Assessing Load Reduction of Endocrine Active Compounds After Disinfection at the O'Brien and Calumet Water Reclamation Plants and the Biological Effects on Fish

A presentation at the

Metropolitan Water Reclamation District of Greater Chicago

February 23, 2018

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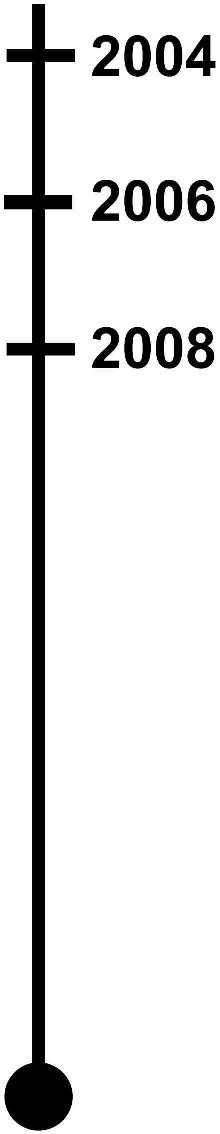
In collaboration with Dalma Martinovic & Paul Edmiston & MWRDGC

2004

2006

Presence and Biological Effects of Endocrine Active Compounds in the North Shore Channel?

Presence and Biological Effects of Endocrine Active Compounds in the North Shore Channel?



Science of the Total Environment 409 (2011) 4720–4728

Contents lists available at ScienceDirect

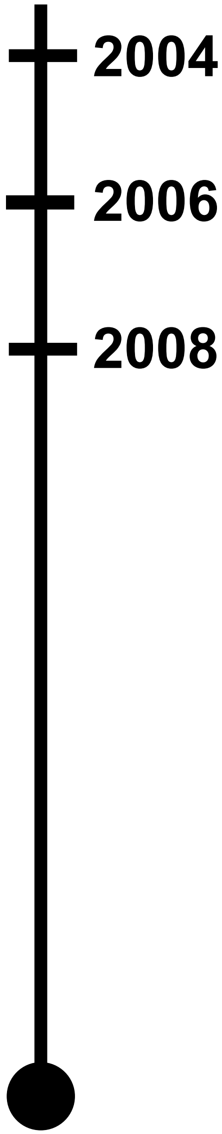
Science of the Total Environment

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

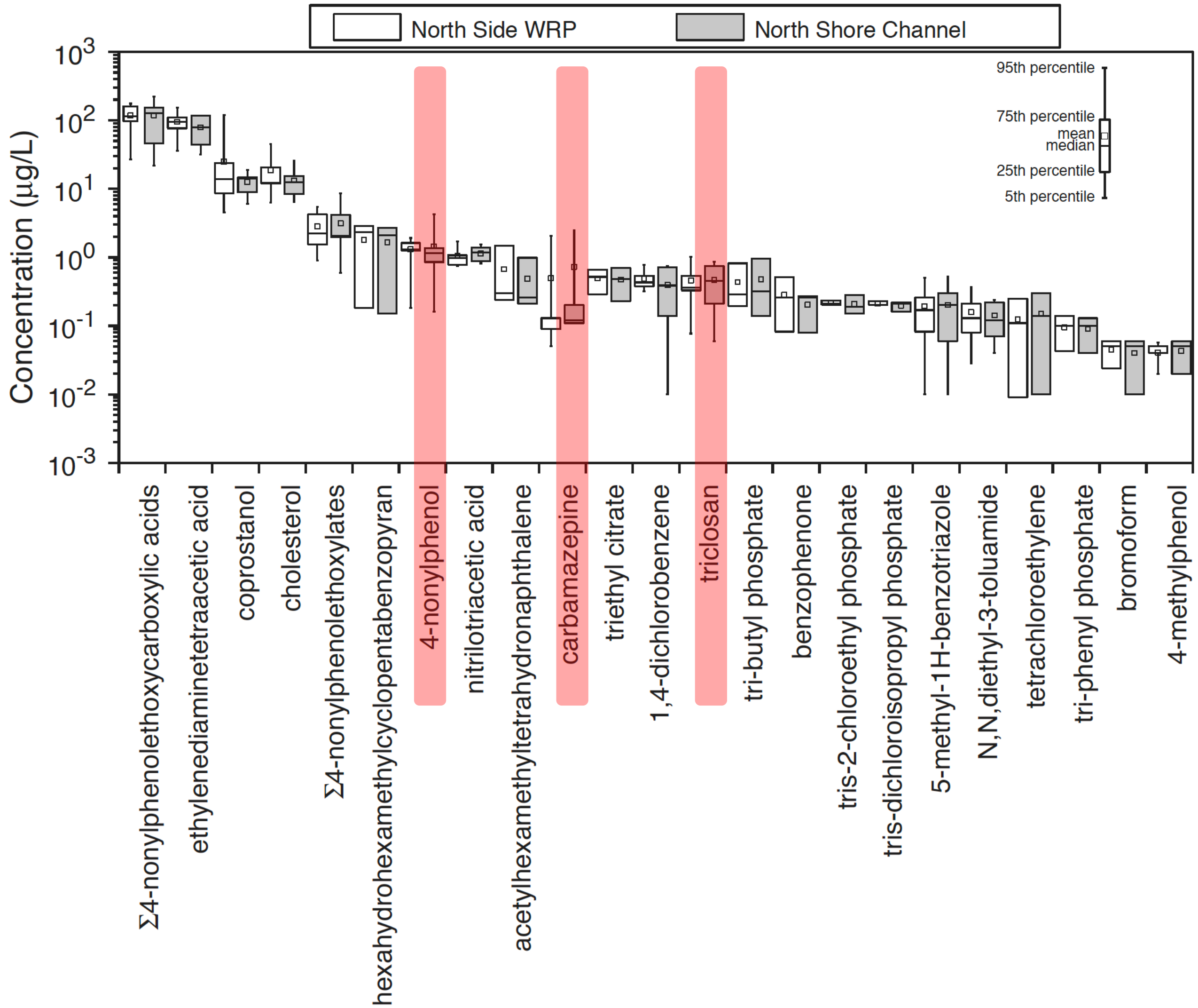
ELSEVIER

Effects of biologically-active chemical mixtures on fish in a wastewater-impacted urban stream

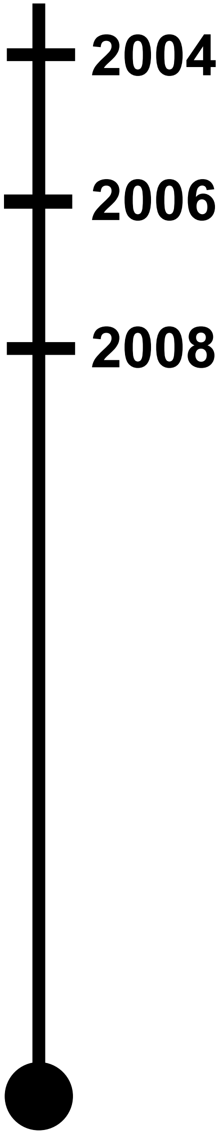
Larry B. Barber [a,*], Gregory K. Brown [a], Todd G. Nettesheim [b], Elizabeth W. Murphy [b], Stephen E. Bartell [c], Heiko L. Schoenfuss [c]



Presence and Biological Effects of Endocrine Active Compounds in the North Shore Channel?



Presence and Biological Effects of Endocrine Active Compounds in the North Shore Channel?



Science of the Total Environment 420 (2012) 191–201

Contents lists available at SciVerse ScienceDirect

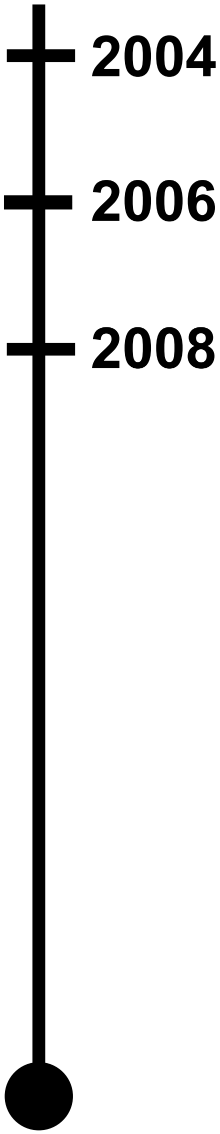
Science of the Total Environment

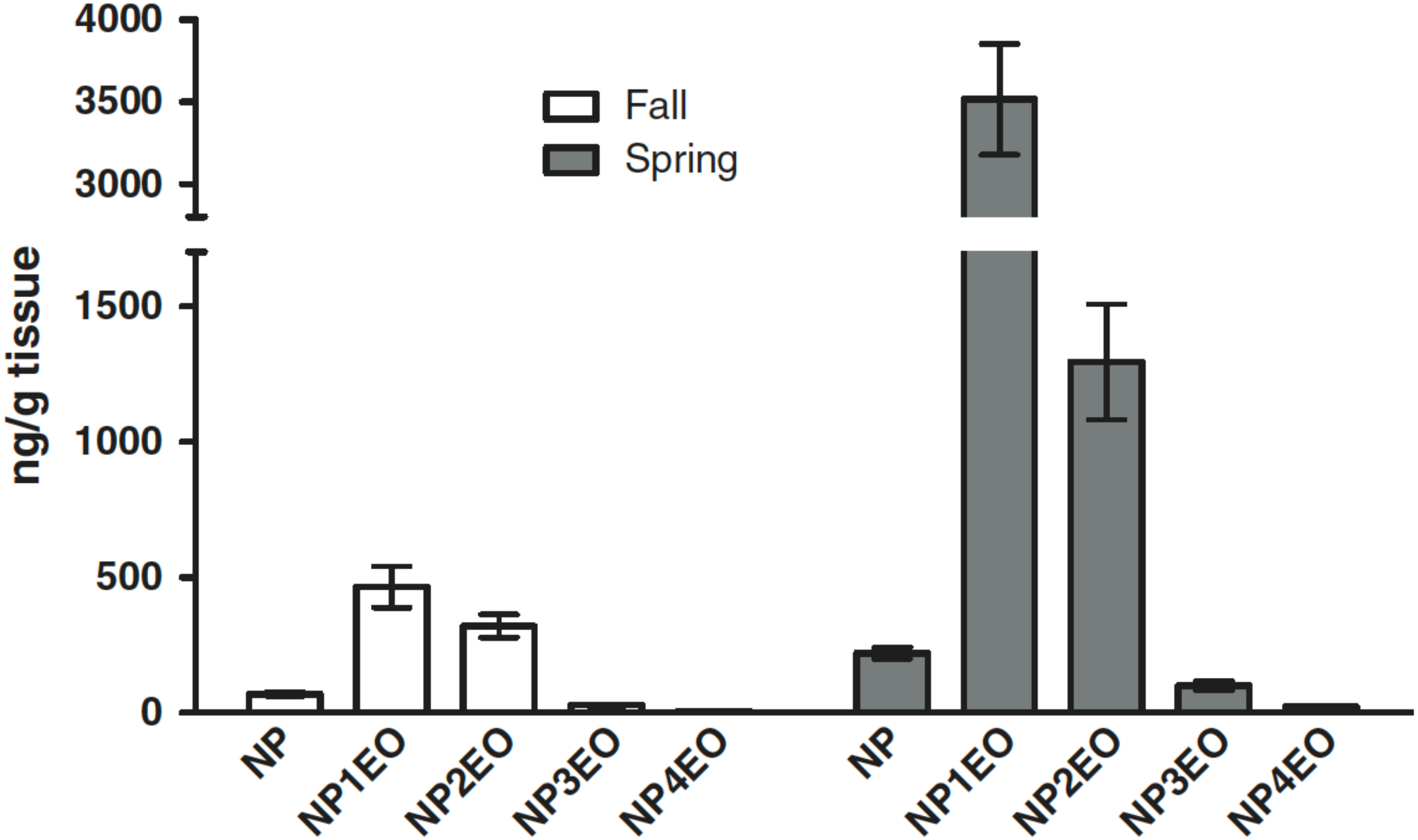
journal homepage: www.elsevier.com/locate/scitotenv

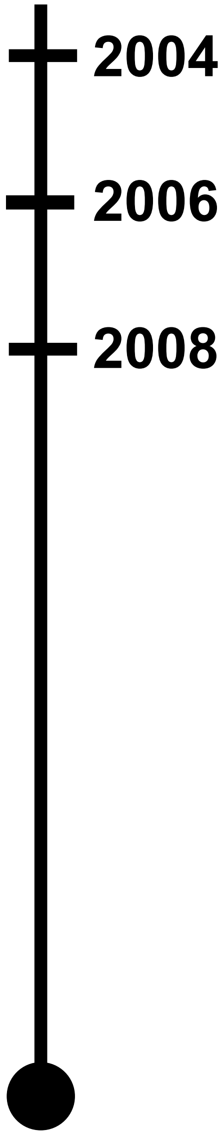
Concentration of organic contaminants in fish and their biological effects in a wastewater-dominated urban stream

Nuria Lozano [a,b], Clifford P. Rice [b,*], James Pagano [c], Larry Zintek [d], Larry B. Barber [e], Elizabeth W. Murphy [f], Todd Nettesheim [f], Tom Minarik [g], Heiko L. Schoenfuss [h]

Presence and Biological Effects of Endocrine Active Compounds in the North Shore Channel?







Presence and Biological Effects of Endocrine Active Compounds in the North Shore Channel?

Plasma vitellogenin results for fish collected from the North Shore Channel and the Outer Chicago Harbor of Lake Michigar
error; frequency, percentage of fish in sample expressing vitellogenin above the detection limit of 0.25 μg/mL].

	Male Fish		
	n	Vitellogenin	Frequency
		μg/mL	%
September 26/27, 2006			
North Shore Channel—largemouth bass	5	0.23 ± 0.10	60
Outer Chicago Harbor—largemouth bass	4	0	0
March 28, 2007			
North Shore Channel—largemouth bass	1	3.3	100
North Shore Channel—common carp	9	38 ± 17	78

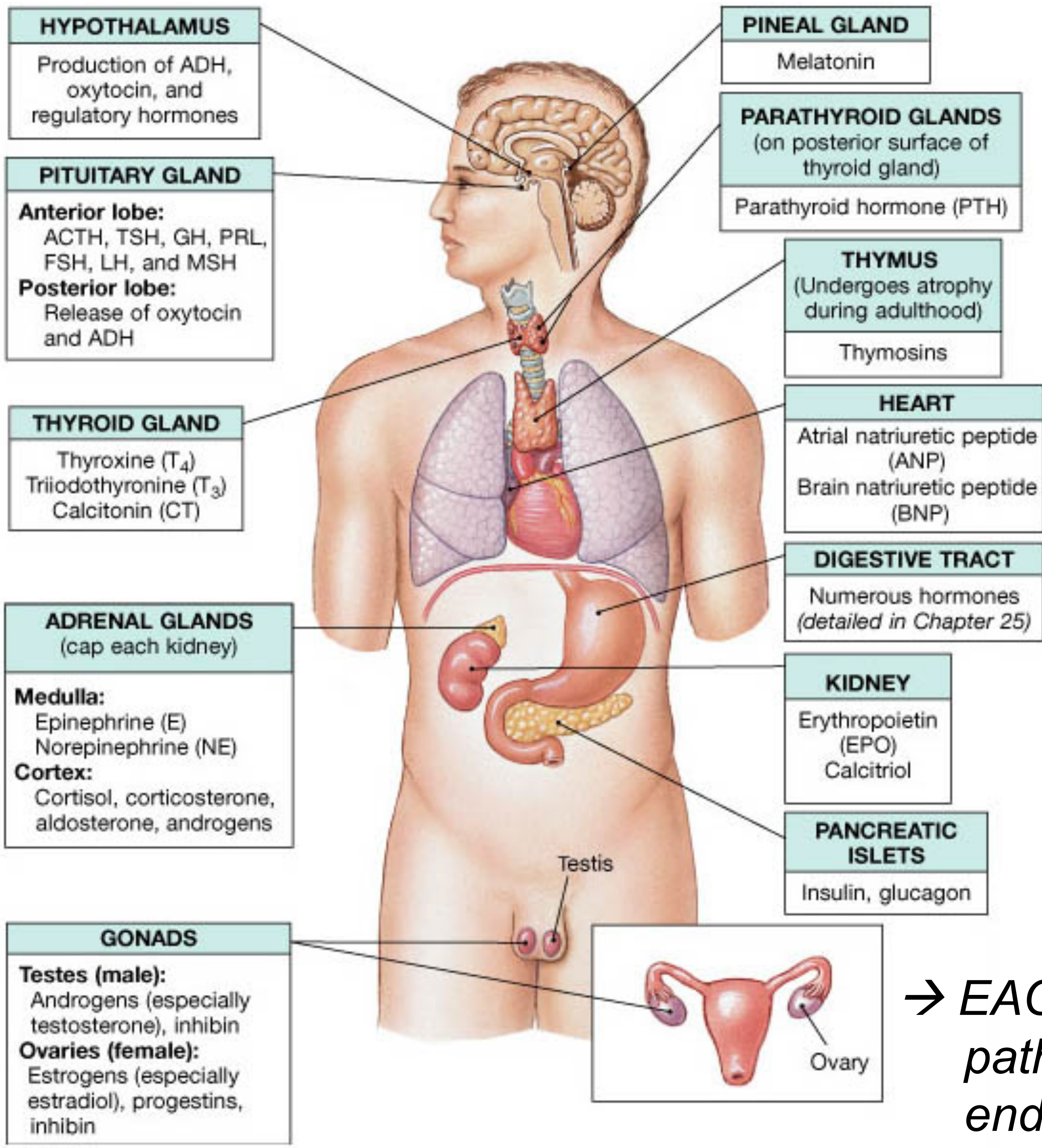
- **Endocrine Active Compounds are present in WRP effluent, water, sediment, and fish tissue.**
- **Resident fish exhibit feminization consistent with exposure to endocrine active compounds.**

What are Endocrine Active Compounds (EAC) ?

