2	0	2	6	3	2
102	2	2	2	6	0	2
108	1	3	not measured	3	2	2
110	2	0	0	2	0	0
rp1	2	0	1	2	0	2
rp2	2	3	0	3	0	0
rp3	3	6	3	4	0	3
rp4	0	0	0	3	0	4
rp5	2	4	not measured	4	0	3
rp6	3	3	2	4	2	4
rp7	2	2	1	3	0	2

Estrogenicity Gradient
not measured
0
1
2
3
4
5
6

Site ID	April 2009	July 2009	October 2009	May 2010	June 2010	September 2010
18	1	0	0	0	not measured	0
24	not measured	not measured	not measured	0	not measured	0
29	2	0	1	0	0	0
35	3	3	3	5	0	1
36	2	0	2	not measured	0	3
37	2	4	1	6	0	2
39	0	2	not measured	3	0	2
40	2	3	not measured	0	0	2
41	0	3	not measured	3	2	not measured
42	not measured	5	not measured	5	4	2
43	3	2	2	3	0	2
46	2	2	0	4	0	3
48	not measured	3	not measured	4	2	0
49	not measured	0	1	2	0	1
50	2	2	0	0	not measured	1
52	2	3	2	0	0	2
55	0	2	2	0	0	0
56	0	not measured	2	2	0	2
57	1	0	2	not measured	0	2
58	2	2	0	2	3	1
59	2	3	2	2	0	not measured
73	2	5	1	6	2	3
74	2	0	not measured	2	0	2
75	1	5	not measured	0	3	2
76	3	6	2	3	0	3
77	0	not measured	1	0	not measured	not measured
78	2	5	2	3	not measured	3
79	5	0	2	0	0	0
80	2	2	1	0	0	0
86	1	3	not measured	not measured	0	3
89	2	0	2	4	0	0
92	2	0	not measured	0	2	3
96	2	2	0	0	0	3
99	0	6	not measured	4	not measured	1
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101	2	0	2	6	3	2
102	2	2	2	6	0	2
108	1	3	not measured	3	2	2
110	2	0	0	2	0	0
rp1	2	0	1	2	0	2
rp2	2	3	0	3	0	0
rp3	3	6	3	4	0	3
rp4	0	0	0	3	0	4
rp5	2	4	not measured	4	0	3
rp6	3	3	2	4	2	4
rp7	2	2	1	3	0	2

Mean Estrogenic Potency

Site ID	Mean ng/L	SD	CV (SD/MEAN)*100
24	0.00	0.00	
18	0.06	0.13	223.61
77	0.32	0.56	173.21
29	0.37	0.59	162.23
49	0.39	0.48	124.79
56	1.03	1.79	173.19
57	1.48	1.74	117.04
55	1.50	2.98	197.84
110	1.66	2.76	165.99
80	1.74	3.23	185.51
74	3.15	4.10	130.37
100	3.27	4.63	141.58
rp1	3.33	3.57	107.36
50	3.58	3.60	100.72
92	3.64	5.30	145.76
36	3.79	5.16	136.03
96	4.48	6.87	153.44
52	4.53	4.95	109.25
40	5.10	8.38	164.30
59	5.17	5.55	107.37
39	5.75	6.12	106.59
58	5.85	5.96	101.86
89	6.20	11.96	192.89
43	7.17	7.53	104.96
rp7	7.30	7.29	99.88
86	8.55	10.45	122.21
rp4	9.42	16.34	173.46
rp2	9.87	14.41	146.03
46	10.49	12.10	115.31
79	11.75	28.19	240.00
108	12.17	10.28	84.48
41	14.82	13.17	88.85
48	15.13	17.89	118.23
75	17.80	27.72	155.70
35	18.97	26.29	138.61
rp5	20.60	23.00	111.63
78	22.04	22.91	103.91
rp6	23.83	14.98	62.87
76	27.52	36.18	131.45
37	28.96	50.44	174.19
rp3	32.76	47.91	146.27
101	35.61	78.23	219.72
102	39.59	86.55	218.62
42	42.90	28.24	65.83
99	52.20	84.51	161.89
73	56.01	96.08	171.54

Low

High

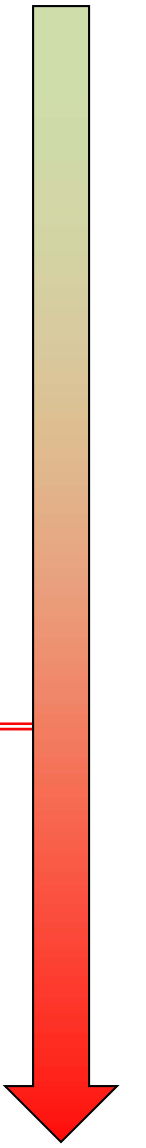
6 of 7 WWTP in upper 50% of estrogenic potency

Temporal Variation

6 of 7 WWTP in lower 2/3 of temporal variability

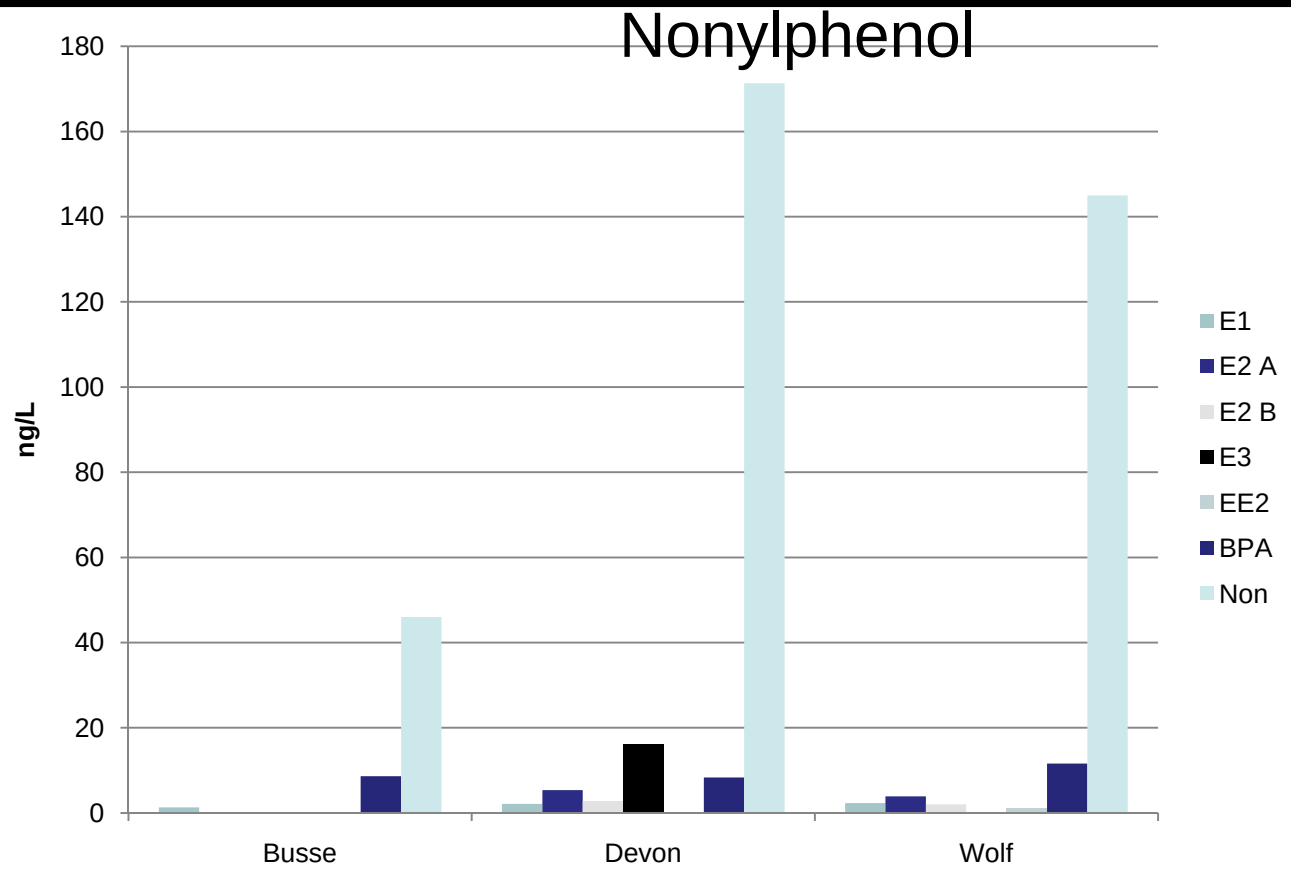
Site ID	Mean ng/L	SD	CV (SD/MEAN)*100
rp6	**23.83**	14.98	62.87
42	**42.90**	28.24	65.83
108	**12.17**	10.28	84.48
41	**14.82**	13.17	88.85
rp7	**7.30**	7.29	99.88
50	**3.58**	3.60	100.72
58	**5.85**	5.96	101.86
78	**22.04**	22.91	103.91
43	**7.17**	7.53	104.96
39	**5.75**	6.12	106.59
rp1	**3.33**	3.57	107.36
59	**5.17**	5.55	107.37
52	**4.53**	4.95	109.25
rp5	**20.60**	23.00	111.63
46	**10.49**	12.10	115.31
57	**1.48**	1.74	117.04
48	**15.13**	17.89	118.23
86	**8.55**	10.45	122.21
49	**0.39**	0.48	124.79
74	**3.15**	4.10	130.37
76	**27.52**	36.18	131.45
36	**3.79**	5.16	136.03
35	**18.97**	26.29	138.61
100	**3.27**	4.63	141.58
92	**3.64**	5.30	145.76
rp2	**9.87**	14.41	146.03
rp3	**32.76**	47.91	146.27
96	**4.48**	6.87	153.44
75	**17.80**	27.72	155.70
99	**52.20**	84.51	161.89
29	**0.37**	0.59	162.23
40	**5.10**	8.38	164.30
110	**1.66**	2.76	165.99
73	**56.01**	96.08	171.54
56	**1.03**	1.79	173.19
77	**0.32**	0.56	173.21
rp4	**9.42**	16.34	173.46
37	**28.96**	50.44	174.19
80	**1.74**	3.23	185.51
89	**6.20**	11.96	192.89
55	**1.50**	2.98	197.84
102	**39.59**	86.55	218.62
101	**35.61**	78.23	219.72
18	**0.06**	0.13	223.61
79	**11.75**	28.19	240.00
24	**0.00**	0.00	