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



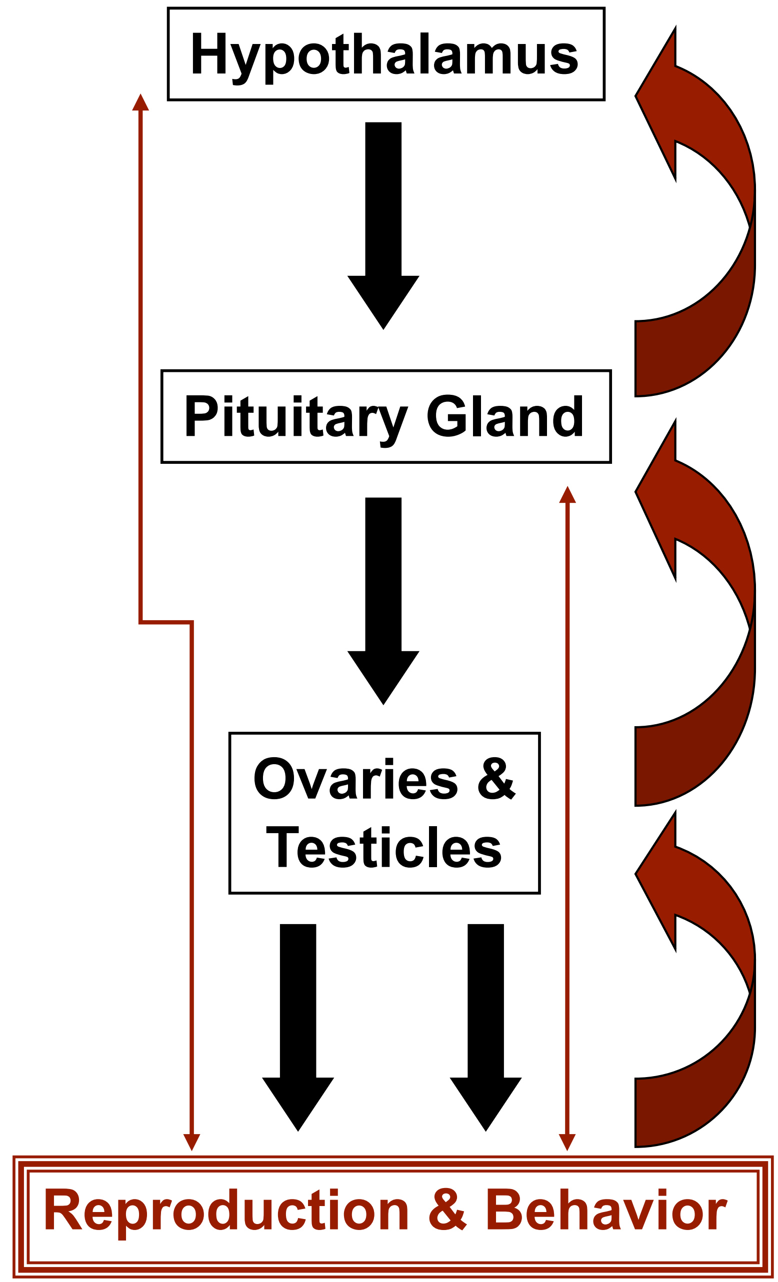
How Do Endocrine Active Compounds Affect Organisms?



→ *EACs can alter biological pathways controlled by the endocrine system.*

How Do Endocrine Active Compounds Affect Organisms?

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



What EACs are found in the Environment ?

•

This USGS Survey Found:

22 Antibiotics

14 Prescription Drugs

5 Nonprescription Drugs

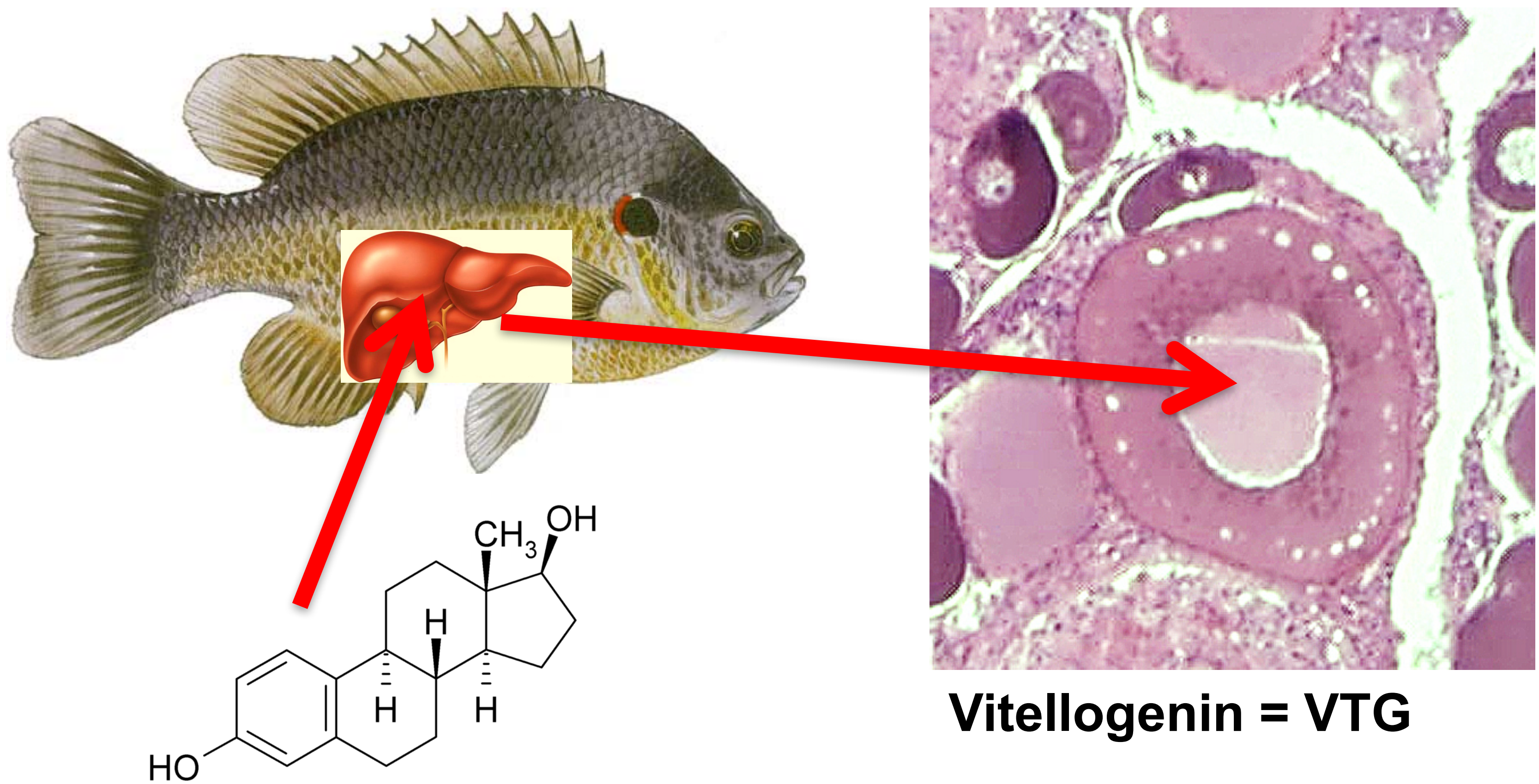
15 Hormones and Steroids

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.

What are Biomarkers for EAC Exposure ?

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

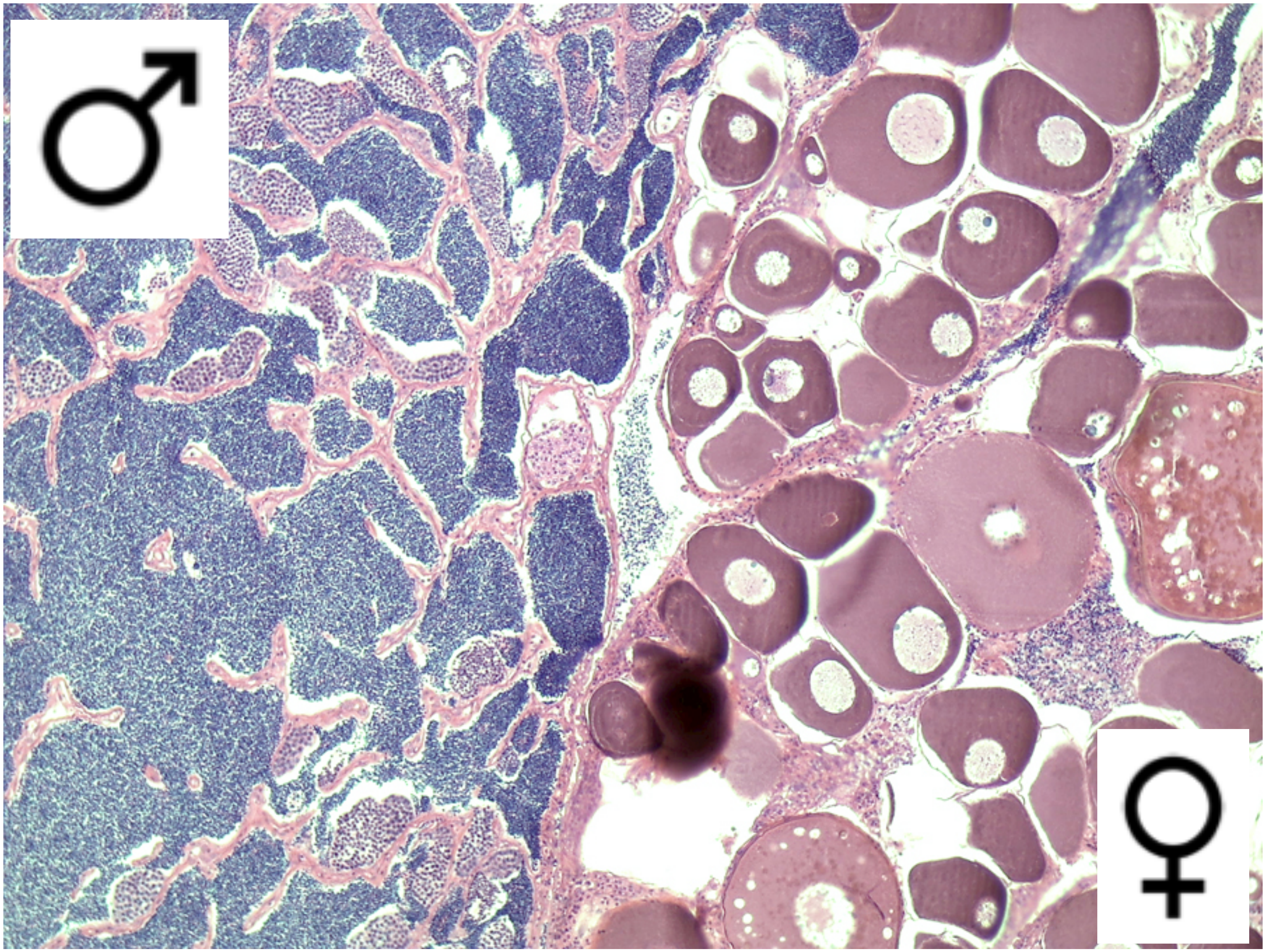


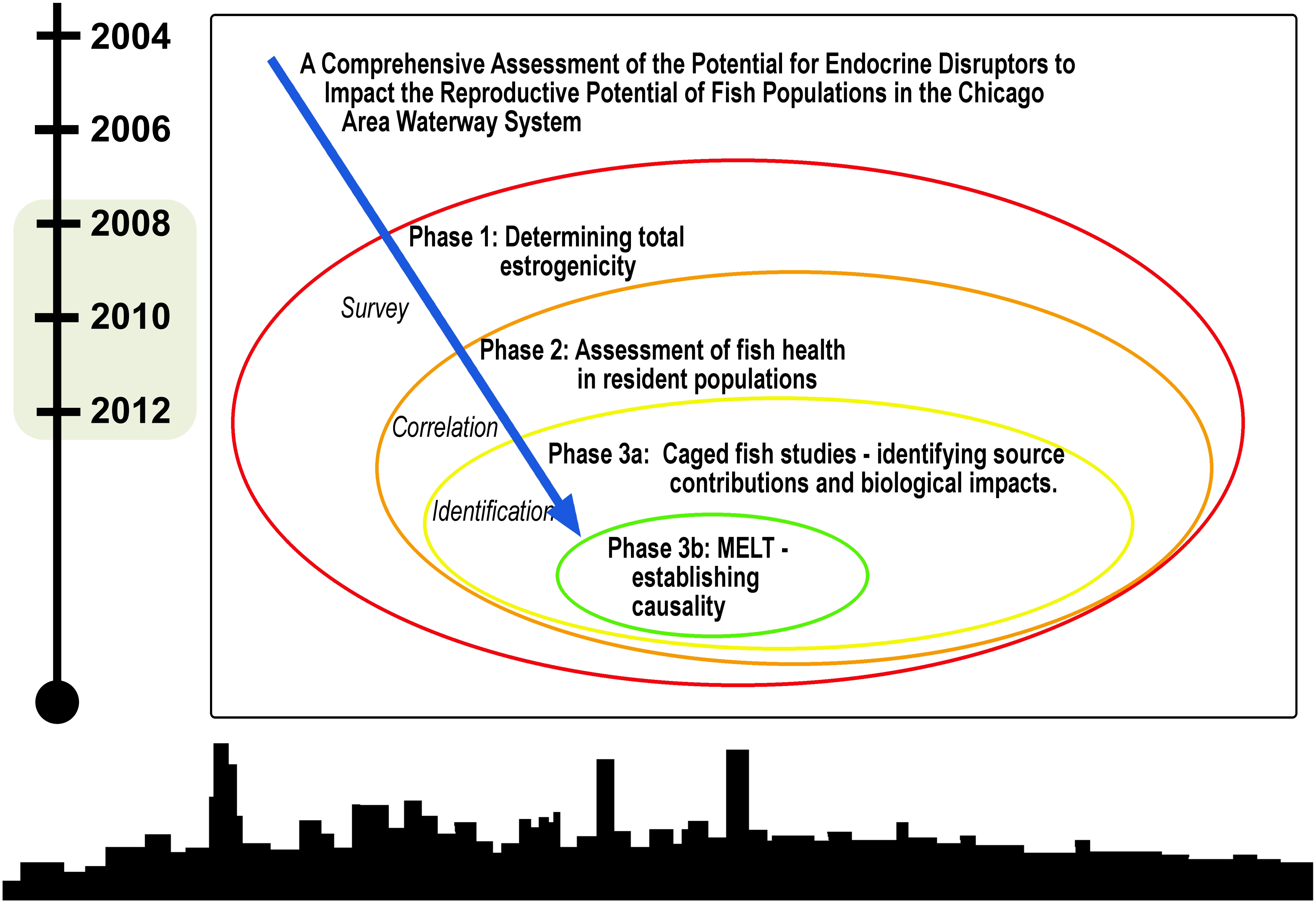
estrogens or estrogen-mimics

Vitellogenin = VTG

What are Biomarkers for EAC Exposure ?