Low

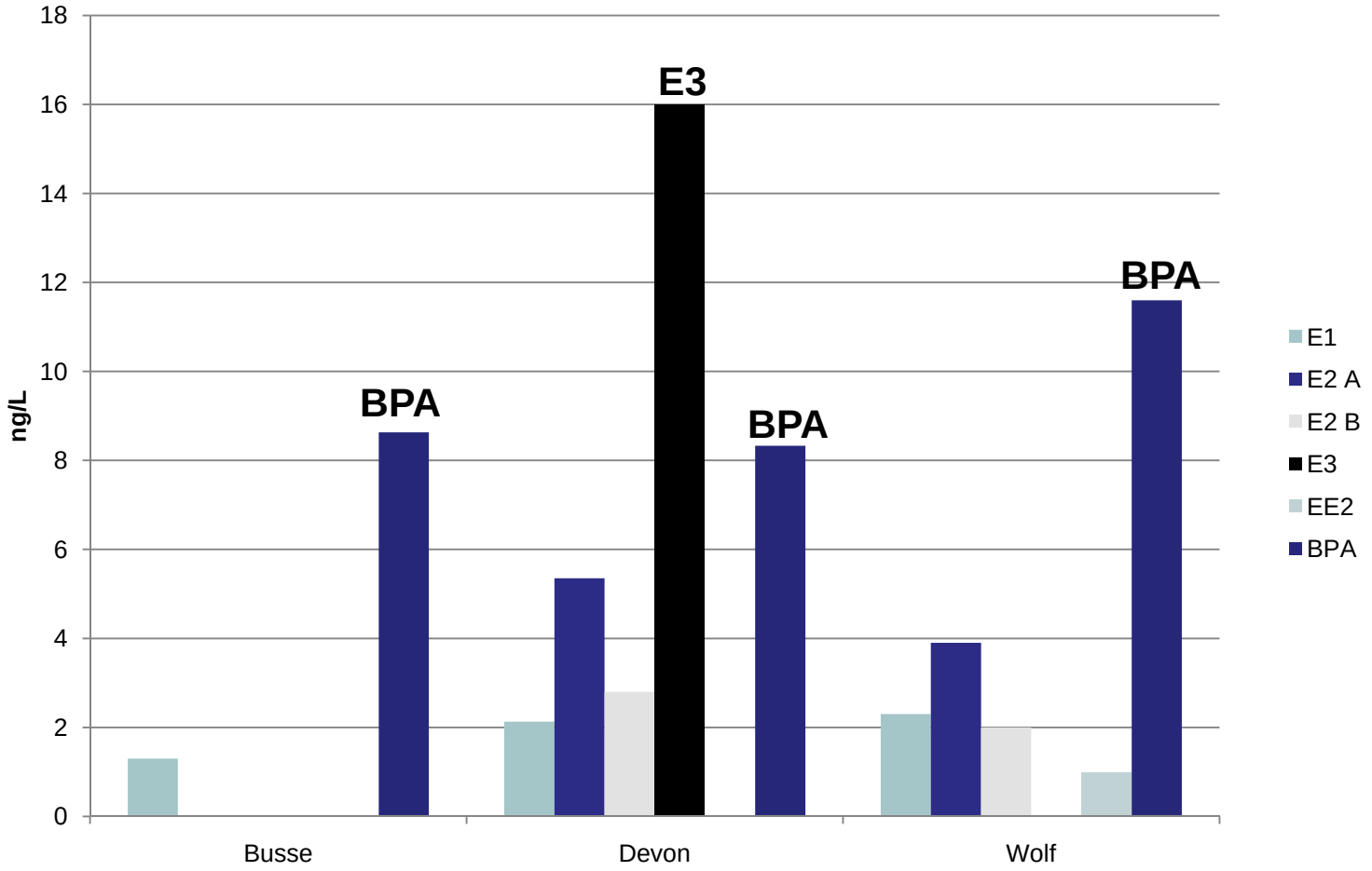


High

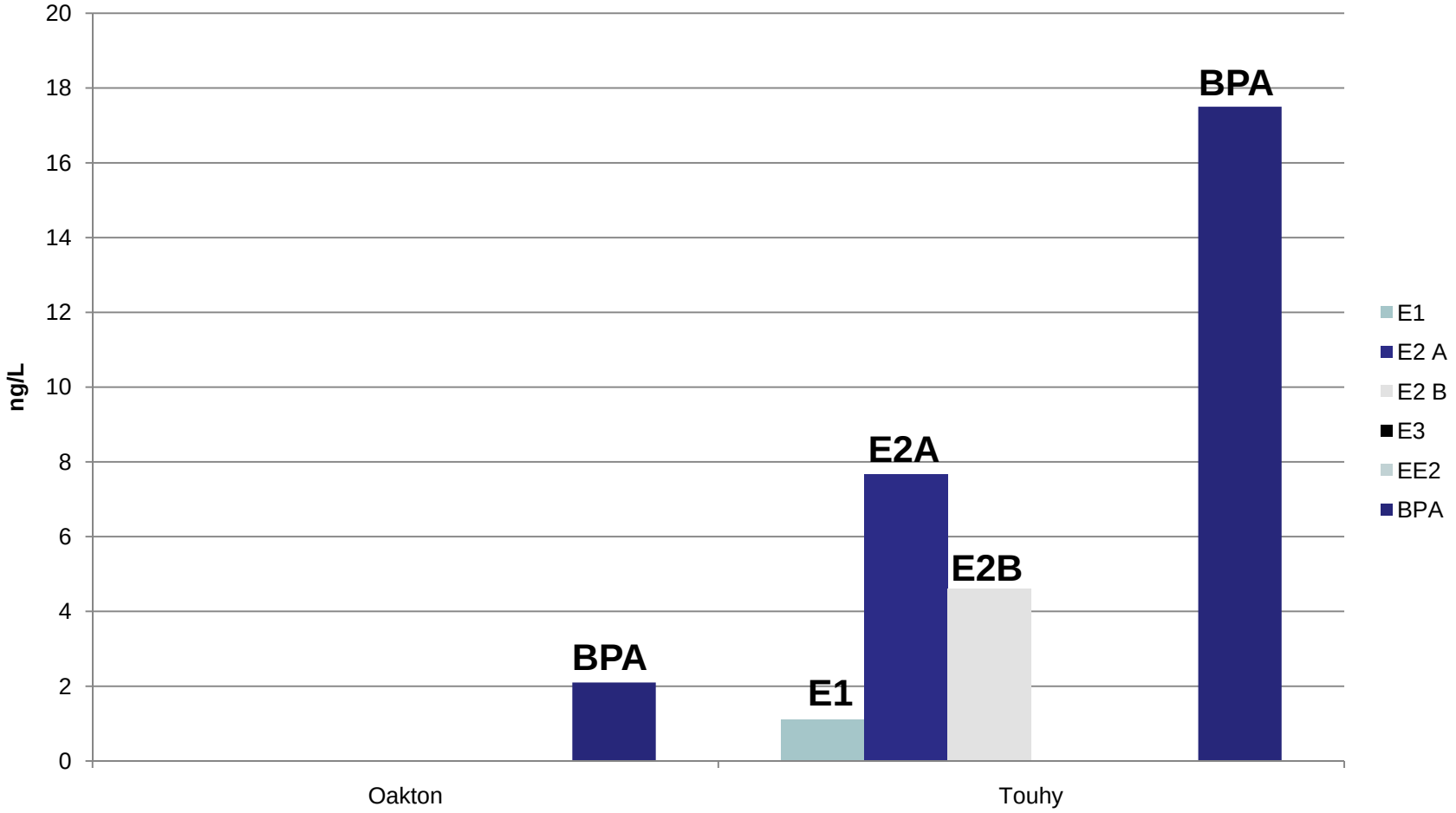
Water Chemistry – Salt Creek



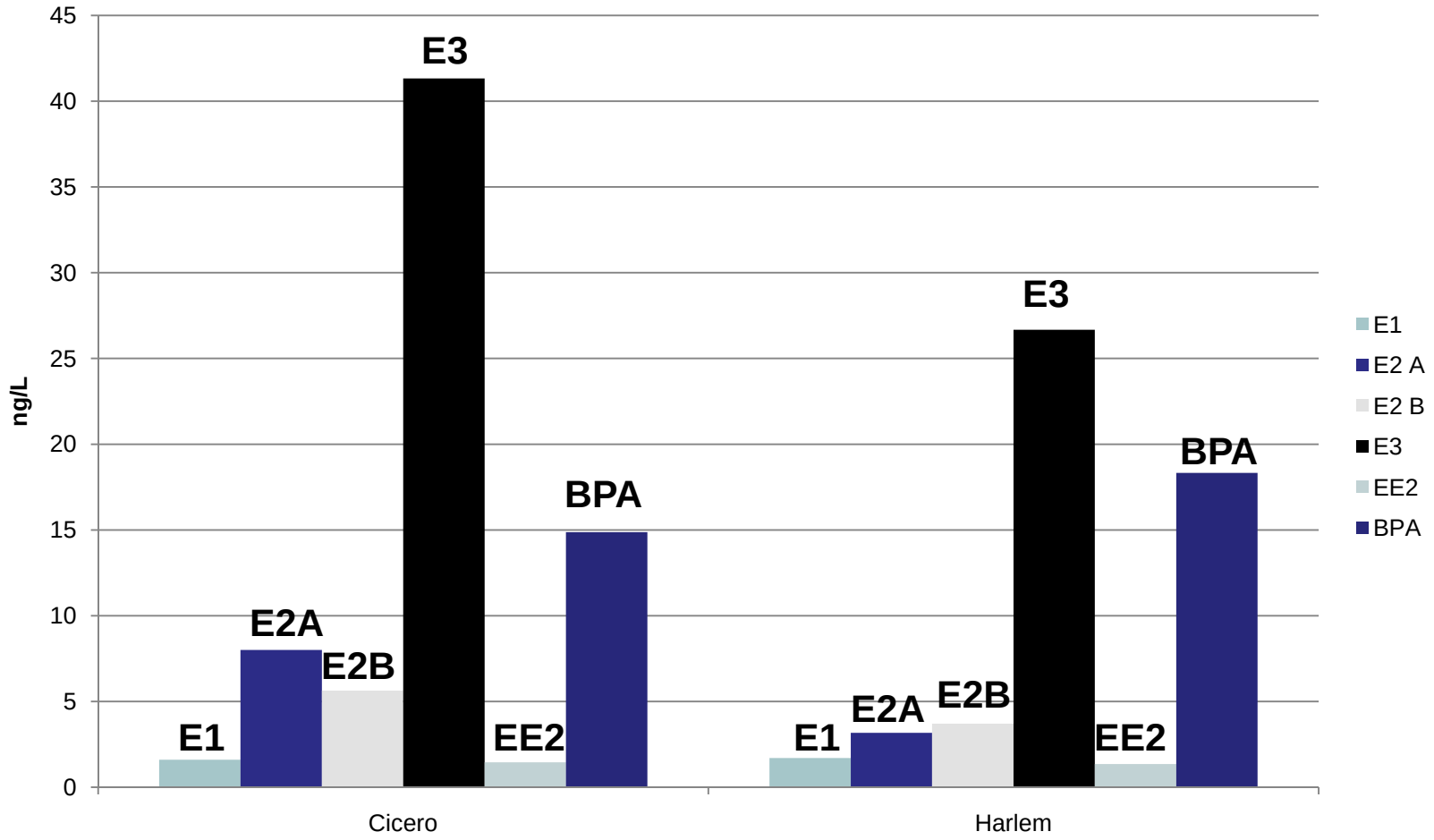
Water Chemistry – Salt Creek



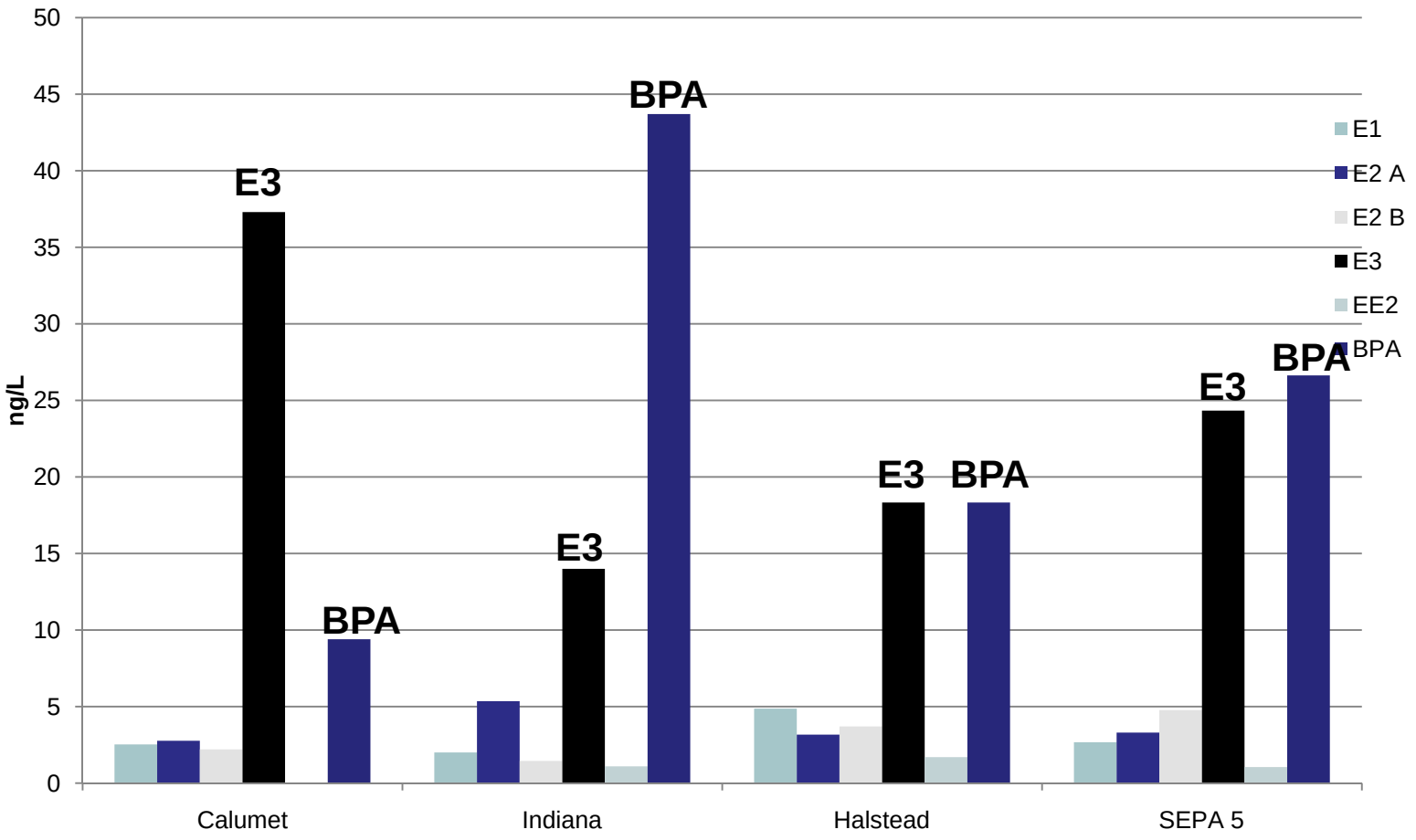
Water Chemistry – North Shore Channel



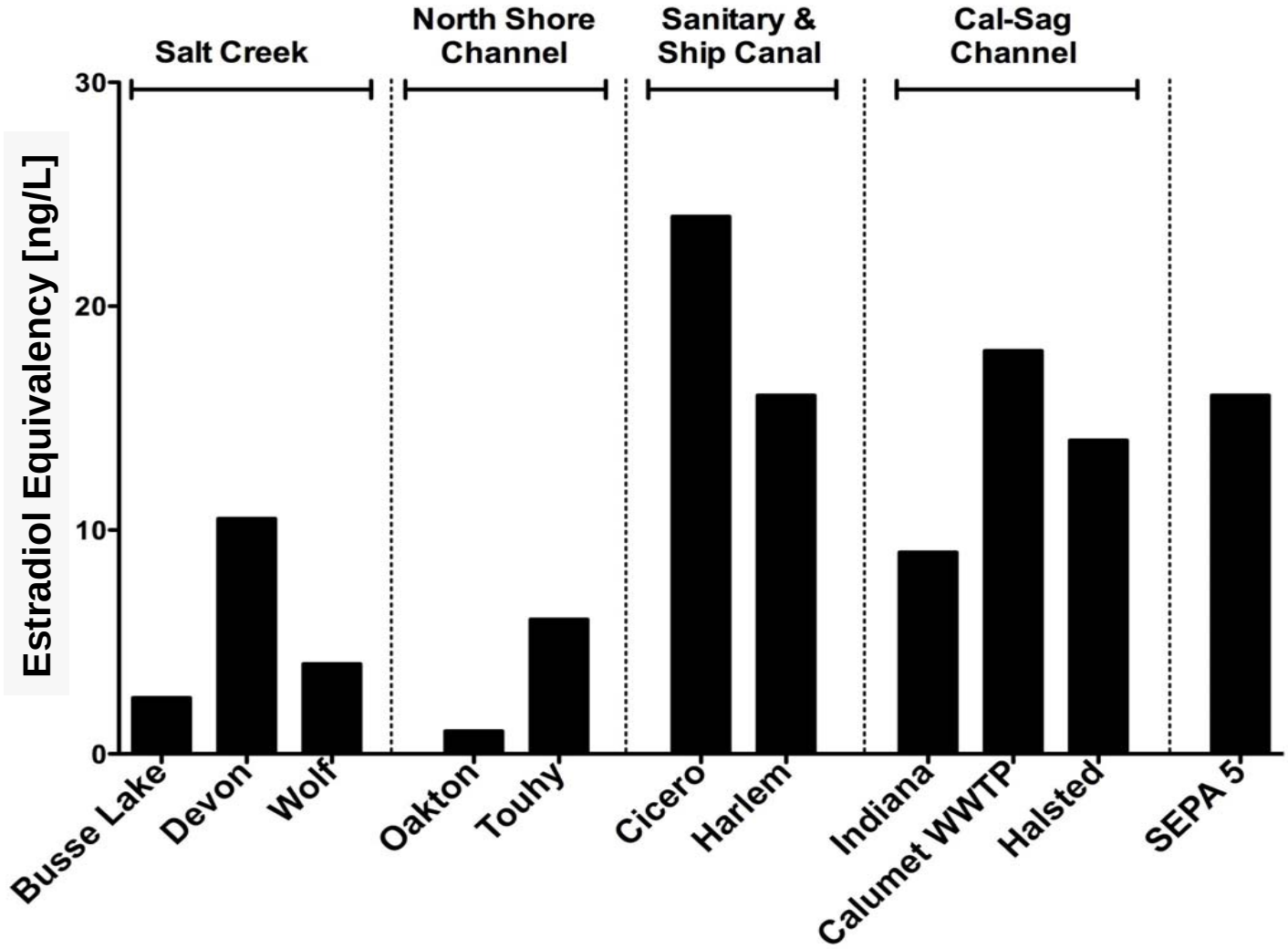
Water Chemistry – Sanitary & Ship Canal



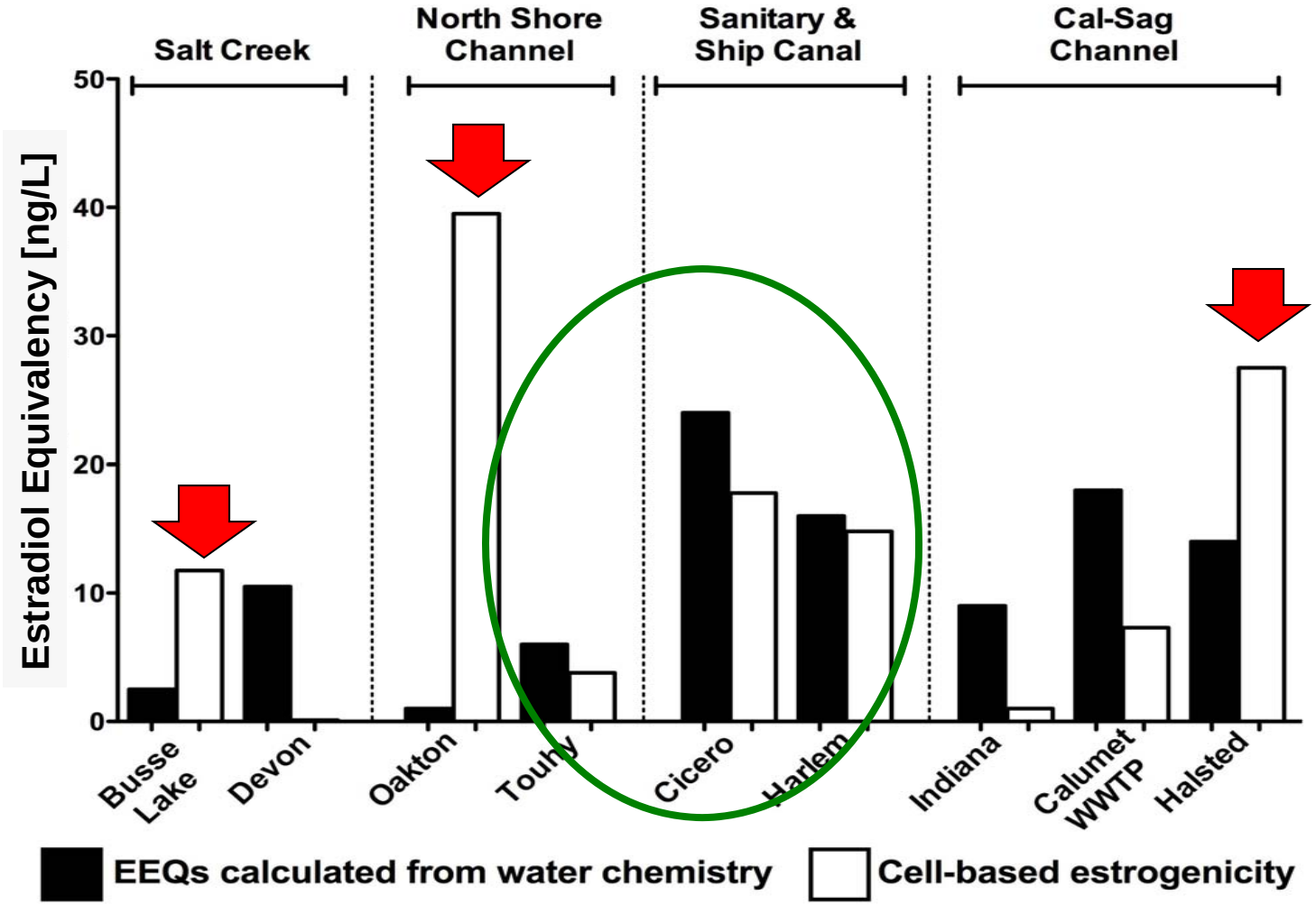
Water Chemistry – Cal Sag Channel



Water Chemistry – Total Estrogenicity



Calculated vs. Cell-based Total Estrogenicity



Water Chemistry - Summary

- A variety of estrogenic endocrine active compounds are found throughout the study areas.
- WWTP effluent contributes to the presence of these compounds.
- Cell-based estrogenicity results and water chemistry suggest additional sources of estrogens in the *Chicago Waterways*.