Histology (microscopic assessment) of organs, especially liver and reproductive organs.





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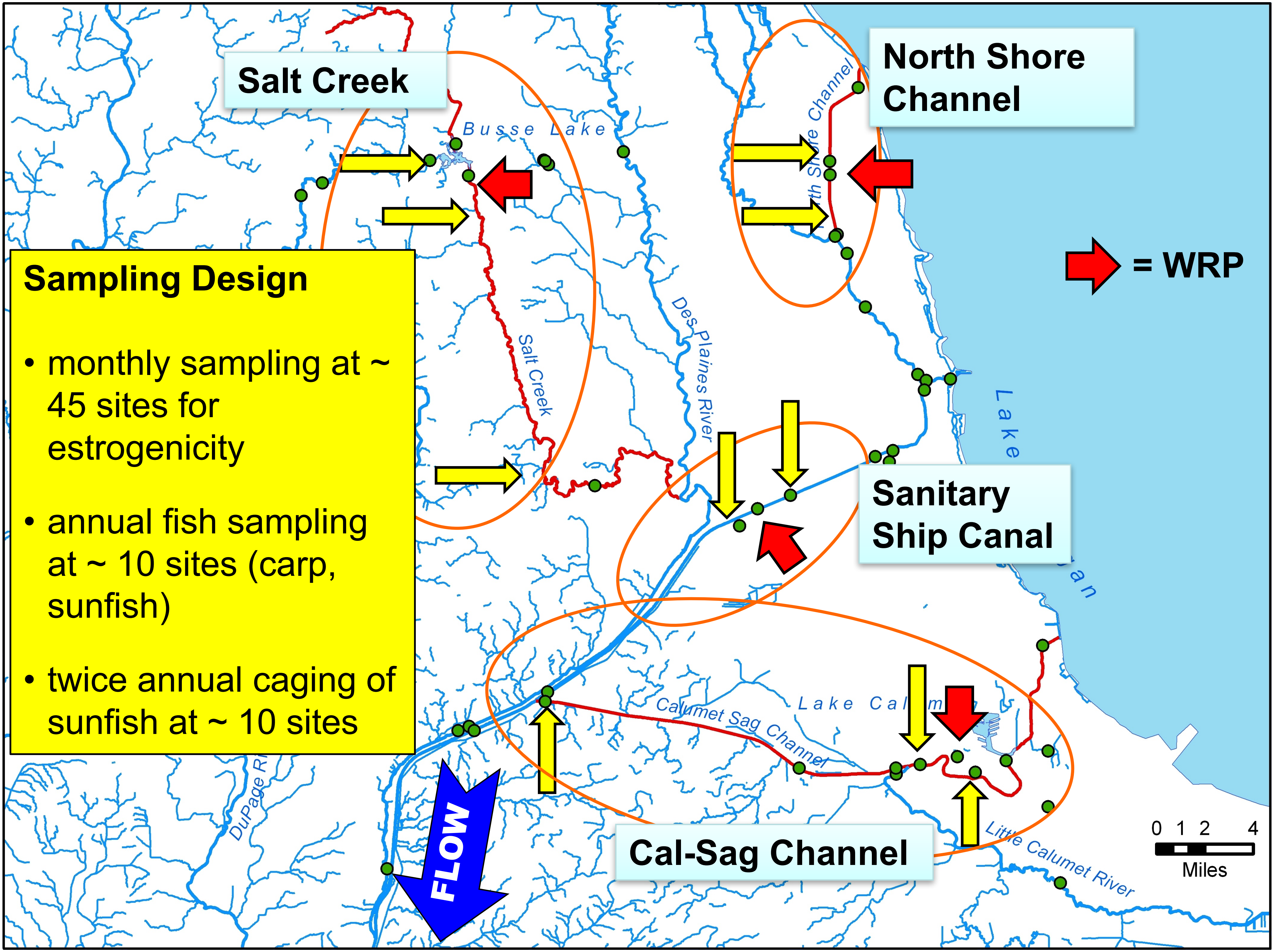
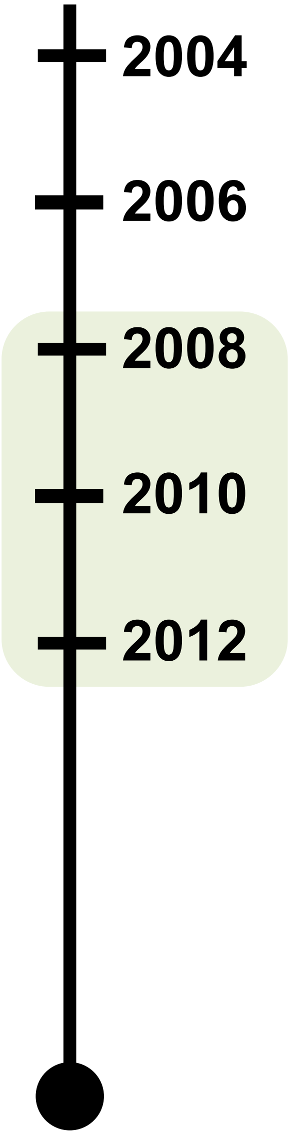
A Comprehensive Assessment of the Potential for Endocrine Disruptors to Impact the Reproductive Potential of Fish Populations in the Chicago Area Waterway System
Phase 1: Determining total estrogenicity
Survey
Phase 2: Assessment of fish health in resident populations
Correlation
Phase 3a: Caged fish studies - identifying source contributions and biological impacts.
Identification
Phase 3b: MELT - establishing causality


Salt Creek
North Shore Channel
Busse Lake
Salt Creek
Des Plaines River
North Shore Channel
= WRP
Lake Michigan
Sanitary Ship Canal
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
Calumet Sag Channel
Lake Calumet
DuPage River
FLOW
Cal-Sag Channel
Little Calumet River
0 1 2 4
Miles



A Comprehensive Assessment of the Potential for Endocrine Disruptors to Impact the Reproductive Potential of Fish Populations in the Chicago Area Waterways

Environment International 61 (2013) 127–137

Contents lists available at ScienceDirect

Environment International

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

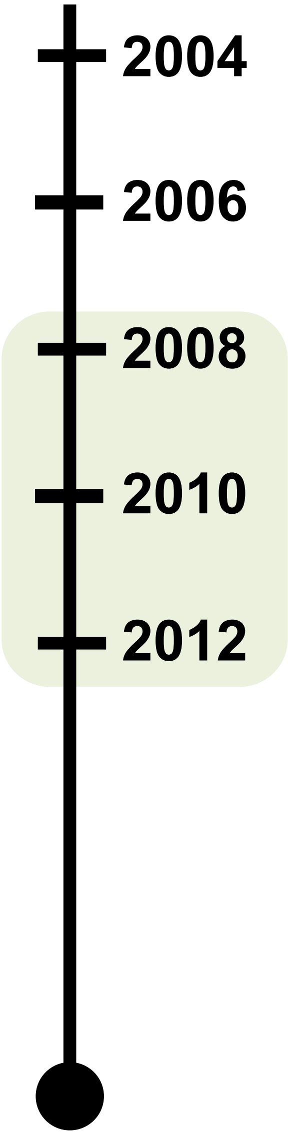
Environmental estrogens in an urban aquatic ecosystem: I. Spatial and temporal occurrence of estrogenic activity in effluent-dominated systems

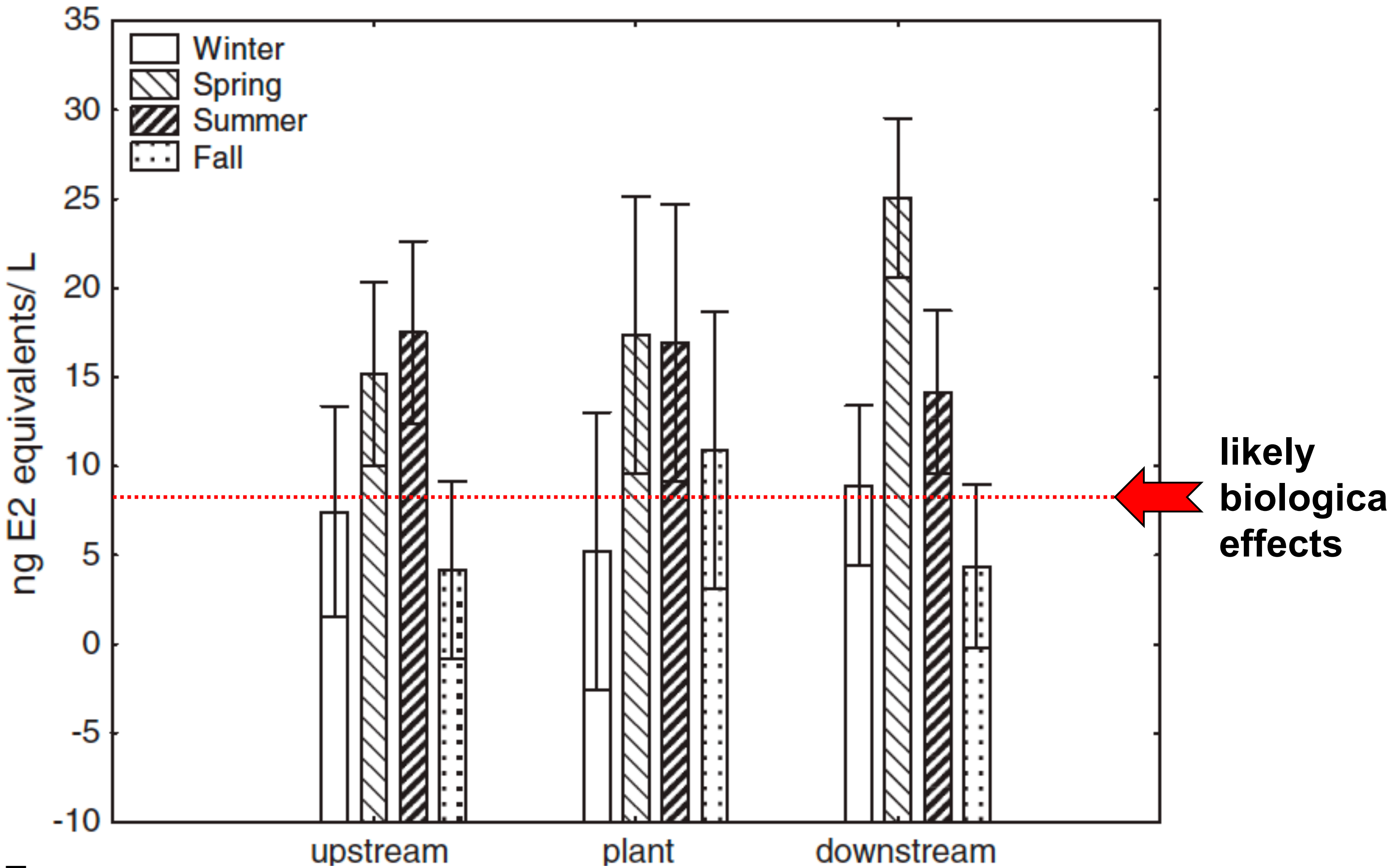
Dalma Martinovic-Weigelt [a,*], Thomas A. Minarik [b], Erin M. Curran [a], Jascha S. Marchuk [a], Matt J. Pazderka [a], Eric A. Smith [a], Rachel L. Goldenstein [a], Christine L. Miresse [a], Thomas J. Matlon [a], Melissa M. Schultz [c], Heiko L. Schoenfuss [d]

Environmental estrogens in an urban aquatic ecosystem: II. Biological effects

Melissa M. Schultz [a], Thomas A. Minarik [b], Dalma Martinovic-Weigelt [c], Erin M. Curran [c], Stephen E. Bartell [d,e], Heiko L. Schoenfuss [e,*]

→ **EACs are common in the Chicago Area Waterways and WRP effluent contributes to their presence.**





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→ Confirmed through our Mobile Exposure Laboratory Trailer (MELT) fathead minnow exposures

JOURNAL OF THE AMERICAN WATER RESOURCES ASSOCIATION
Vol. 50, No. 2 AMERICAN WATER RESOURCES ASSOCIATION April 2014

ON-SITE EXPOSURE TO TREATED WASTEWATER EFFLUENT HAS SUBTLE EFFECTS ON MALE FATHEAD MINNOWS AND PRONOUNCED EFFECTS ON CARP[1]

Thomas A. Minarik, Justin A. Vick, Melissa M. Schultz, Stephen E. Bartell, Dalma Martinovic-Weigelt, Daniel C. Rearick, and Heiko L. Schoenfuss[2]