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Widespread occurrence of intersex in black basses (*Micropterus* spp.) from U.S. rivers, 1995–2004

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ABSTRACT

Intersex occurrence in freshwater fishes was evaluated for nine river basins in the United States. Testicular oocytes (predominantly male testes containing female germ cells) were the most prevalent form of intersex observed, even though similar numbers of male ($n = 1477$) and female ($n = 1633$) fish were examined. Intersex was found in 3% of the fish collected. The intersex condition was observed in four of the 16 species examined (25%) and in fish from 34 of 111 sites (31%). Intersex was not found at all sites from the same site but was most prevalent in largemouth bass (*Micropterus salmoides*; 39% of males) and smallmouth bass (*M. dolomieu*; 33% of males). The percentage of intersex fish per site was 14–100% for largemouth bass and 14–73% for smallmouth bass. The incidence of intersex was greatest in the southeastern United States, with intersex largemouth bass present at all sites in the Apalachicola-Chattahoochee-Flint and Pee Dee River Basins. Total mercury, *trans*-nonachlor, *p,p'*-DDE, *p,p'*-DDD, and total PCBs were the only detected chemical contaminants at all sites, regardless of whether intersex was present. The genotype of the intersex fish was not determined, the microscopic appearance of testicular oocytes, presence of mature sperm, and the concentrations of sex steroid hormones and vitellogenin in male largemouth bass were males. Few reproductive endpoints differed significantly among male and intersex fish; vitellogenin concentration in males was not a good indicator of intersex presence. Histological evaluation of the intersex condition to reproductive function will require a more quantitative measure of severity (e.g. severity index) rather than presence or absence of the condition. The baseline incidence of intersex in gonadal tissue in black basses and other freshwater fishes is unknown, but intersex prevalence appears to be related to collection season, age, and endocrine active compounds in the environment. Intersex was commonly found in largemouth bass older than five years and was most common in 1–3-year-old male largemouth bass. The cause(s) of intersex in these species is also unknown, and it remains to be determined whether the intersex we observed in largemouth and smallmouth bass developed during sex differentiation during early life stages, during exposure to environmental factors during adult life stages, or both.

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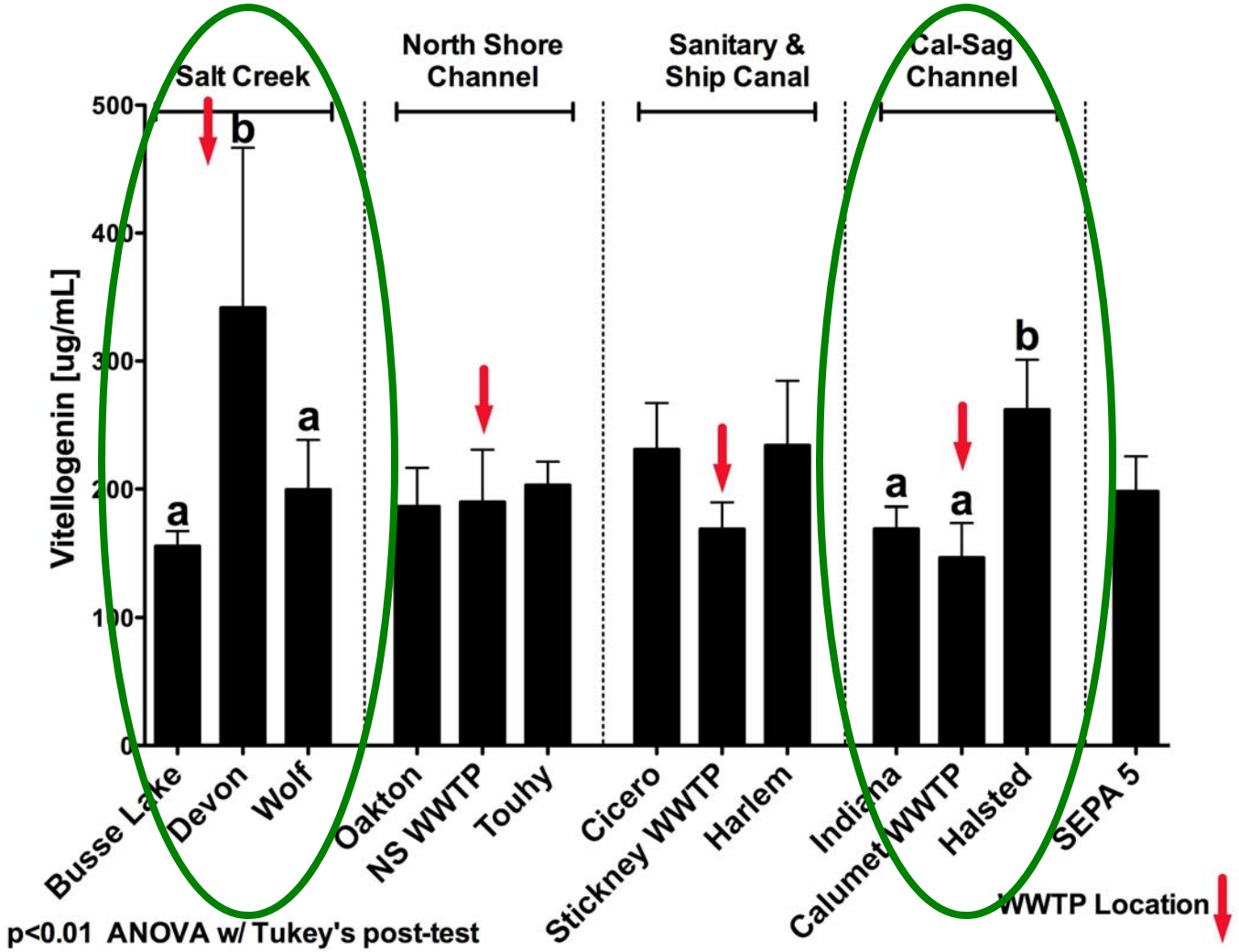
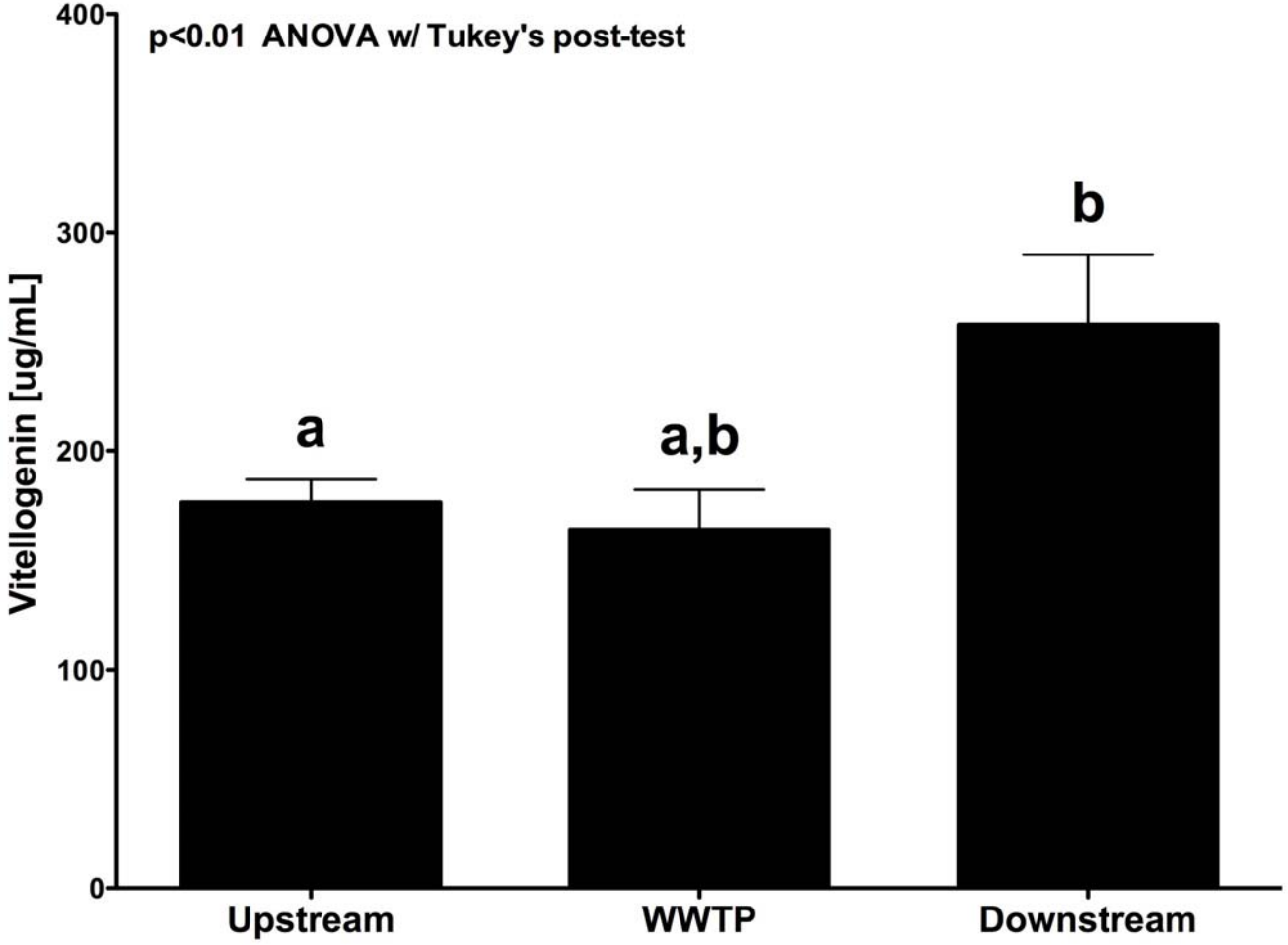
									Fall	
									Caged	
								rp	Sunfish	n
								/f		m/f
								/22	38	244
										79
									34	79
									35	102
								/19	40	221
									37	37
								5	39	252
									40	155
									31	31
									4	130
										215
										57
Channel	Halsted	11/11	12/28	12/24	23/17	4/7	21/22	14/2		265
	SEPA5		13/18	20/20	27/12	14/18	17/10	22		237
	Baseline	20/20			20/20	13/24			20/2	2,104