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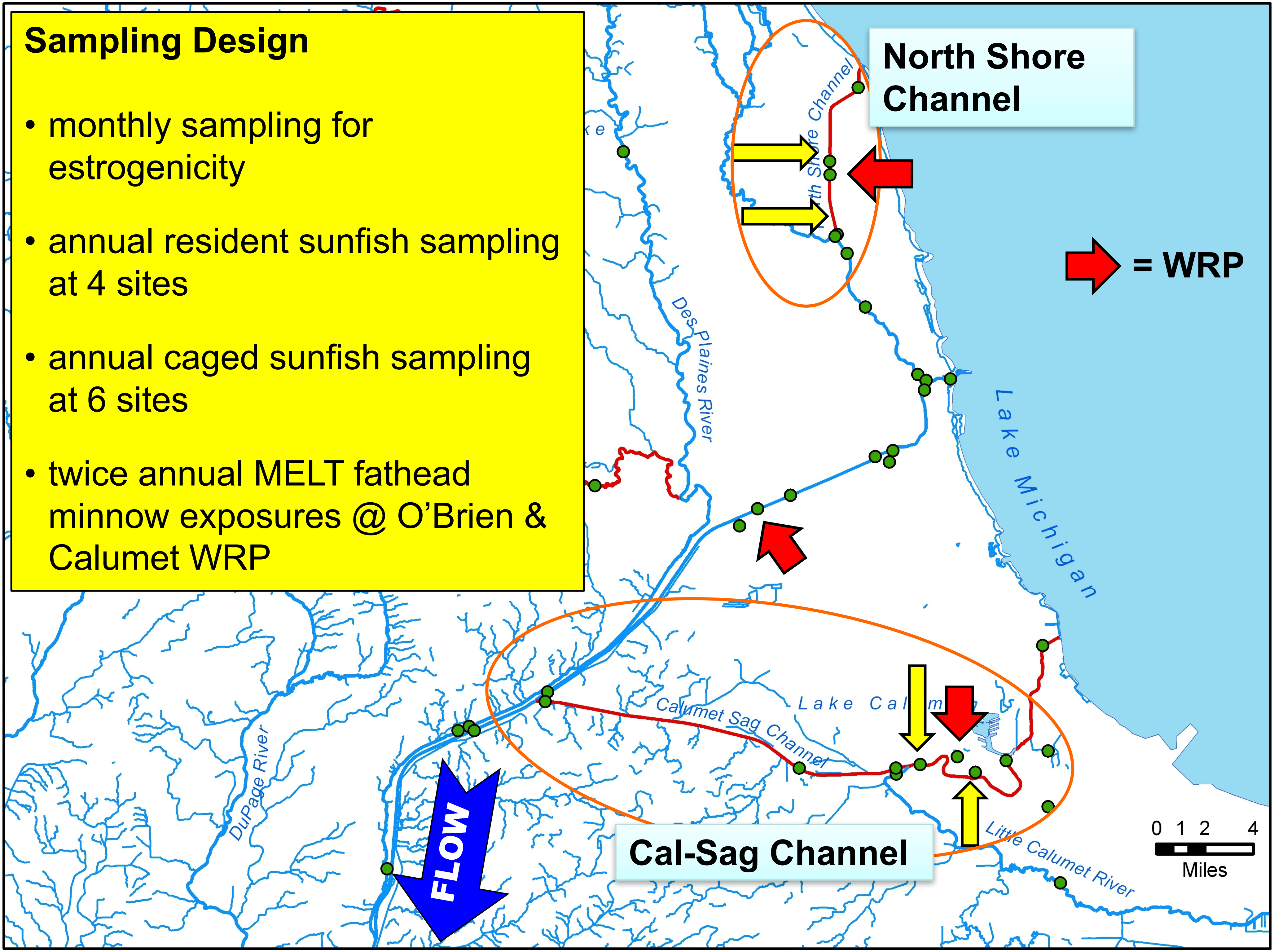
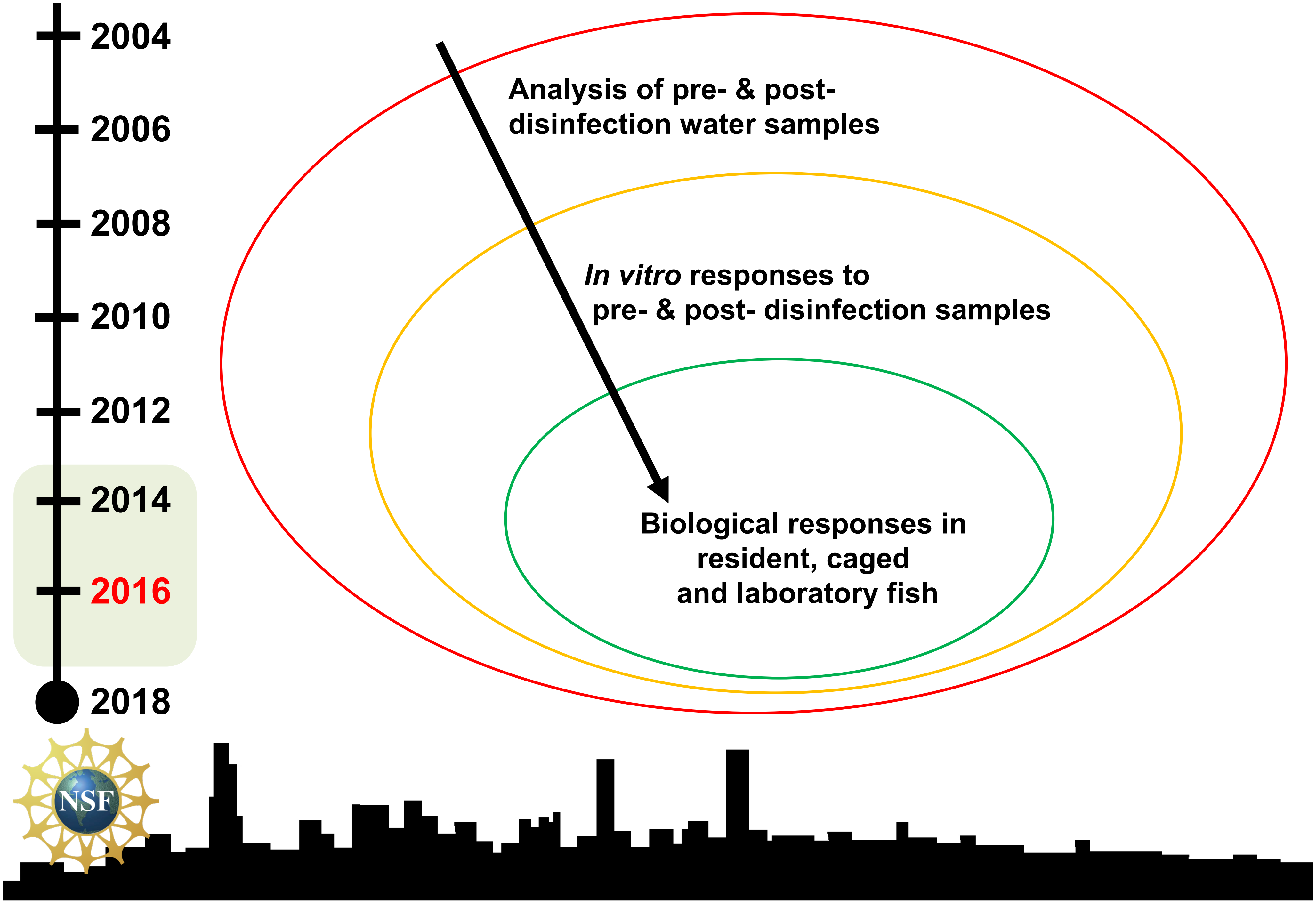
→ **Effluent treatment upgrades to disinfection at Terrence O'Brien (UV) and Calumet (chlorination/ dechlorination) WRP.**

Assessing Load Reduction and Biological Recovery After 500 MGD Treatment Upgrades in an Effluent-Dominated Aquatic Ecosystem

Sampling Design

- monthly sampling for estrogenicity
- annual resident sunfish sampling at 4 sites
- annual caged sunfish sampling at 6 sites
- twice annual MELT fathead minnow exposures @ O'Brien & Calumet WRP

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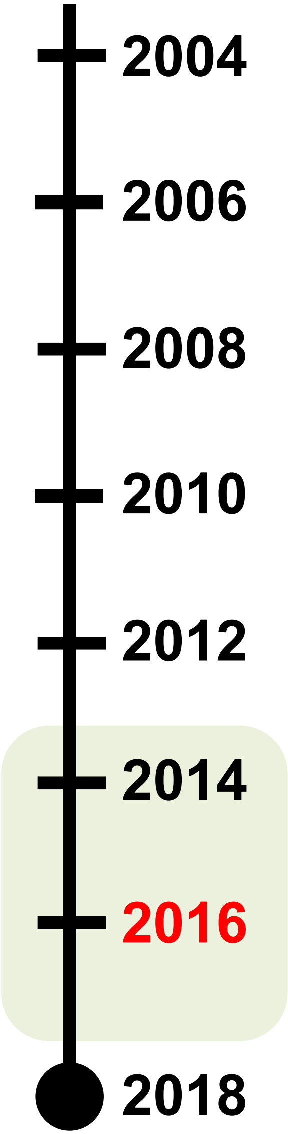
Analysis of pre- & post-disinfection water samples
In vitro responses to pre- & post- disinfection samples
Biological responses in resident, caged and laboratory fish
NSF



Analysis of pre- & post-disinfection water samples:

- Water sample collections 2014 through 2017
- MELT water sample collects during fish exposures
- Benchtop confirmatory experiments

estrone (E1)	estrogenic hormone
estradiol (E2)	estrogenic hormone
bupropion	antidepressant
carbamezipine	anti-epileptic
citalopram	antidepressant
duloxetine	antidepressant
fluoxetine	antidepressant
norfluoxetine	metabolite
norsertraline	metabolite
paroxetine	antidepressant
sertraline	antidepressant
venlafaxine	antidepressant

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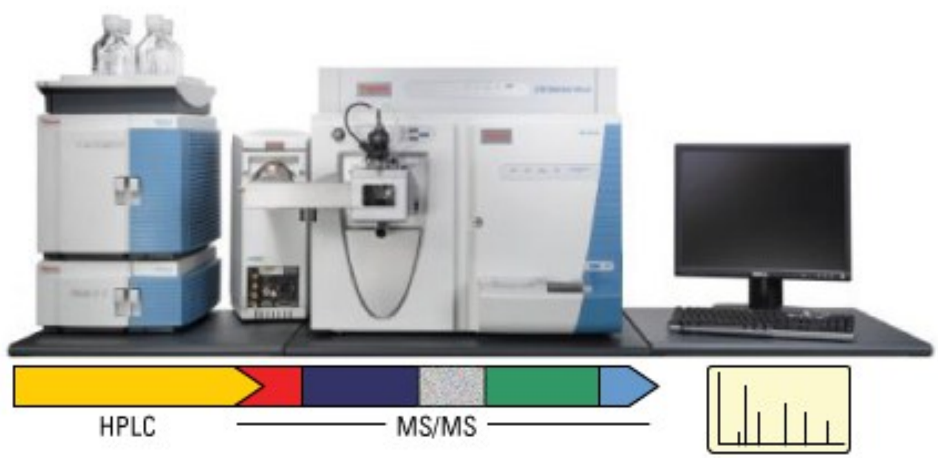
Analytical methodology

Samples were filtered to remove suspended solids.

Isotopically labeled internal standards were added for each pharmaceutical compound.

Compounds were extracted and concentrated using solid phase extraction.

Measurement using HPLC-MS/MS.



O'Brien WRP- UV Disinfection: Pharmaceuticals Pre- and Post-Disinfection, 2016-2017

Compound	Samples Detected§	Average Concentration (ng/L) Pre-disinfection	Post-disinfection	Percent Change	*p*
estrone (E1)	1	9 ± 2	9 ± 2	-6	0.768
estradiol (E2)	1	2 ± 3	nd	-	-
bupropion	15	120 ± 50	100 ± 40	-1	0.247
carbamezipine	15	230 ± 150	170 ± 70	-29	0.096
citalopram	15	130 ± 40	120 ± 40	-7	0.389
duloxetine	10	12 ± 15	4 ± 3	-65	0.098
fluoxetine	15	13 ± 17	20 ± 35	53	0.239
norfluoxetine	7	3 ± 4	4 ± 6	66	0.026
norsertraline	15	210 ± 140	180 ± 150	-6	0.726
paroxetine	4	8 ± 1	2 ± 2	-68	0.249
sertraline	16	60 ± 90	24 ± 16	-62	0.113
venlafaxine	16	240± 440	160 ± 60	-37	0.256

§Total sampling events= 16; *p* = probability value.

provisional data – do not cite

Bench Scale Testing of Transformation: UV

Pharmaceutical	% Decrease after 2 min		% Decrease after 15 min	
	Deionized Water	Wastewater Effluent	Deionized Water	Wastewater Effluent
estrone				
4-nonylphenol	90	93	100	100
bupropion	3.96	17.8	76.21	92.2
carbamazepine	-2.2	0	2.9	0
citalopram	33.4	0	93.0	86.1
duloxetine	100	0	100	100
fluoxetine	98.3	100	100	87.6
norfluoxetine	100	17.6	100	0
norsertraline	31.1	23.4	95.8	7.53
paroxetine	46.9	100	94.6	100
sertraline	99.2	100	95.6	100
venlafaxine	4.6	34.5	24.0	99.9

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Comparison to Effluent Data to Bench Testing

O'Brien WRP: UV Disinfection, 7-Day MELT Composite Samples: Spring 2017

Compound	Concentration (ng/L)		Percent	
	Pre	Post	Change	*p*
estrone	9 ± 2	9 ± 2	-6	
estradiol	2.3 ± 3	nd		
bupropion	100 ± 3	83 ± 2	-17	<0.001
carbamazepine	102 ± 5	100 ± 20	-	0.243
citalopram	84 ± 2	68 ± 2	-19	<0.001
duloxetine	4.9 ± 0.4	2.9 ± 0.1	-39	<0.001
fluoxetine	81 ± 22	52 ± 3	-35	0.049
norfluoxetine	7 ± 4	6 ± 1	-39	0.187
norsertraline	195 ± 100	153 ± 18	-21	<0.001
paroxetine	2.6 ± 0.2	2.1 ± 0.2	-	0.113
sertraline	22 ± 0.3	17 ± 1	-19	0.001
venlafaxine	89 ± 1	75 ± 2	-16	0.001

→ *Good match between compounds observed to decrease in effluent and those determined to be transformed in laboratory.*

Calumet WRP- Hypochlorite Disinfection: Pharmaceuticals Pre- and Post-Disinfection

Compound*	Samples Detected[§]	Average Concentration (μg/L) Pre-disinfection	Post-disinfection	Percent Change	*p*
estrone (E1)	1	9 ± 1	8 ± 1	-12	0.155
estradiol (E2)	0	nd	nd		
bupropion	16	90 ± 100	60 ± 40	-30	0.368
carbamazepine	16	160 ± 50	150 ± 50	-1	0.805
citalopram	16	82 ± 70	29 ± 20	-64	0.012
duloxetine	16	3 ± 3	2 ± 2	-53	0.005
fluoxetine	16	320 ± 550	130 ± 140	-59	0.212
norfluoxetine	10	63 ± 68	18 ± 31	-71	0.020
norsertraline	16	270 ± 240	220 ± 180	-12	0.629
paroxetine	4	3 ± 1	2 ± 1	-17	0.447
sertraline	16	39 ± 65	13 ± 6	-66	0.130
venlafaxine	16	100 ± 40	67 ± 28	-33	0.002

[§]Total sampling events = 16; *p* = probability value.

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Bench Scale Testing of Transformation: Chlorination

Pharmaceutical	% Reacted after 30 minutes of Chlorination (Di Water)	% Reacted after 30 minutes of Chlorination (Wastewater)	% Recovery after 2 minutes of Dechlorination (Di Water)	% Recovery after 2 minutes of Dechlorination (Wastewater)
estrone				
4-nonylphenol				
bupropion	99.8	100	5.7	0.0793
carbamazepine	26.8	1.12	70.9	83.7
citalopram	100	99.9	45.7	0.42
duloxetine	100	100	15.7	15.2
fluoxetine	95.8	97.7	78.7	58.2
norfluoxetine	100	100	83.5	84.1
norsertraline	100	40.8	0	100
paroxetine	100	100	0	0
sertraline	100	91.9	33.1	100
venlafaxine	100	100	0	17.8

provisional data – do not cite

Comparison to Effluent Data to Bench Testing

Calumet WRP: Chlorination 7-Day MELT Composite Samples: Spring 2017

Compound	Concentration (ng/L) Pre	Post	Percent Change	*p*
estrone	9 ± 1	8 ± 1	-12	0.156
estradiol	nd	nd		
bupropion	47.6 ± 0.3	32.3 ± 0.3	-32	<0.001
carbamazepine	73 ± 2	67 ± 3	0	0.016
citalopram	43.9 ± 0.3	31.9 ± 0.4	-27	<0.001
duloxetine	3.3 ± 0.1	2.4 ± 0.1	- 22	0.001
fluoxetine	36 ± 5	39 ± 3	0	0.179
norfluoxetine	1.8 ± 1	5 ± 4	138	0.158
norsertraline	190 ± 9	130 ± 4	-31	<0.001
paroxetine	2 ± 1	0.5 ± 0.5	-71	0.050
sertraline	8 ± 7	9 ± 1	0	0.465
venlafaxine	53 ± 2	41 ± 0.5	-23	0.001

→ *Good match between compounds observed to decrease in effluent and those determined to be transformed in laboratory.*

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Summary - Water Chemistry

1. **UV Disinfection**
   i. Reduction in load of some endocrine active compounds
   ii. Reactions are dependent on dissolved oxygen
   iii. Reaction products are mixtures of oxidized compounds
2. **Chlorination**
   i. Reduction in load of some endocrine active compounds
   ii. Reaction products may include chlorinated products
3. **Benchtop Validation Experiments**
   i. Good match to environmental conditions

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Predictive & Cell-Based *In Vitro* Toxicology

- Evaluate measured chemical concentrations against national toxicity data base ("Tox21")
  - **E**xposure **A**ctivity **R**atio *>1 likely biological effect*
- Evaluate *in vitro* molecular response to pre- and post-disinfected effluent
  - Biological pathway analysis
  - Global clustering analysis

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Predictive & Cell-Based Toxicology: Exposure Activity Ratio

Biological Process	O'Brien (EAR)	
	Pre-disinfection	UV disinfection
Cell Cycle	<0.0001	<0.0001
Cell Morphology	<0.0001	<0.0001
CYP	<0.0001	<0.0001
DNA Binding	<0.0001	<0.0001
GPCR	<0.0001	0
Ion Channel	<0.0001	0
Miscellaneous Protein	0	0
Nuclear Receptor	**57.3563**	**95.6101**
Oxidoreductase	<0.0001	<0.0001
Steroid Hormone	0.0012	0.0023
Transporter	<0.0001	0

→ *nuclear receptors are likely to be activated by O'Brien effluent pre- and post- disinfection.*

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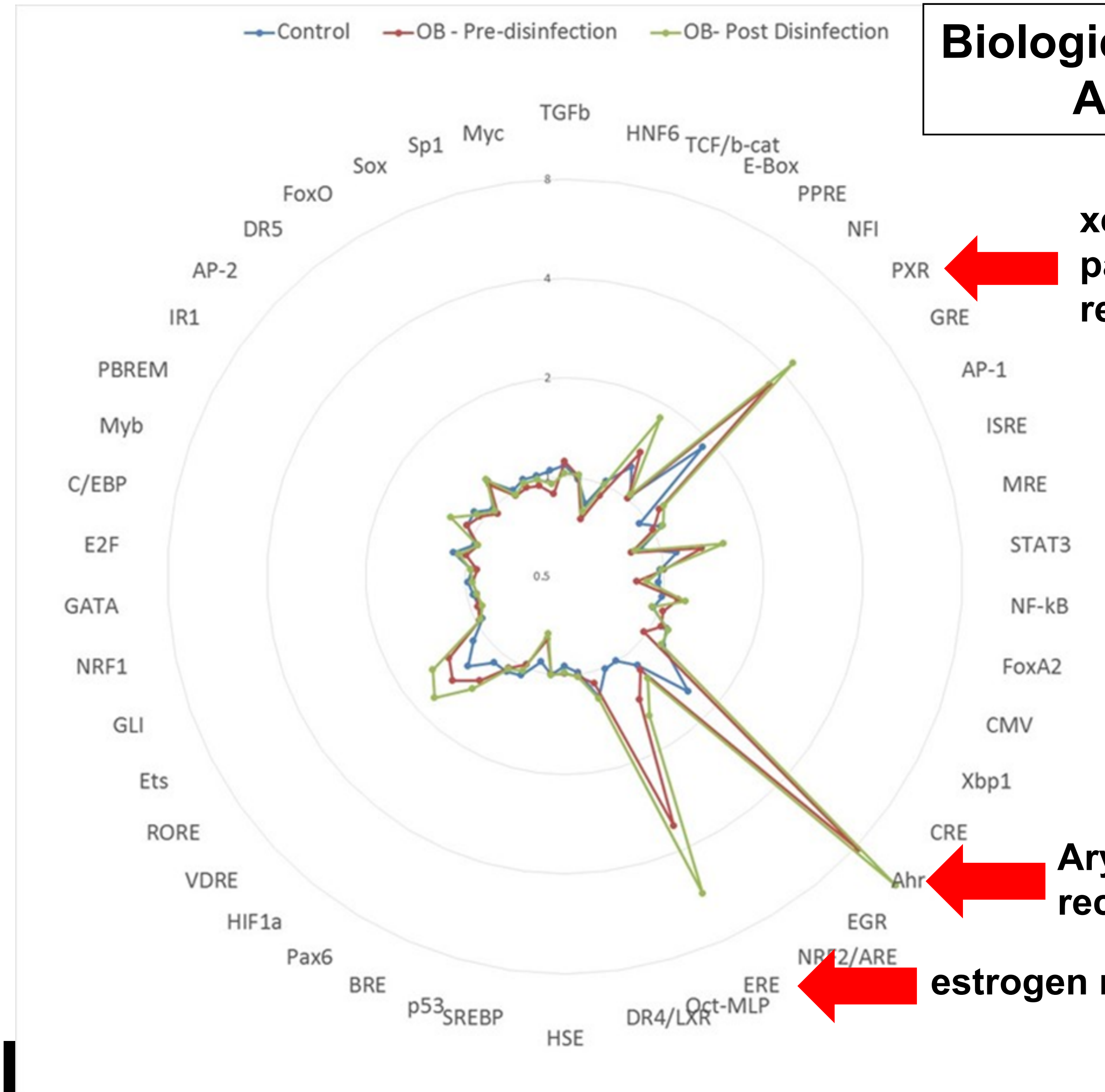
Biological Pathway Analysis



xenobiotic pathway receptor

Aryl-hydrocarbon receptor

estrogen receptor

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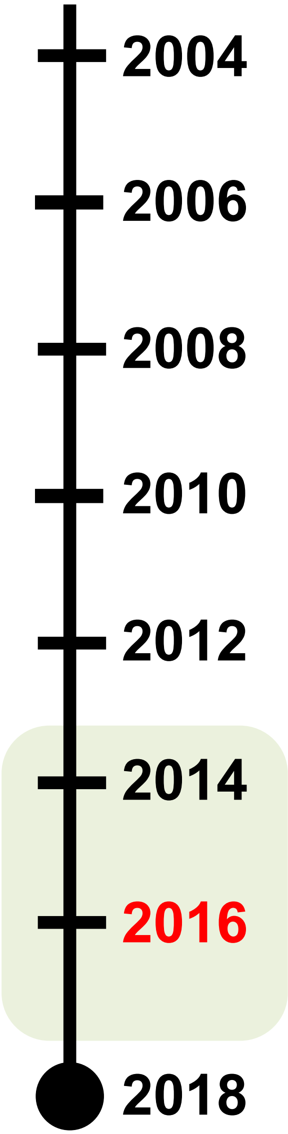

Predictive & Cell-Based Toxicology: Exposure Activity Ratio

Biological Process	Calumet (EAR)	
	Pre-disinfection	UV disinfection
Cell Cycle	<0.0001	<0.0001
Cell Morphology	<0.0001	<0.0001
CYP	<0.0001	<0.0001
DNA Binding	<0.0001	<0.0001
GPCR	0	0
Ion Channel	0	0
Miscellaneous Protein	0	0
Nuclear Receptor	0.0082	0.2082
Oxidoreductase	0	0
Steroid Hormone	0	<0.0001
Transporter	0	0

→ *nuclear receptors are not likely to be activated by Calumet effluent pre- or post- disinfection.*

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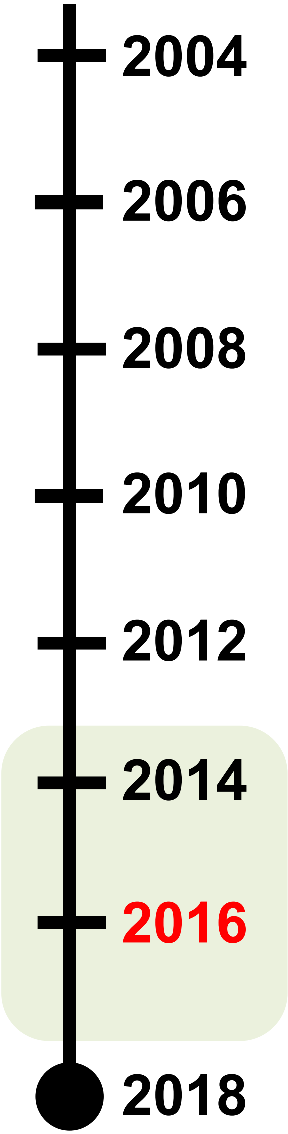

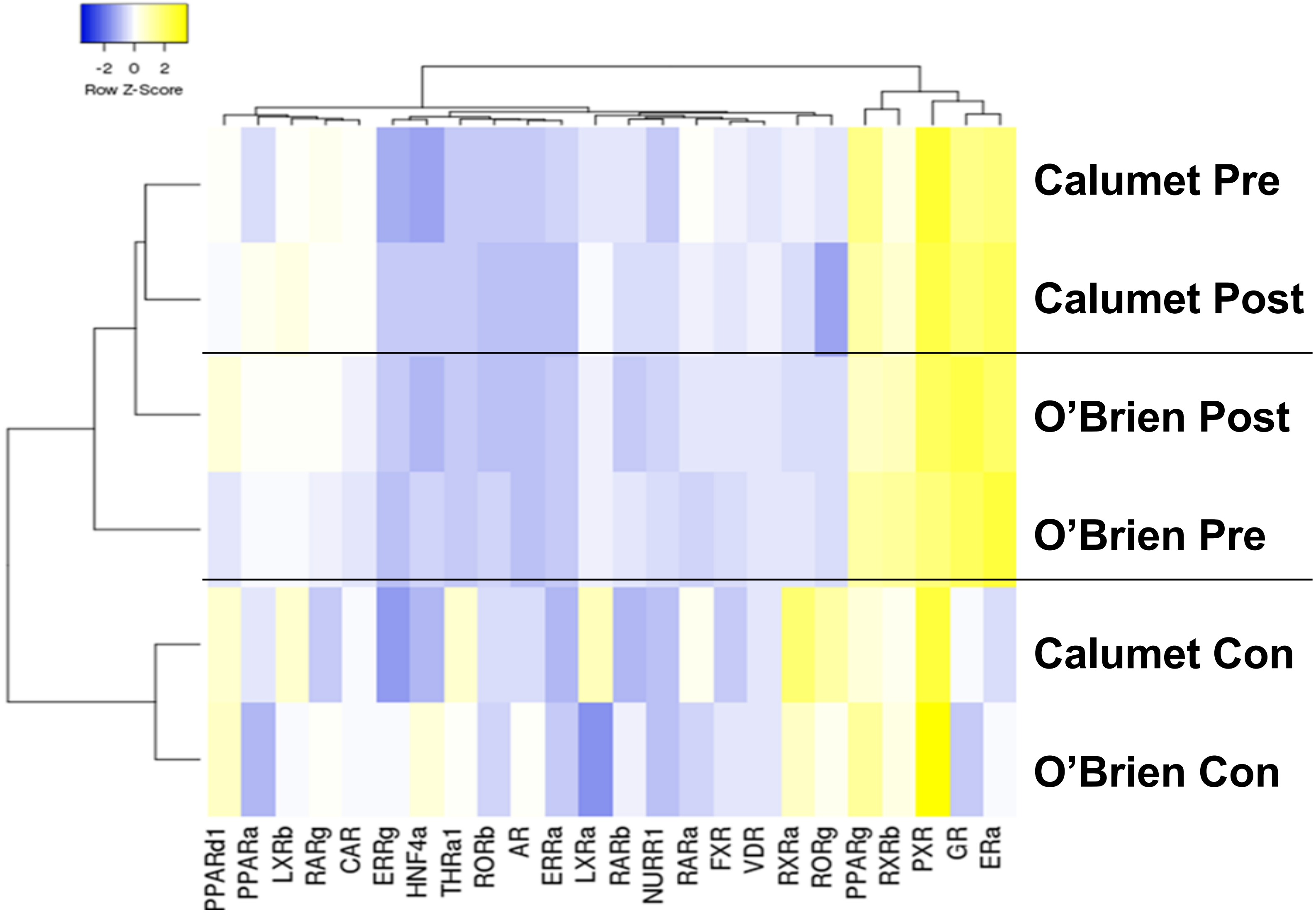

→ *global clustering analyses indicated similarity in pre- and post-disinfection biological activity profiles (in vitro)*

provisional data – do not cite

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Summary - *In Vitro* Toxicology

1. **Exposure Activity Ratio (EAR)**
   i. Nuclear receptors likely activated by O'Brien but not Calumet effluent
2. **Pathway Analysis**
   i. PXR, Ahr and ERE activation matches chemistry
3. **Global Cluster Analysis**
   i. similarity in pre- and post-disinfection biological activity profiles (*all analyses*)

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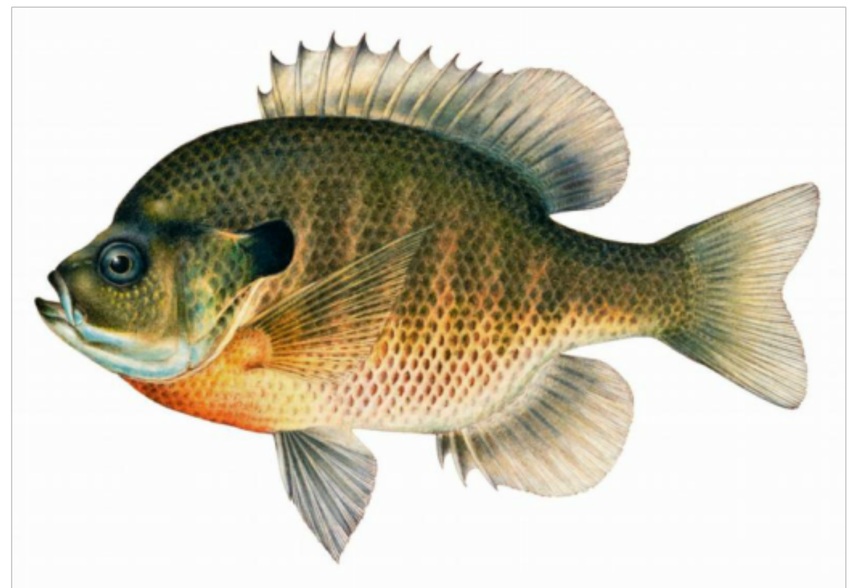
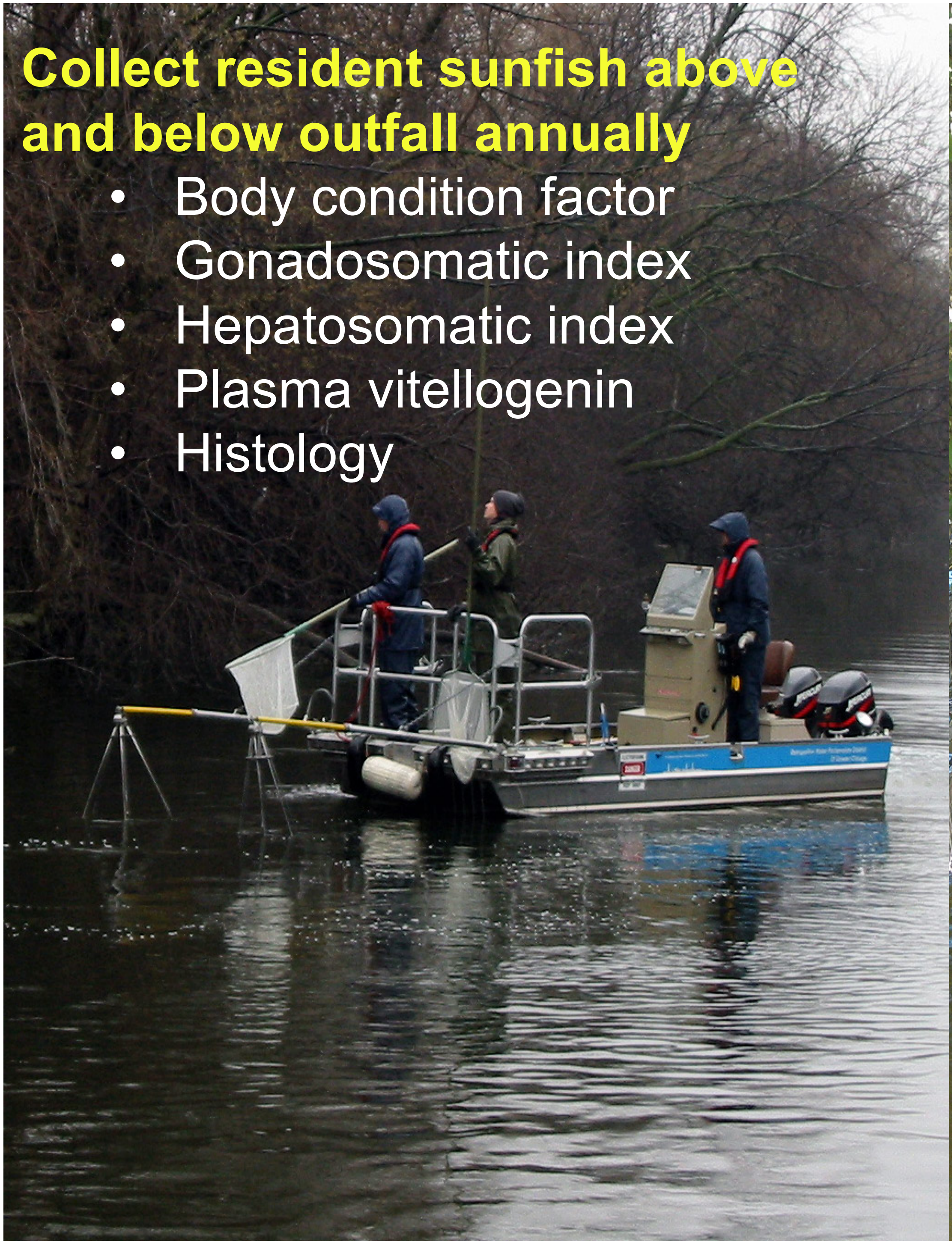
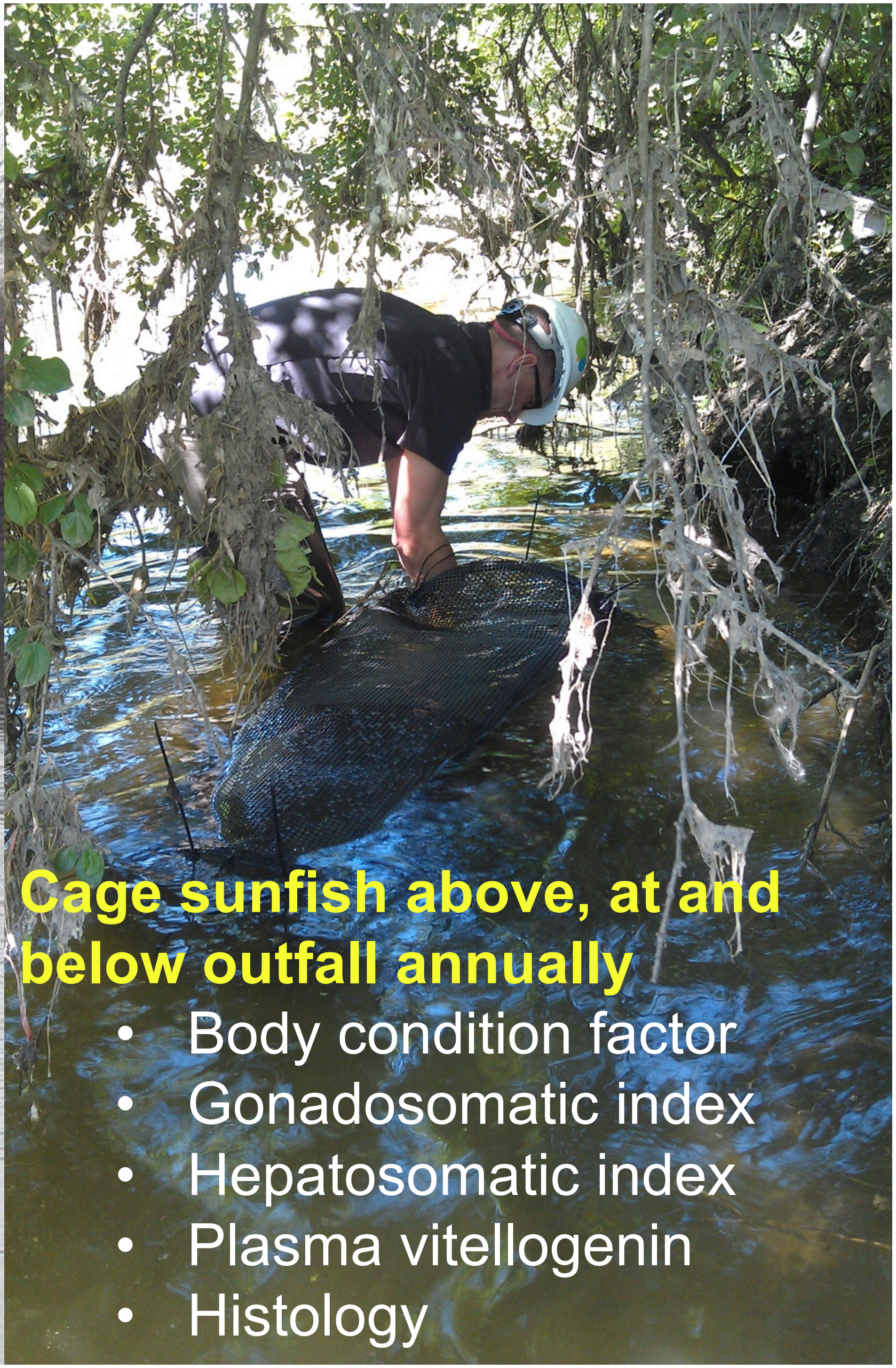
Biological Responses in Fish