caged sunfish | wild-caught fish | not attempted

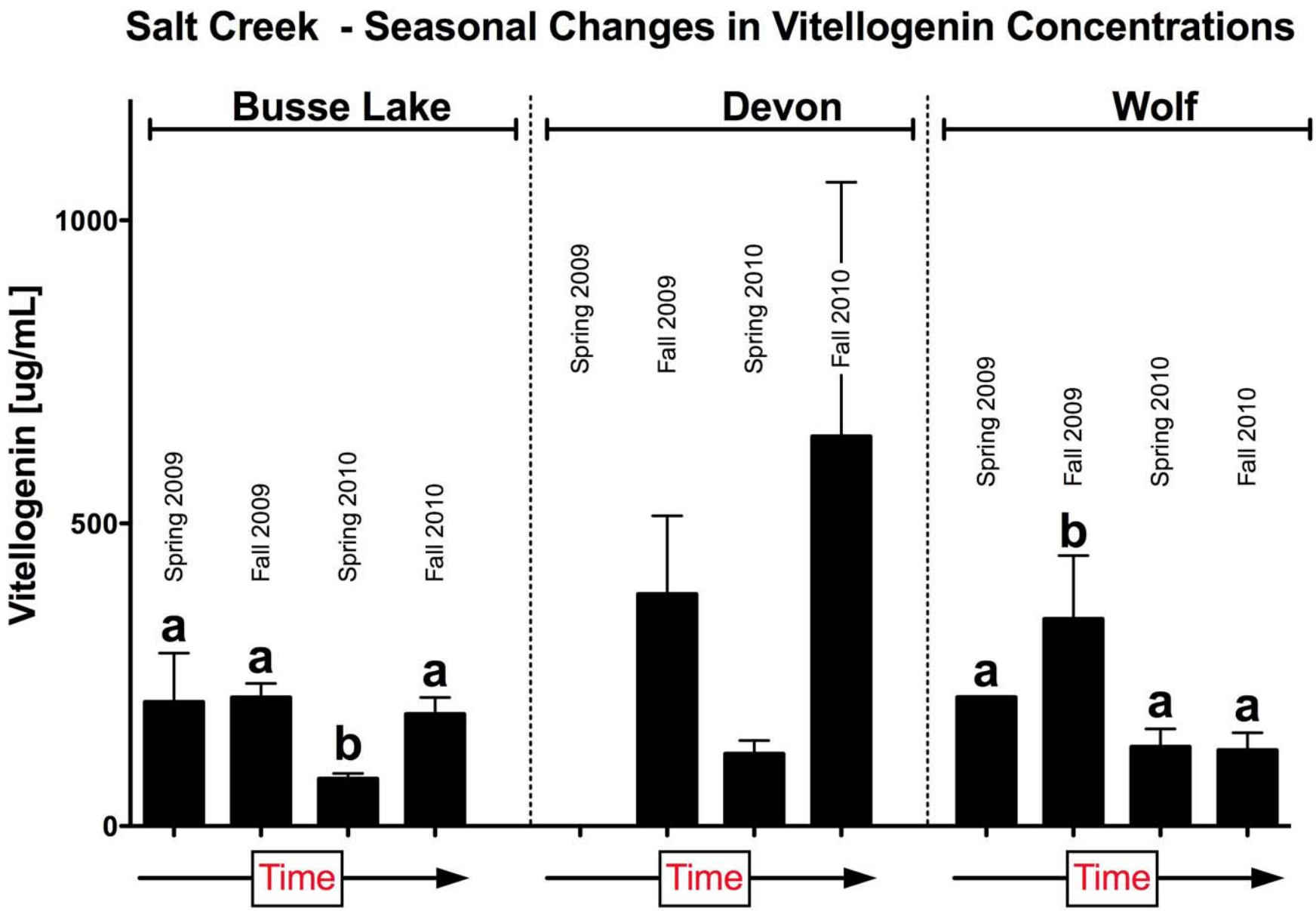
Fish Analyses – Vitellogenin in Caged Sunfish