- Evaluate resident sunfish annually in spring (2014-17)
- Evaluate caged sunfish annually in spring (2014-17)
- Conduct fathead minnow laboratory exposure experiments annually in spring and fall (2014-17)

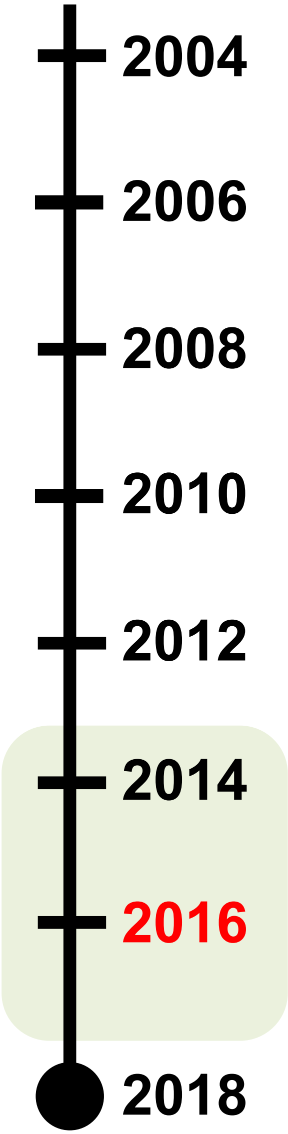


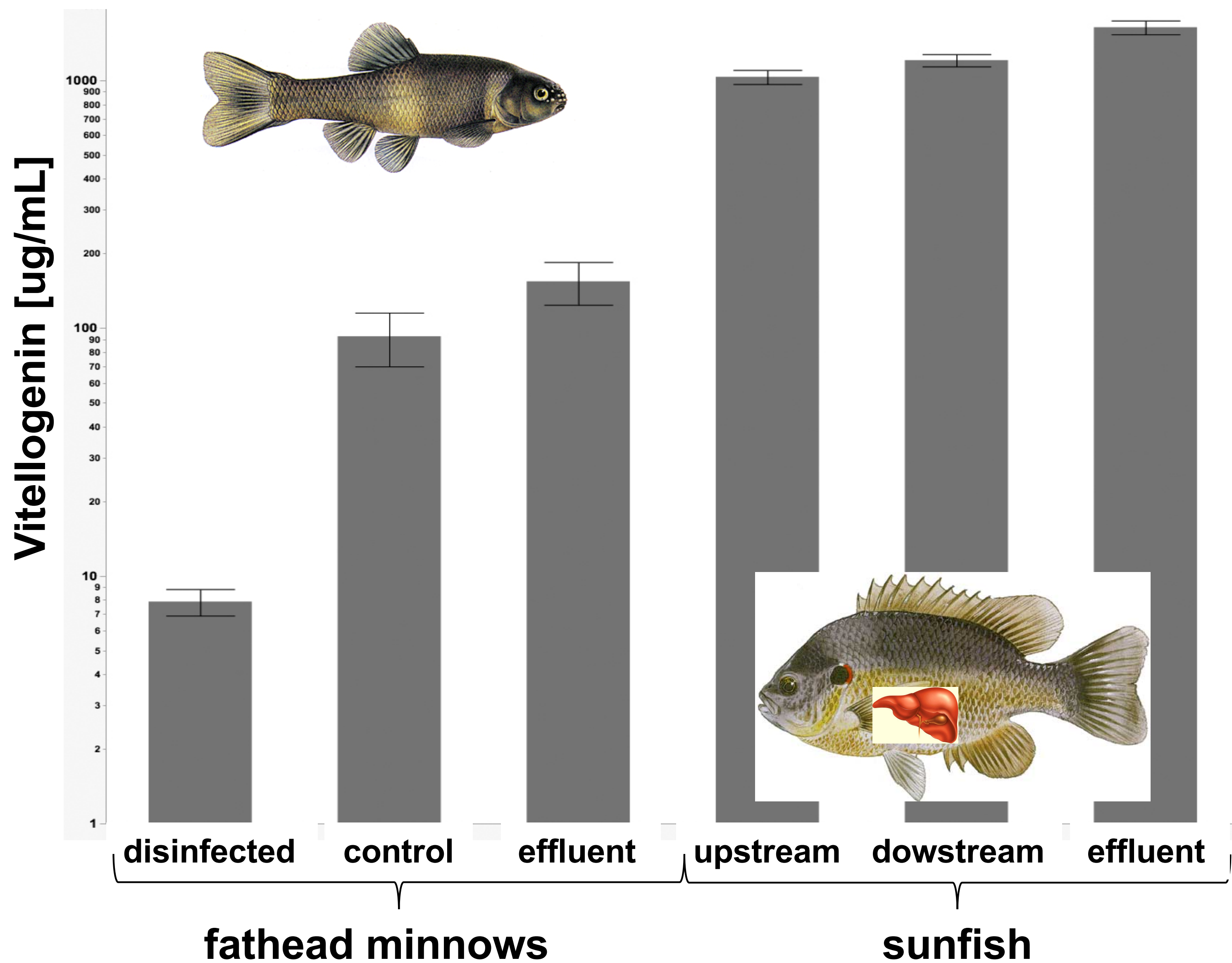
Biological Responses in Fish





Male Fathead Minnows vs. Male Sunfish - Vitellogenin

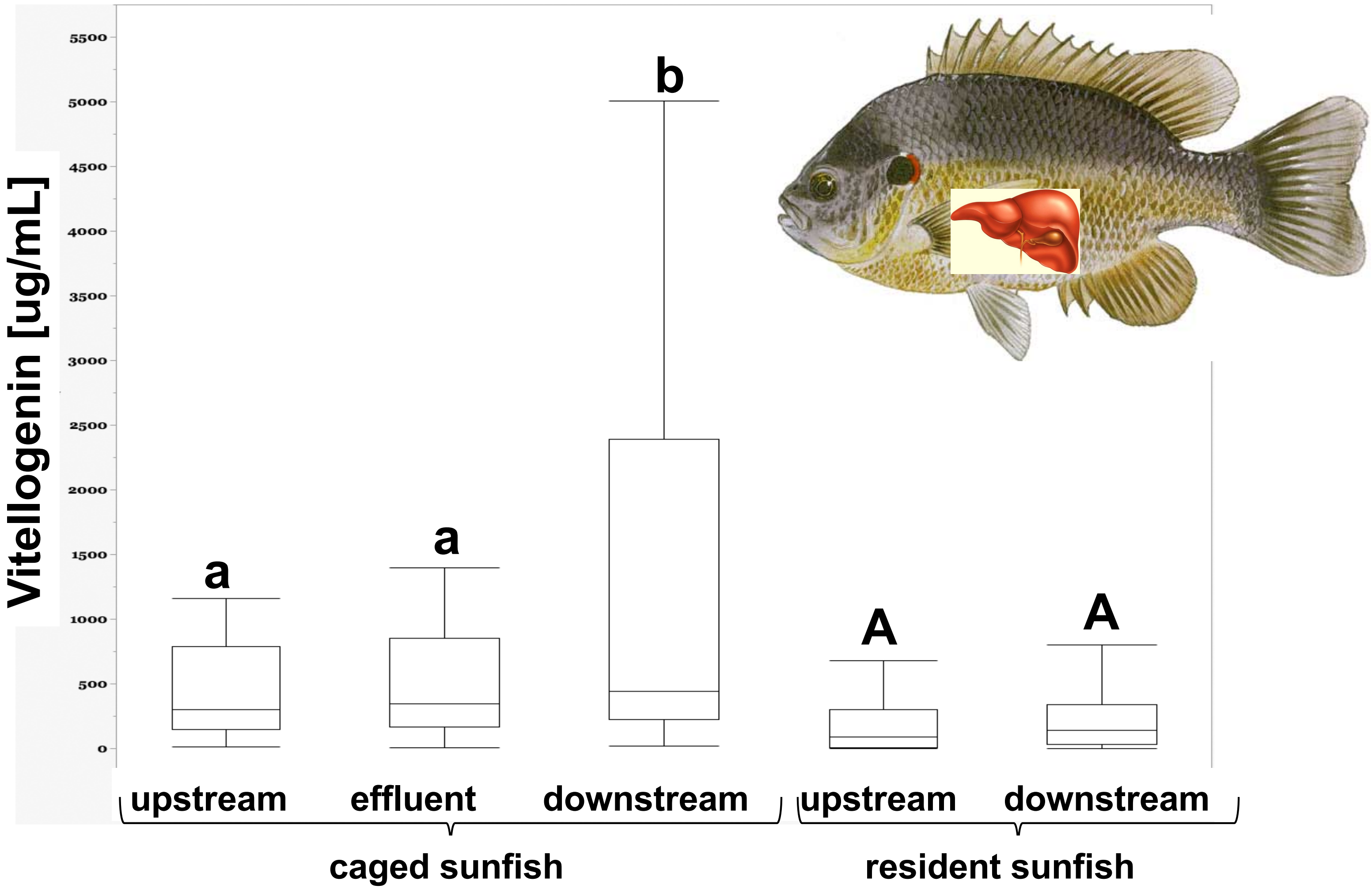




provisional data – do not cite

Caged & Resident Male Sunfish - Vitellogenin

2004
2006
2008
2010
2012
2014
2016
2018



→ *no immediate effect of disinfection on vitellogenin induction in sunfish.*

provisional data – do not cite

MELT Exposures – Fathead Minnows

2004
2006
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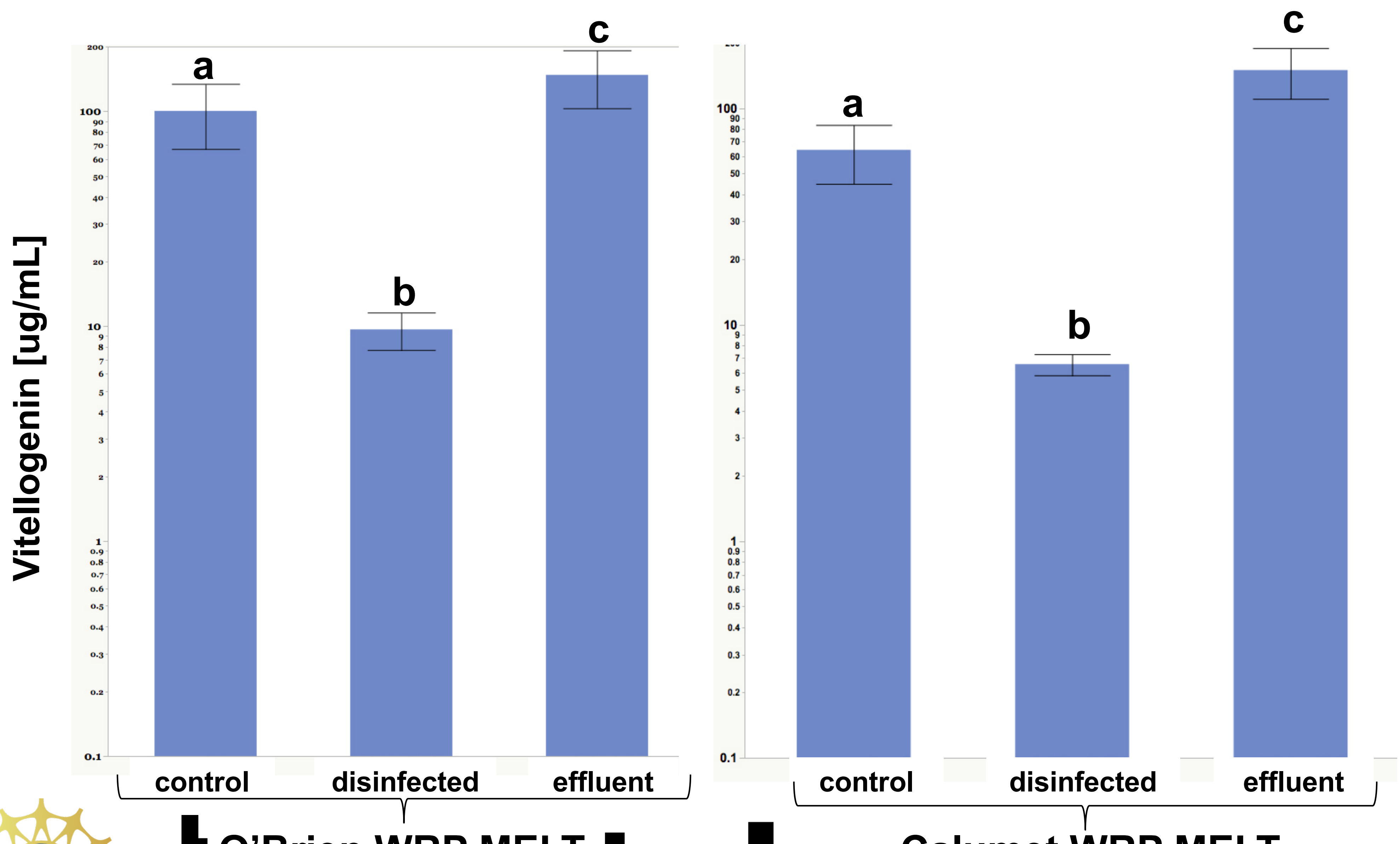
2012
2014
2016
2018

MELT Exposures – Vitellogenin



provisional data – do not cite

MELT Exposures - GSI

2004
2006
2008
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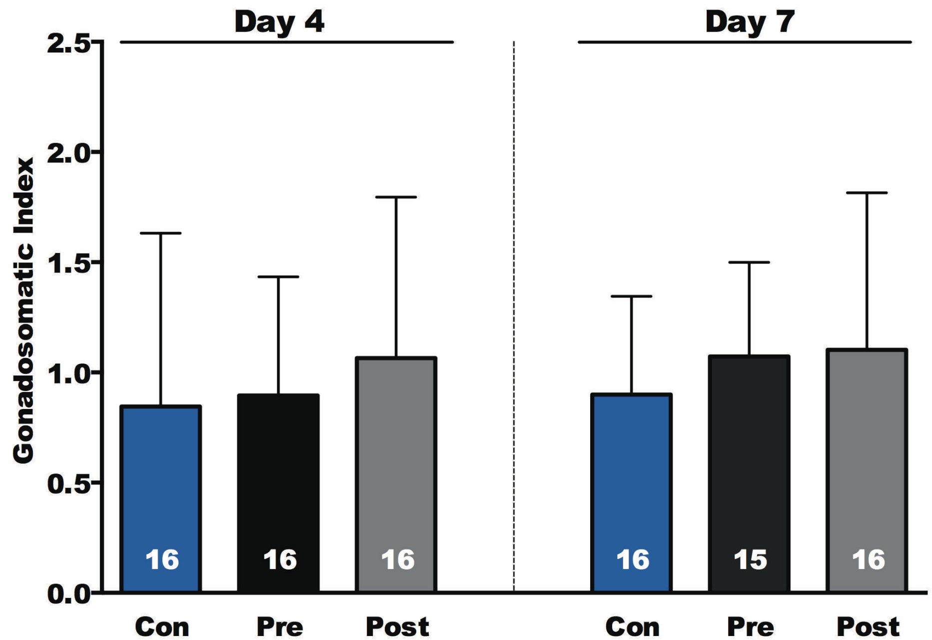
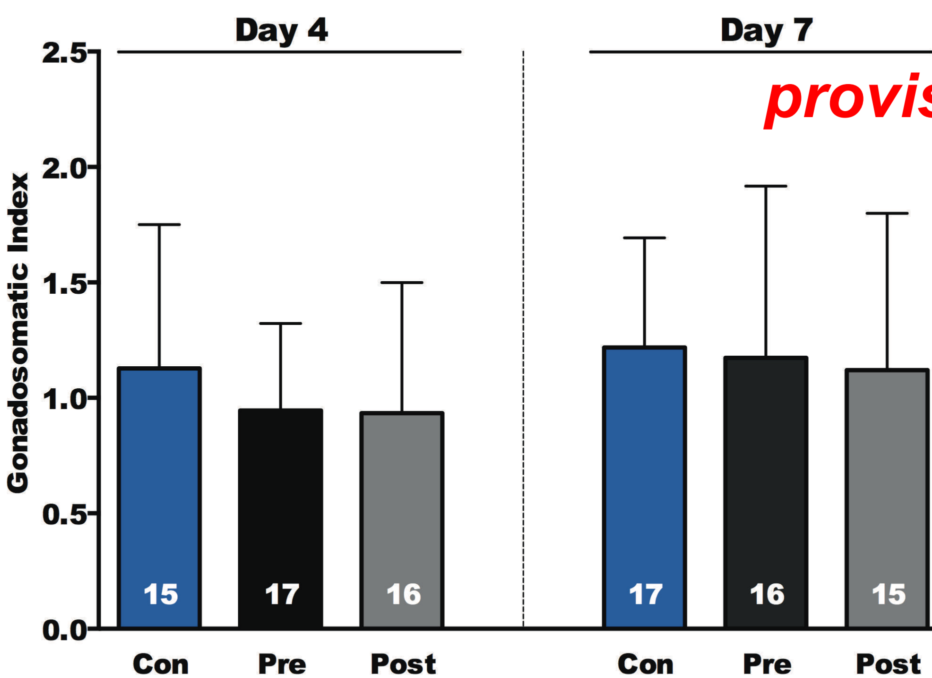
O'Brien Wastewater Reclamation Plant (UV-Disinfection)



Calumet Wastewater Reclamation Plant (Chlorination)



Statistical analysis: ANOVA with Holm-Sidak post-test

provisional data – do not cite

- *Disinfection does not alter the gonadosomatic index.*

MELT Exposures – Testis Histology

2004
2006
2008
2010
2012
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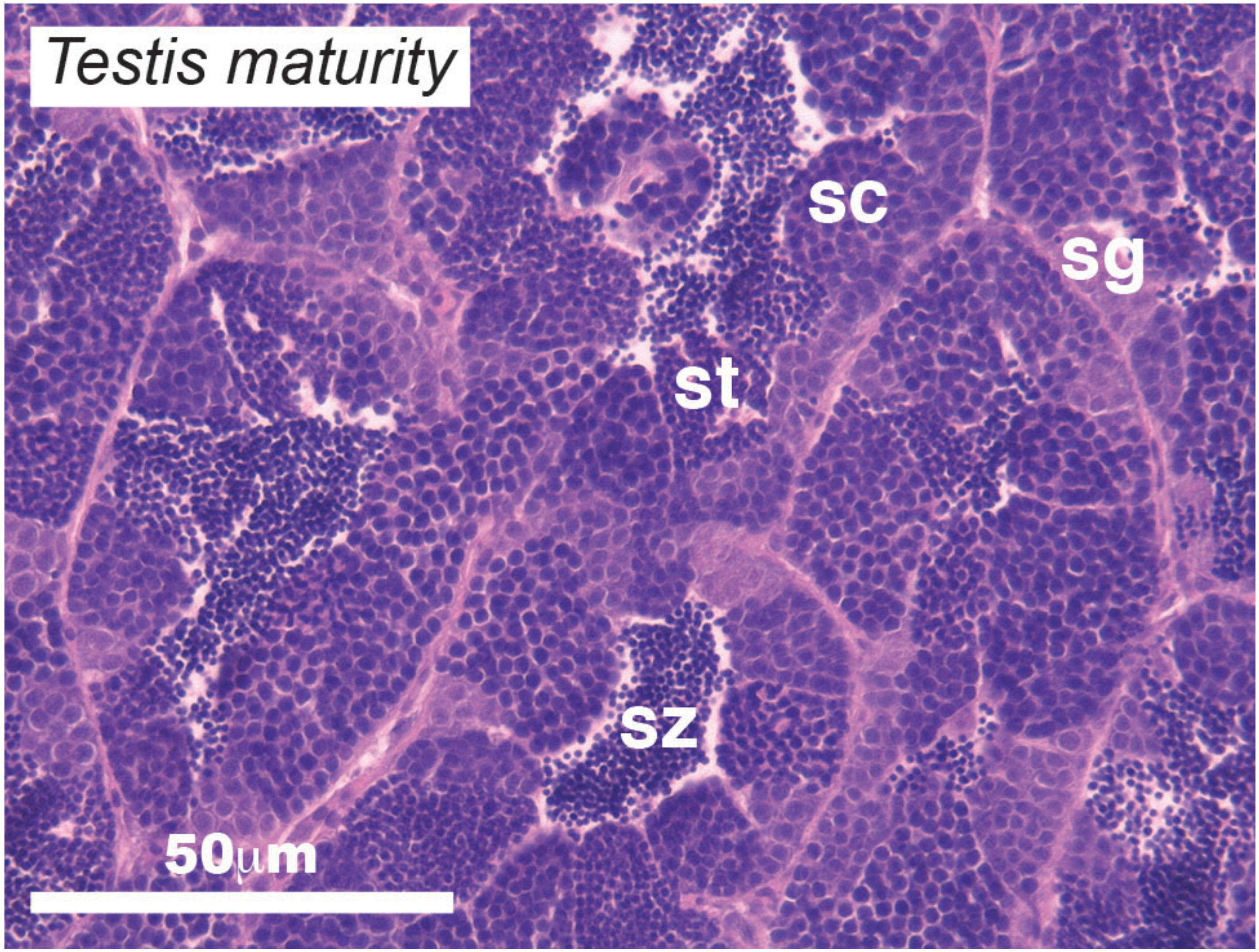
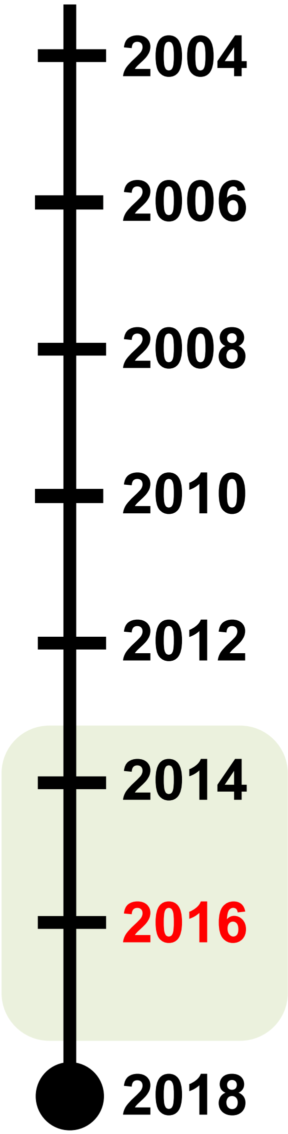
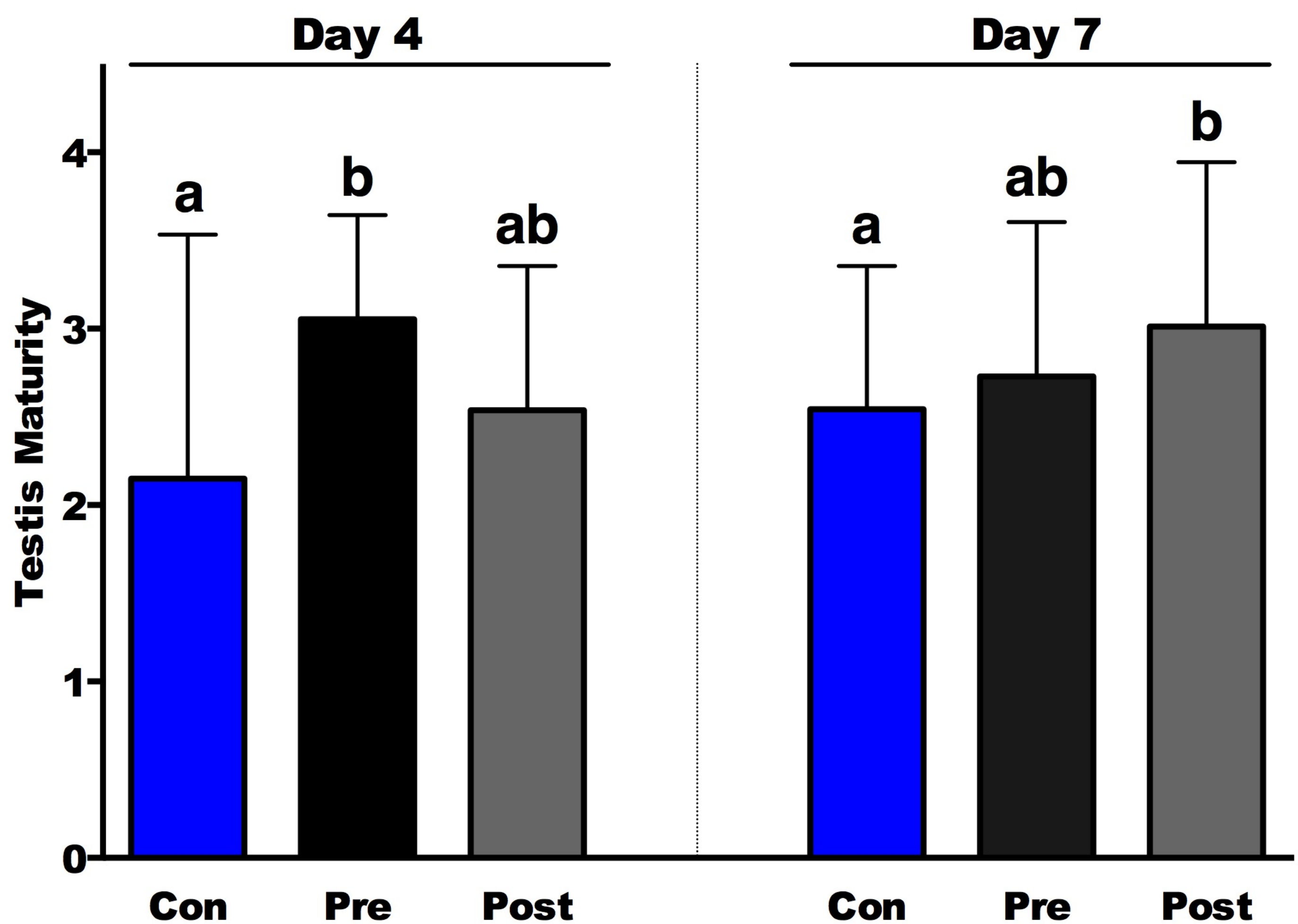
2016
2018



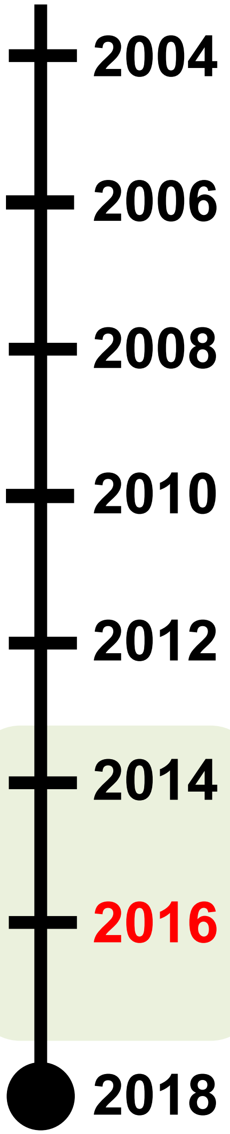


O'Brien Wastewater Reclamation Plant (UV-Disinfection)