Fish Analyses



Fish Analyses



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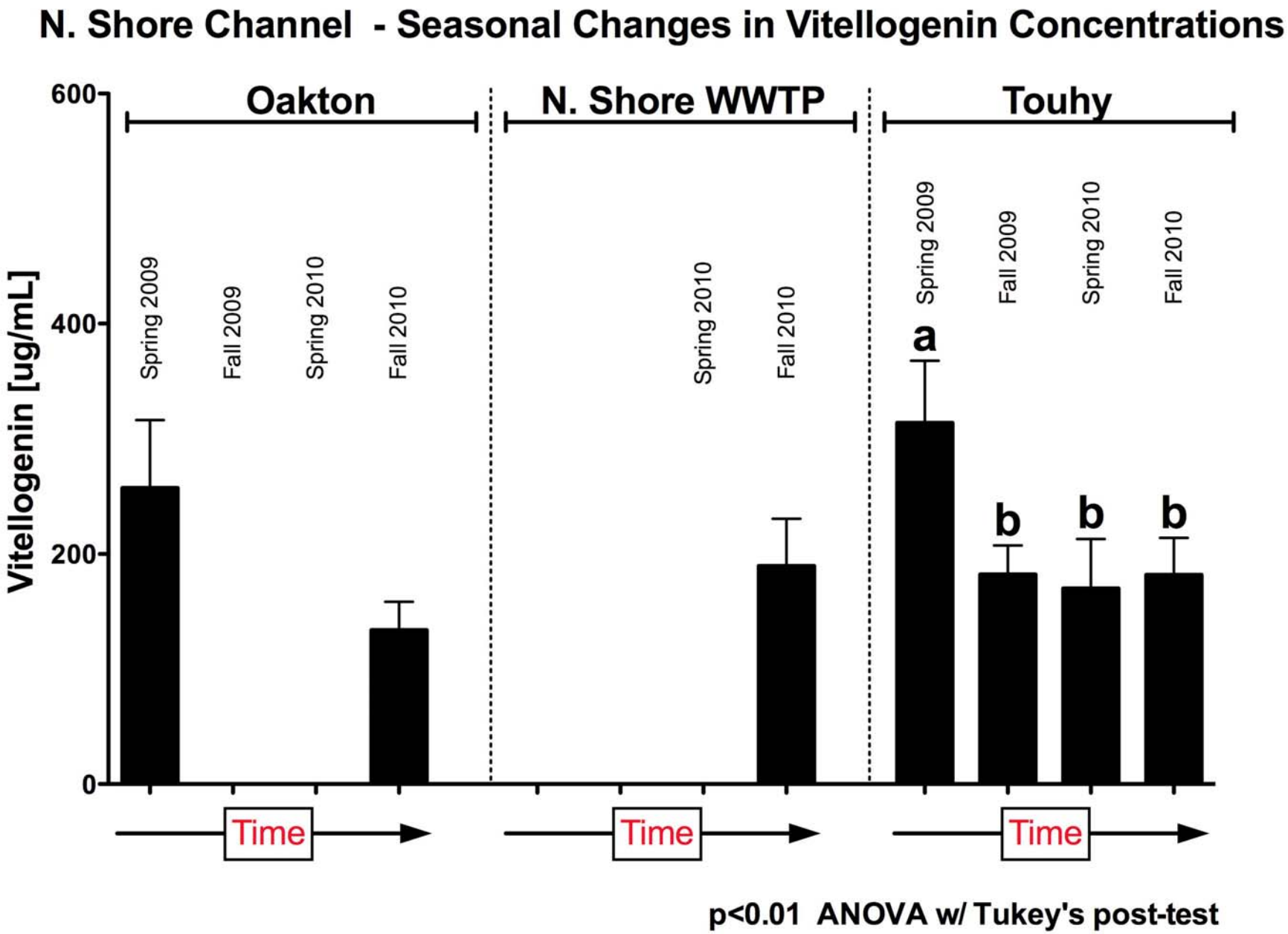
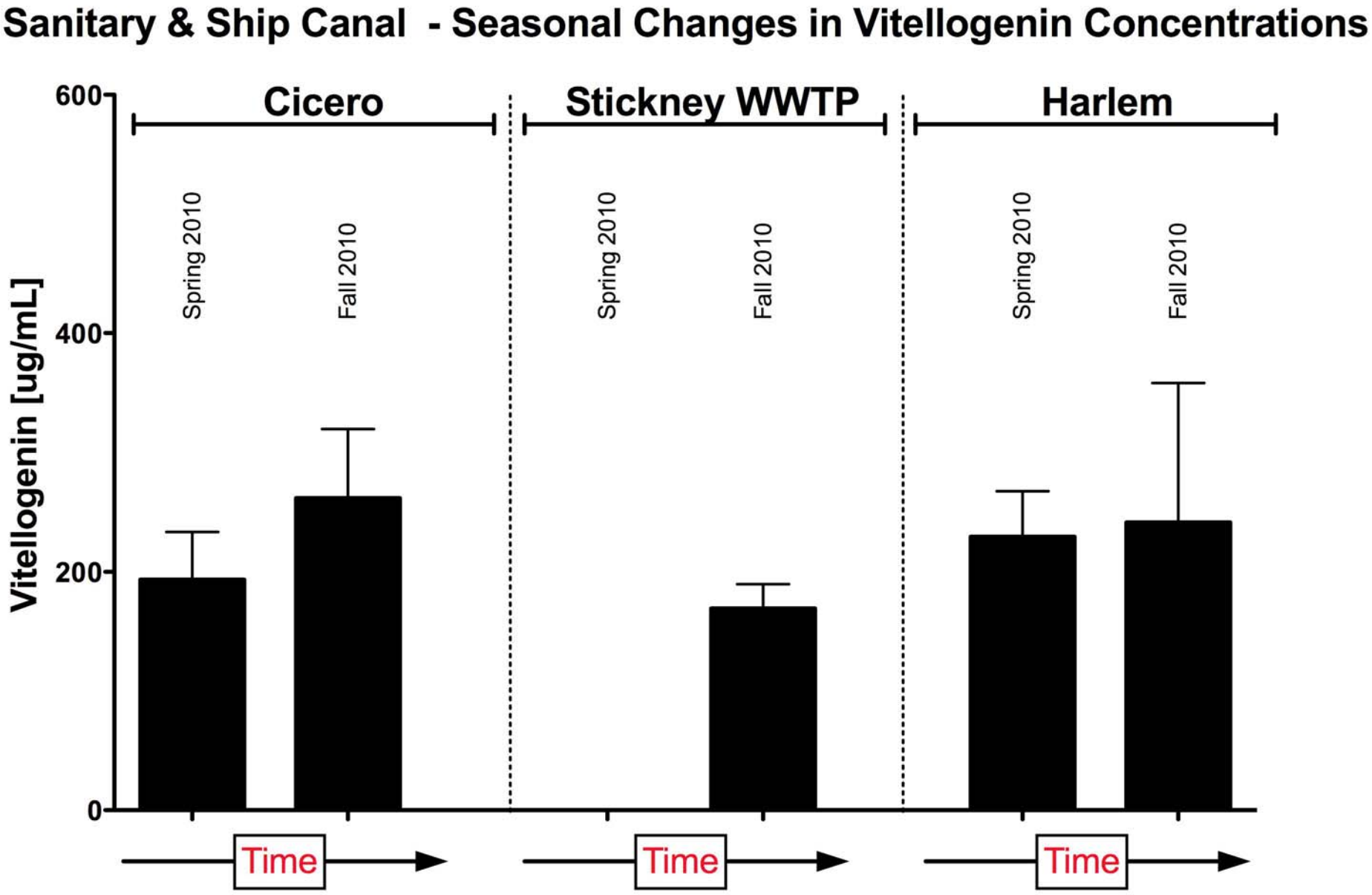
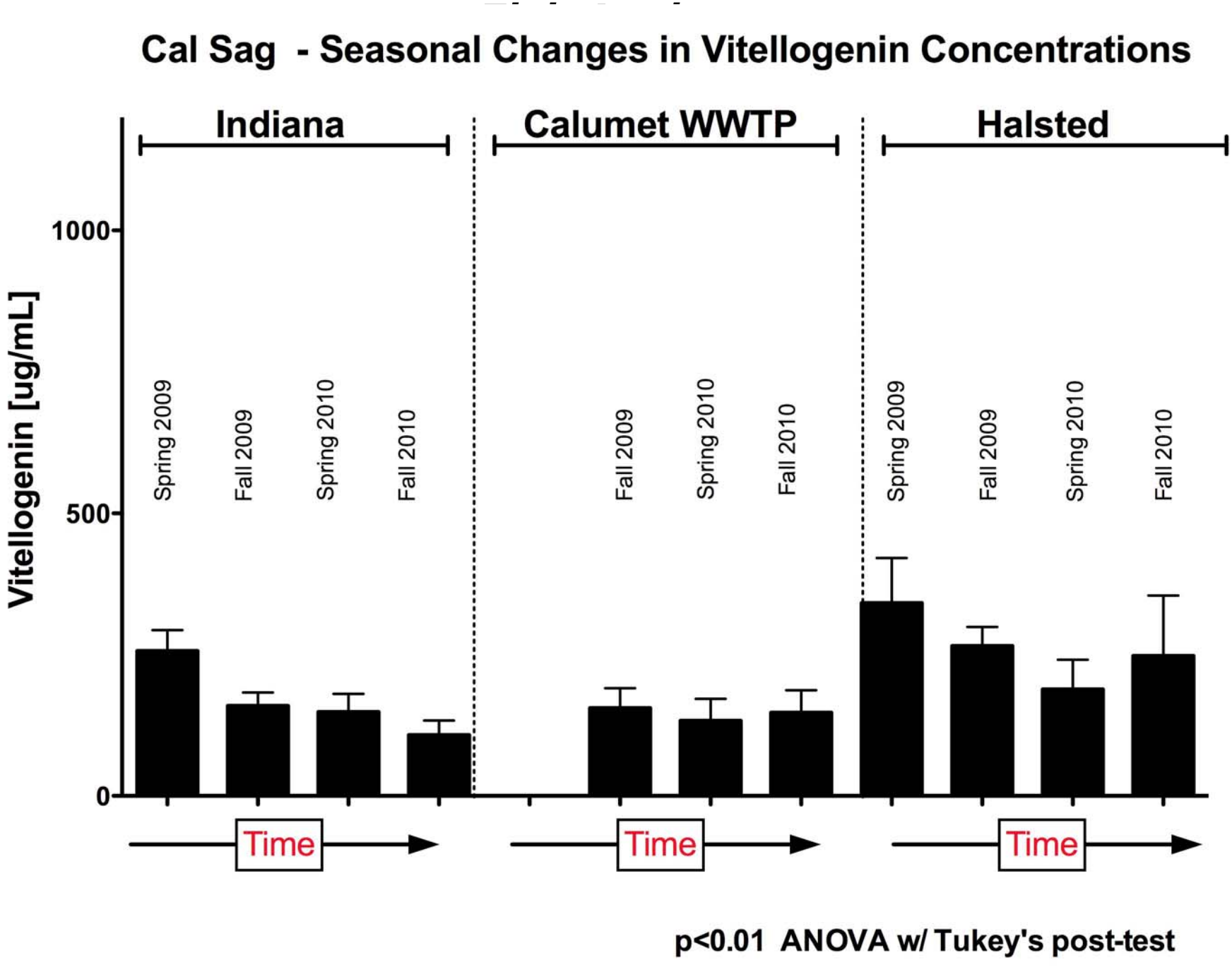
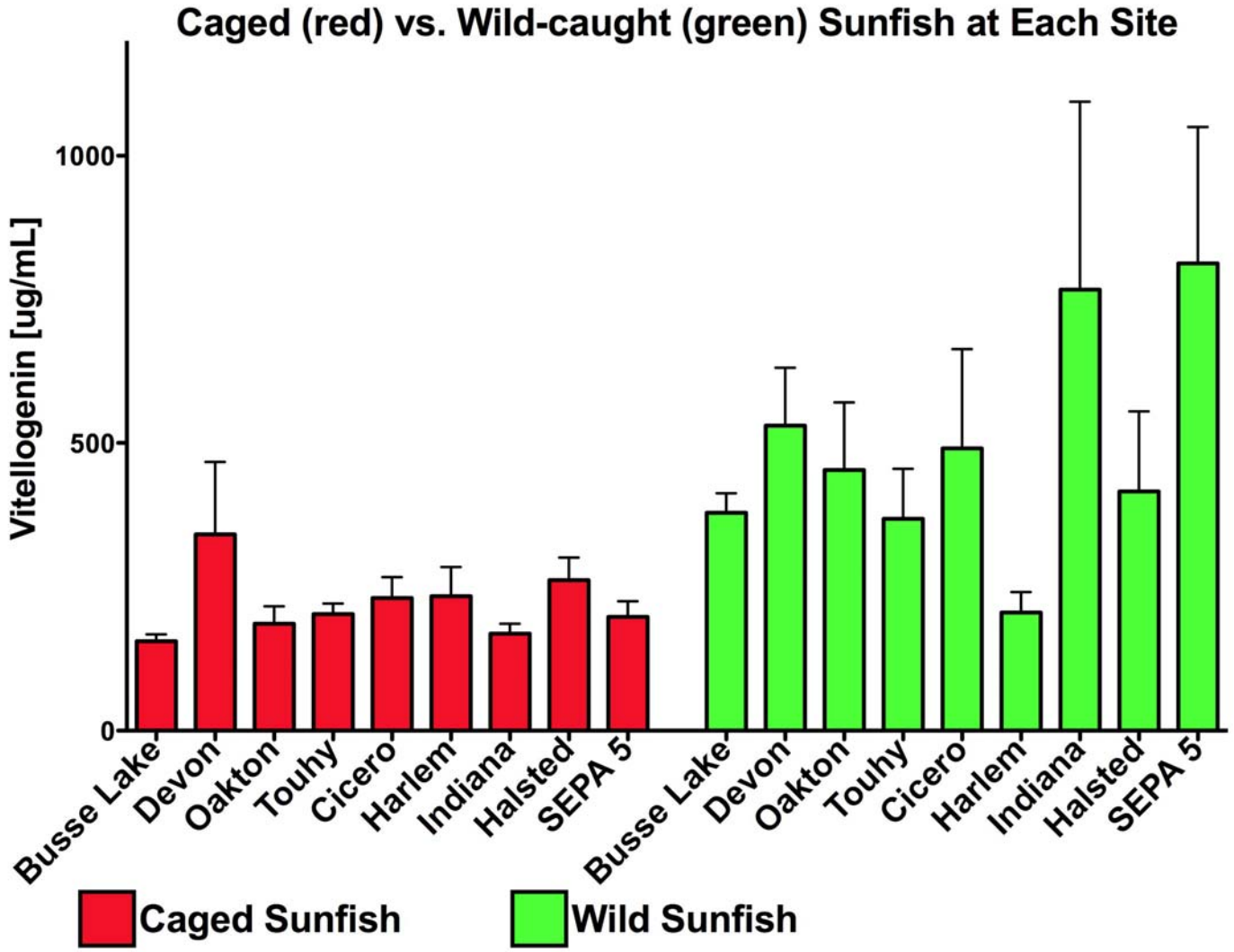


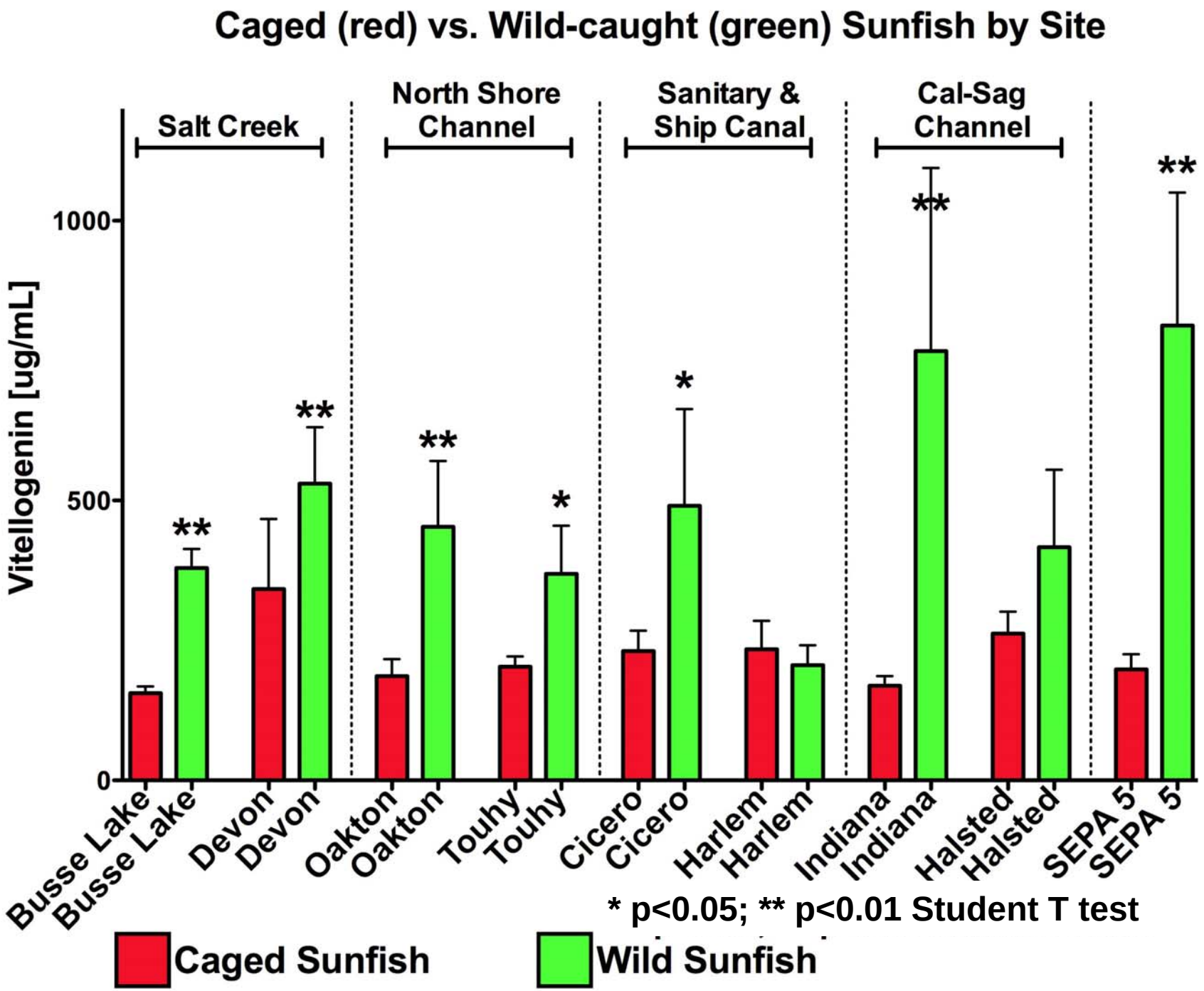
Fish Analyses



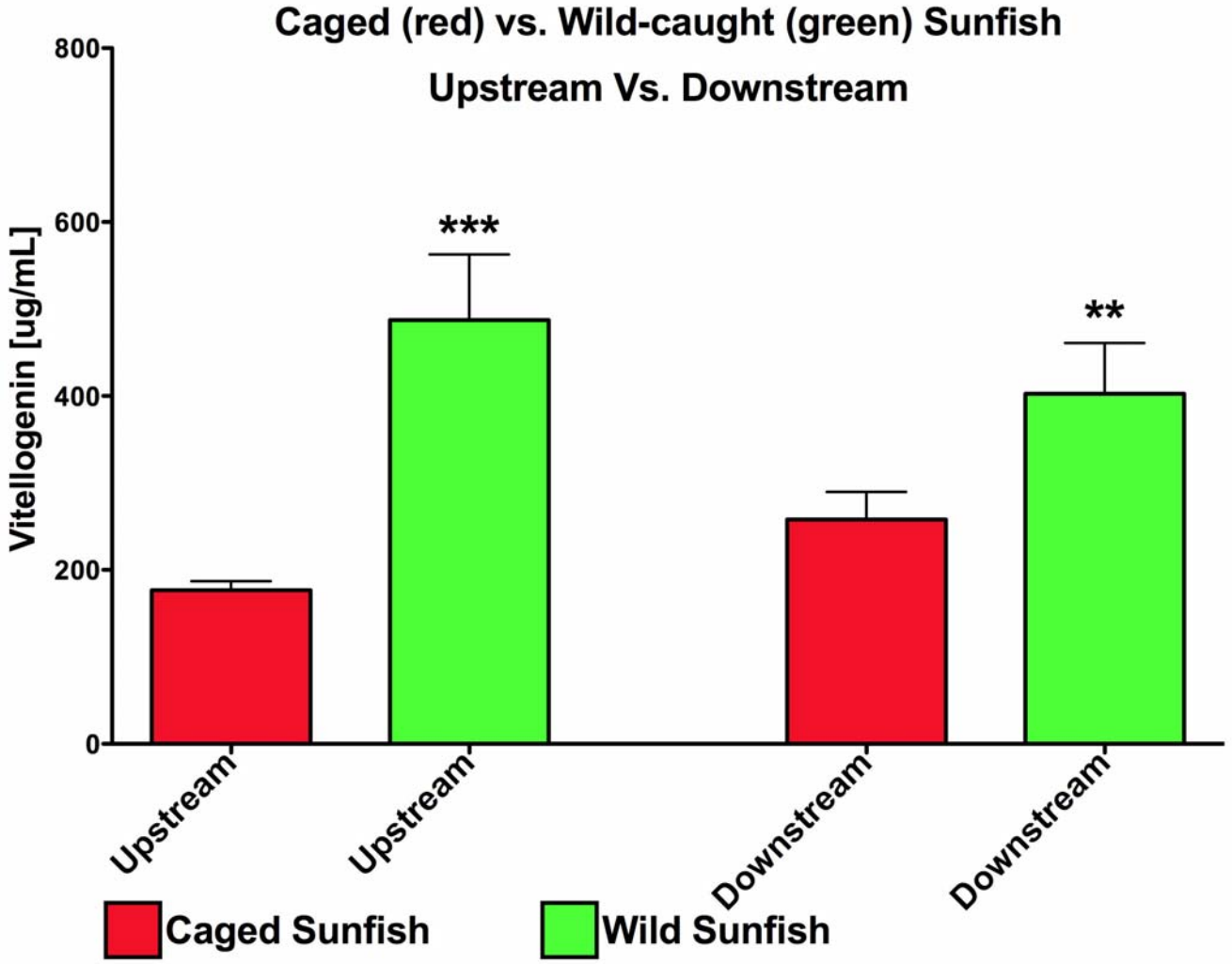

Cal Sag - Seasonal Changes in Vitellogenin Concentrations
Indiana
Calumet WWTP
Halsted
Vitellogenin [ug/mL]
1000
500
0
Spring 2009
Fall 2009
Spring 2010
Fall 2010
Fall 2009
Spring 2010
Fall 2010
Spring 2009
Fall 2009
Spring 2010
Fall 2010
Time
Time
Time
p<0.01 ANOVA w/ Tukey's post-test

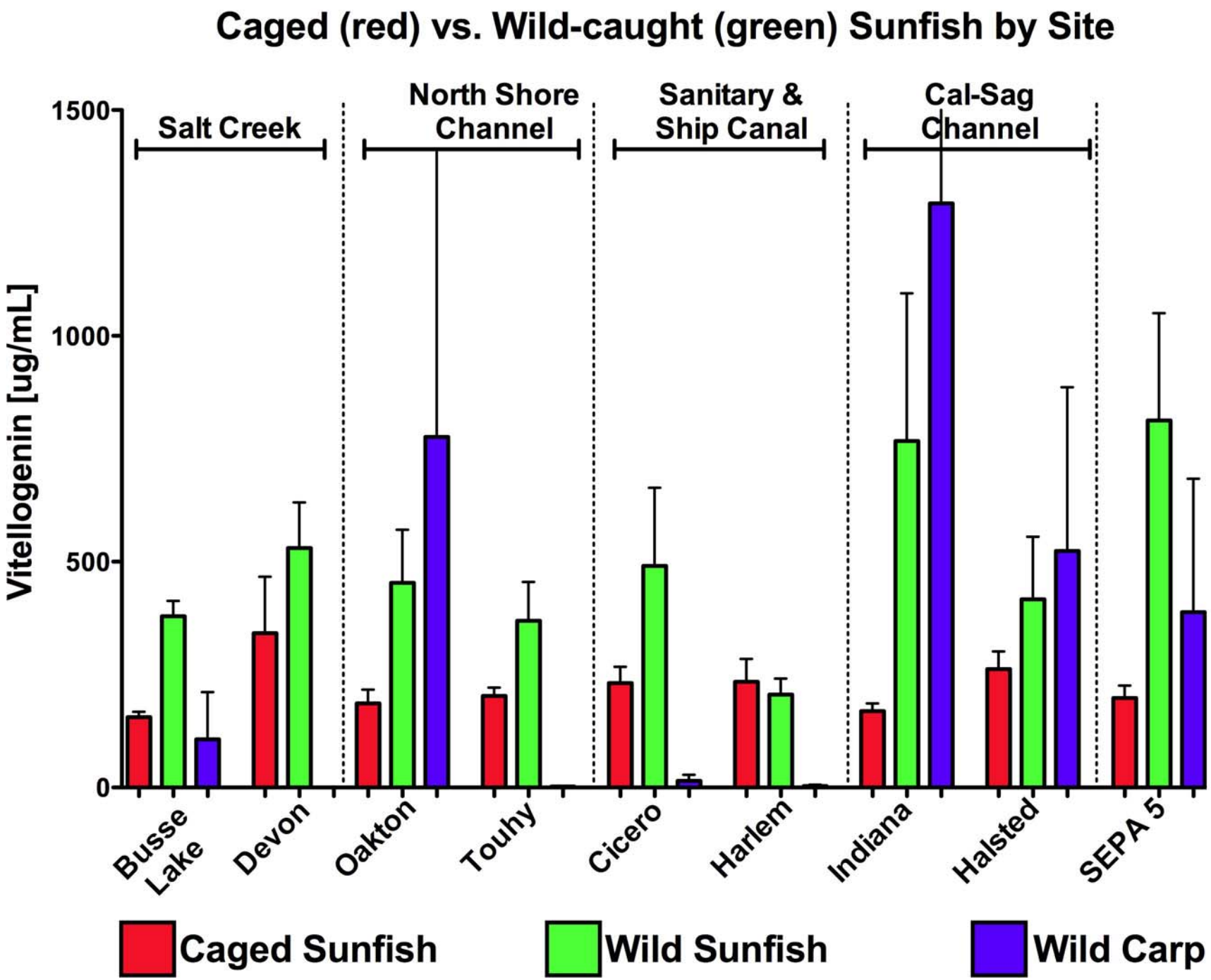
Fish Analyses



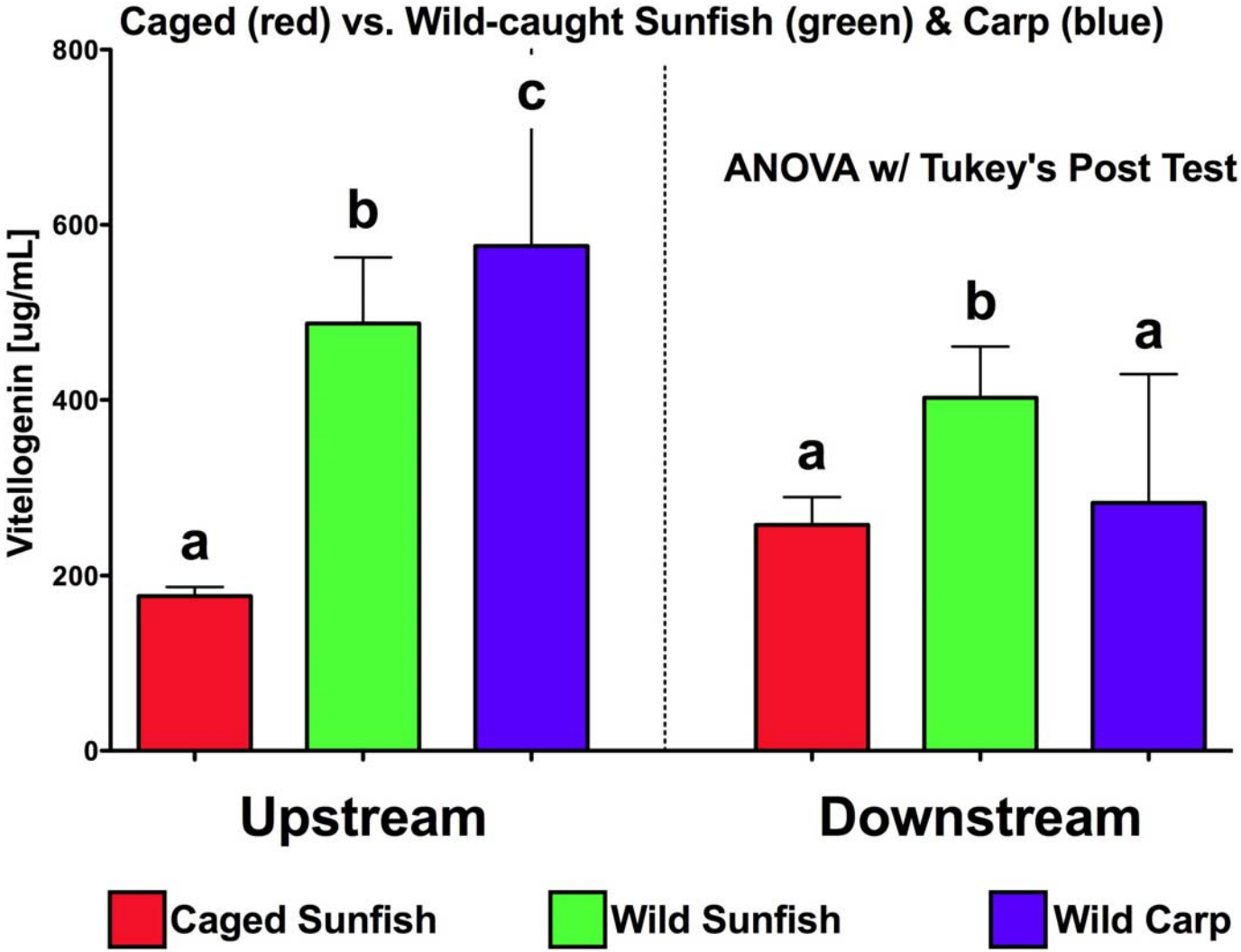

Caged (red) vs. Wild-caught (green) Sunfish by Site
Salt Creek
North Shore Channel
Sanitary & Ship Canal
Cal-Sag Channel
Vitellogenin [ug/mL]
1000
500
0
Busse Lake
Busse Lake
Devon
Devon
Oakton
Oakton
Touhy
Touhy
Cicero
Cicero
Harlem
Harlem
Indiana
Indiana
Halsted
Halsted
SEPA 5
SEPA 5
* p<0.05; ** p<0.01 Student T test
Caged Sunfish
Wild Sunfish

Fish Analyses



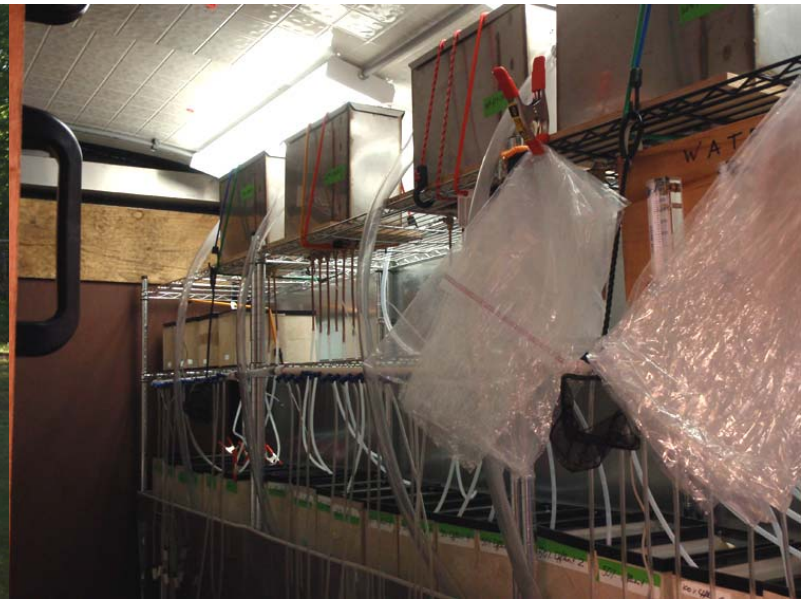

Caged (red) vs. Wild-caught (green) Sunfish by Site
Vitellogenin [ug/mL]
1500
1000
500
0
Salt Creek
North Shore Channel
Sanitary & Ship Canal
Cal-Sag Channel
Busse Lake
Devon
Oakton
Touhy
Cicero
Harlem
Indiana
Halsted
SEPA 5
Caged Sunfish
Wild Sunfish
Wild Carp

Fish Analyses

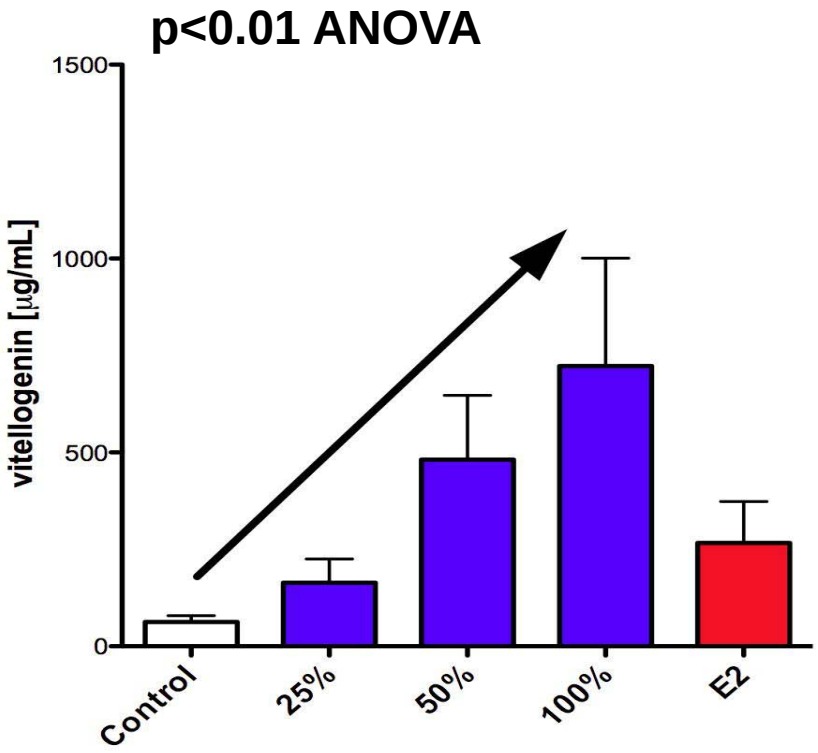


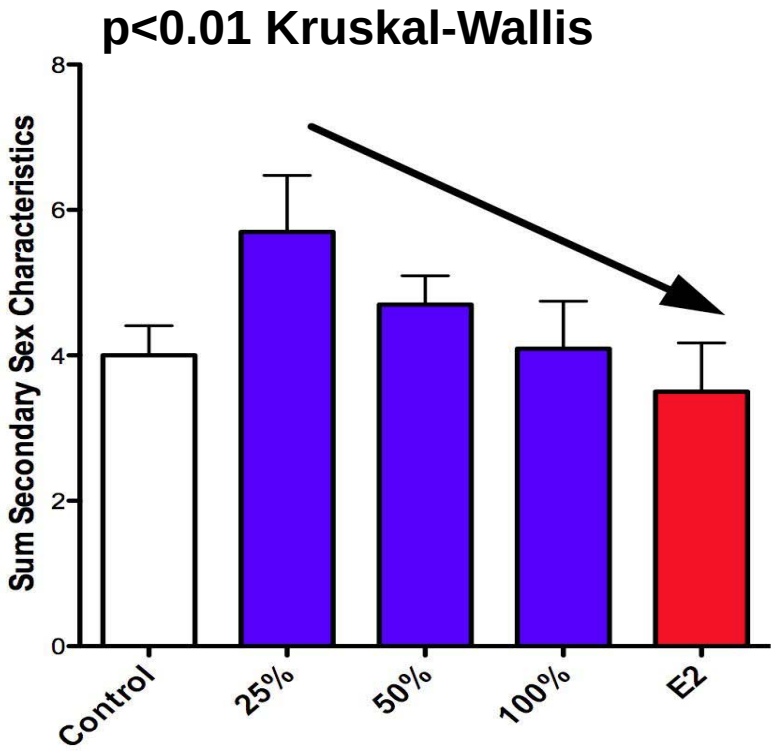
Mobile Exposure Laboratory Deployment - MELT

- → *Establishing causal relationships between observed endocrine disruption and sources in the Chicago Waterways.*
- → *Deployments at Stickney (Sept'10)*
- → *0%, 25%, 50%, 100% effluent and 30 ng/L estradiol positive control*
- → *12 day exposures of mature male fathead minnows*



Mobile Exposure Laboratory Deployment - MELT





Fish Analyses - Summary

- In congruence with estrogenicity and water chemistry, fish in the study area are exposed to estrogenic endocrine active compounds.

- Resident fish exhibit greater expression of biomarkers of exposures - indicative of longer exposure periods.

- Seasonality in estrogenic responses is visible but not easily resolved – awaits further analysis.

- A signal of WWTP effluents is visible in the fish data, however, it is overlaid by other exposure sources – these need to be explored.

Final Thoughts…..

- Urban aquatic ecosystems are exposed to biologically active compounds through multiple pathways – including treated wastewater effluent.

  → Understanding other pathways is essential.

- Fish are impacted by these exposures to a varying degree.

  → Identifying vulnerable habitats, species, and life stages will add critical knowledge.

Final Thoughts…..

- Remediation will require a comprehensive approach that involves education as much as technology.

  → The public is willing to contribute if given the opportunity.

Final Thoughts…..

- **Academic - Industry** collaborations can provide effective mechanisms to identify and remediate environmental concerns.

→ The St. Cloud State University – MWRDGC collaboration has been outstanding – Thank You!

Protecting Our Water Environment

Metropolitan Water Reclamation District of Greater Chicago

Many thanks ……

- *Industrial Waste Division and Wastewater Research for sample collection*
- *Analytical Laboratory Division for sample analyses*
- *Microbiology for laboratory support*
- *All MWRDGC staff*

Spring 2011
Laboratory Retreat
Ney Nature Center