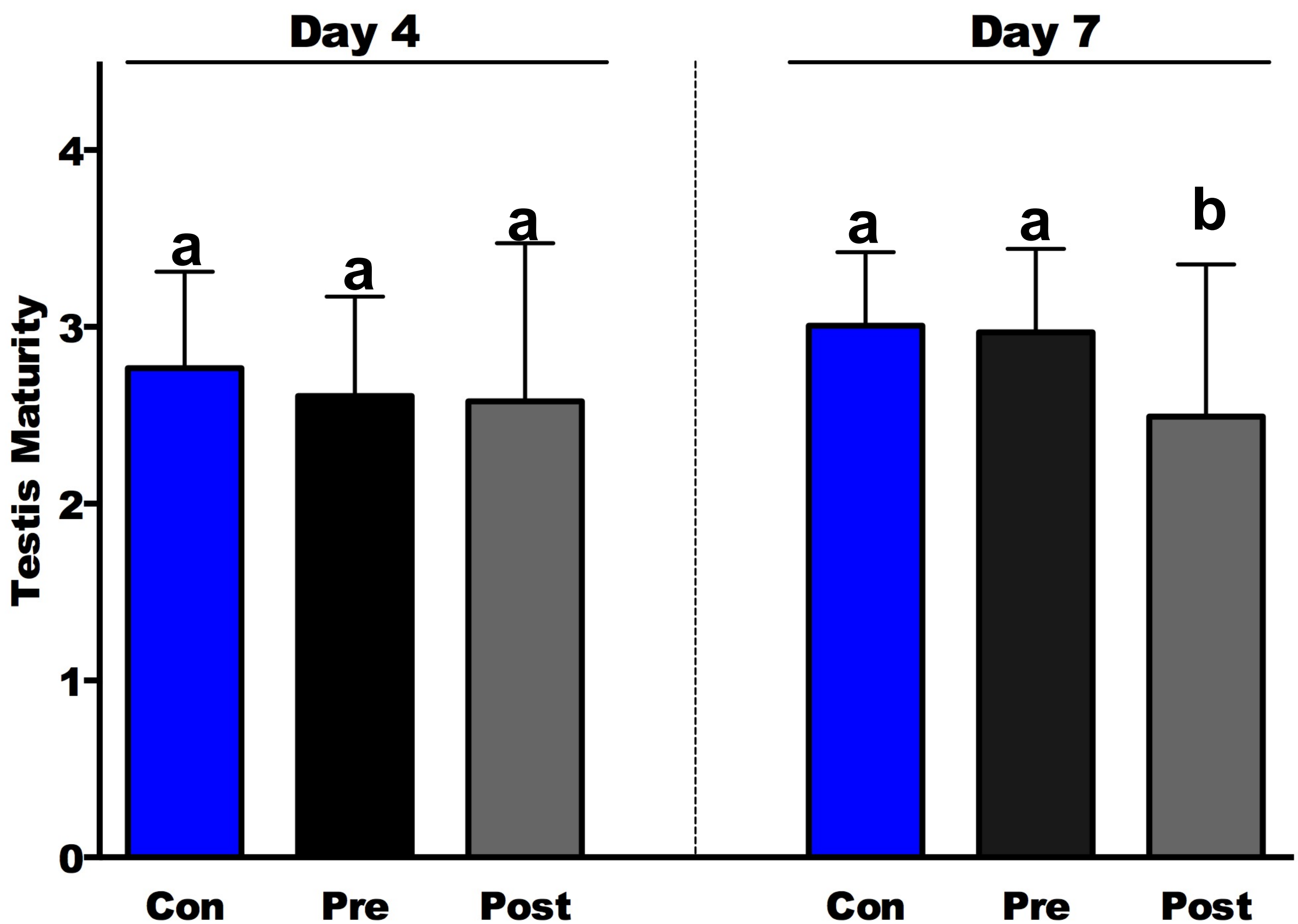


ANOVA with Tukey's post-test

provisional data – do not cite



Calumet Wastewater Reclamation Plant (Chlorination)



→ *UV disinfection improves maturity over seven days*

→ *Chlorination reduces maturity over seven days*

provisional data – do not cite

MELT Exposures – Liver Histology

2004
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2008
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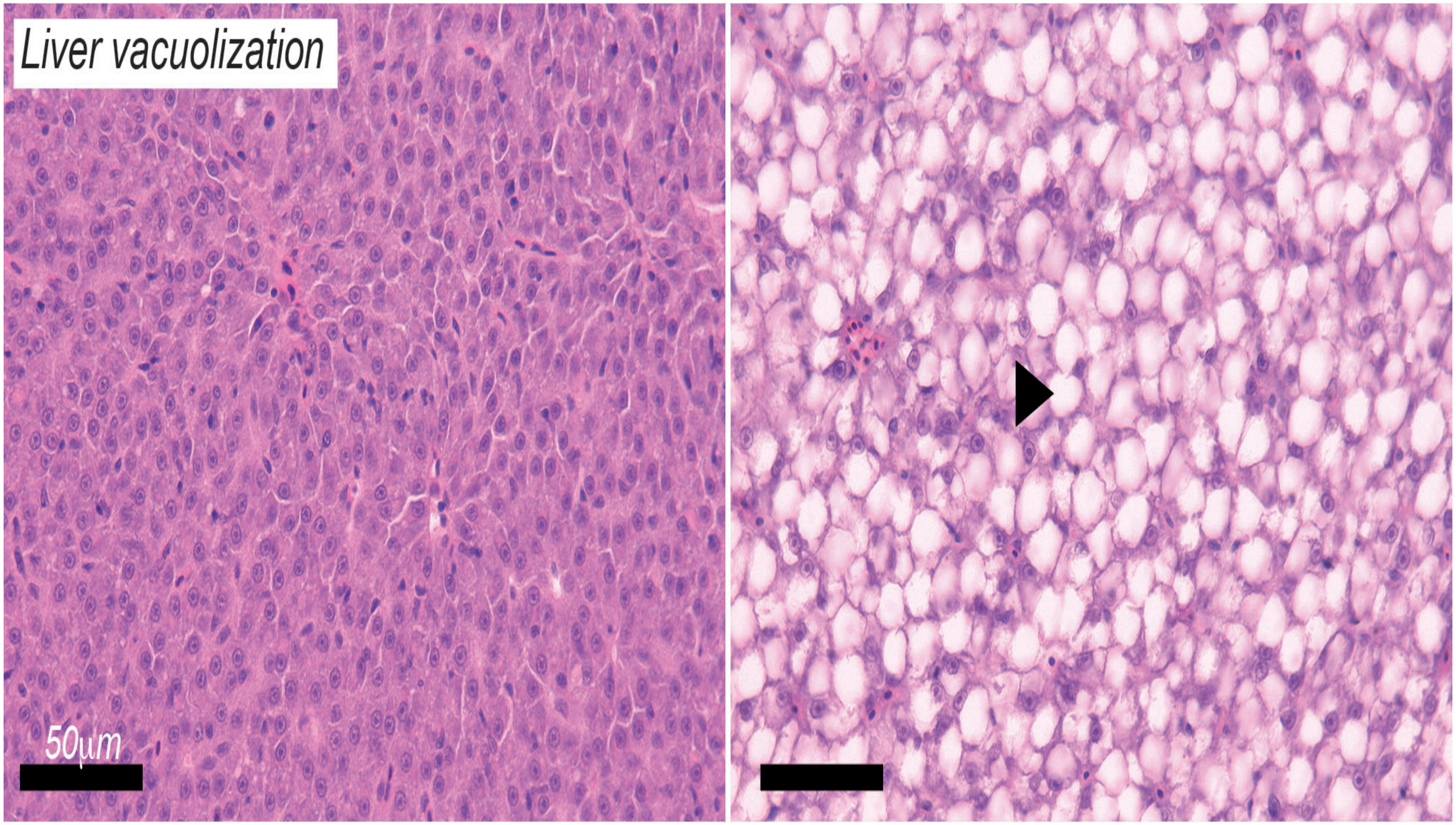
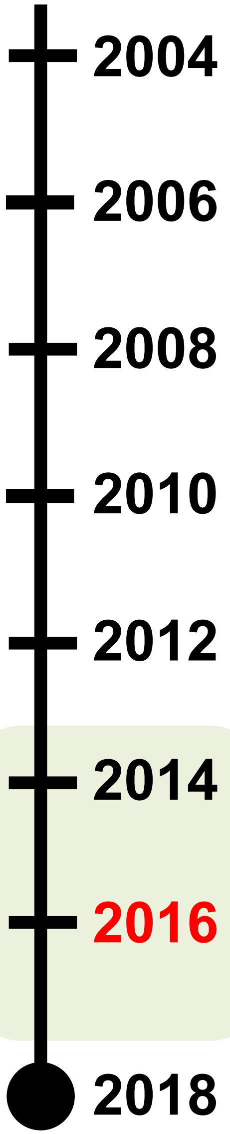
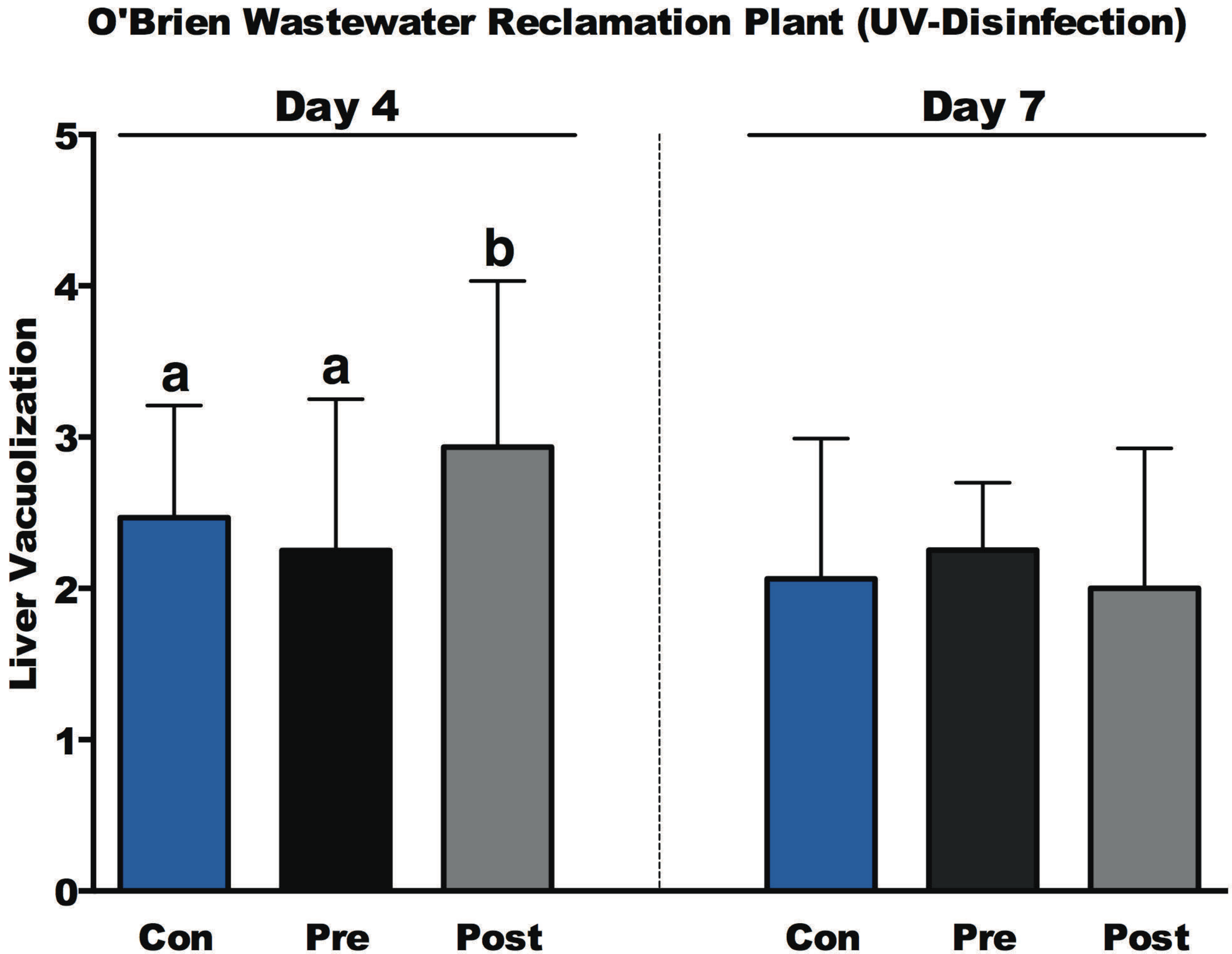
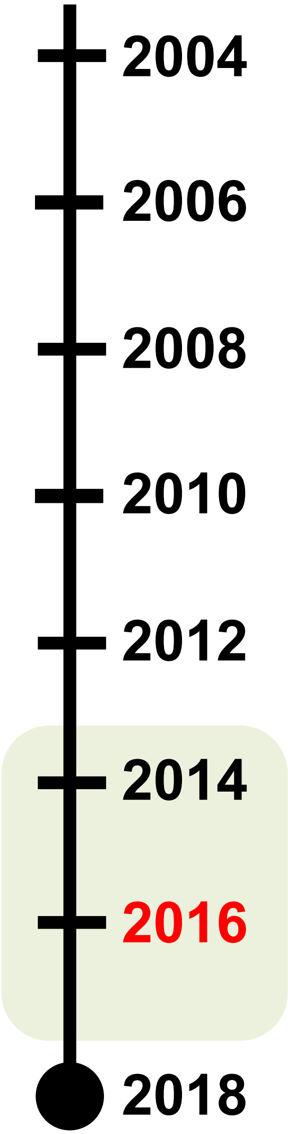
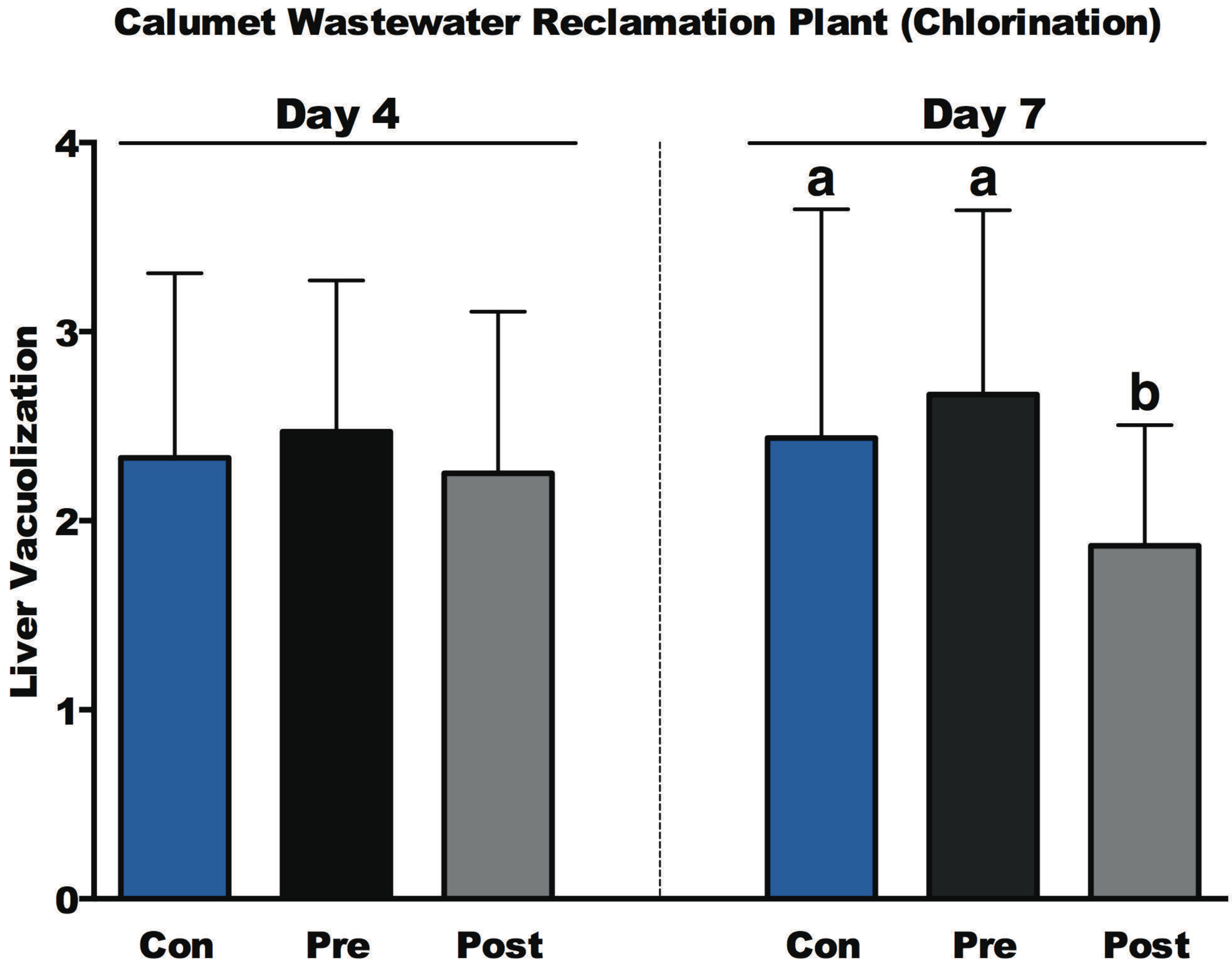






provisional data – do not cite





→ *UV disinfection does not alter liver vacuolization*

→ *Chlorination reduces liver vacuolization over seven days*

provisional data – do not cite

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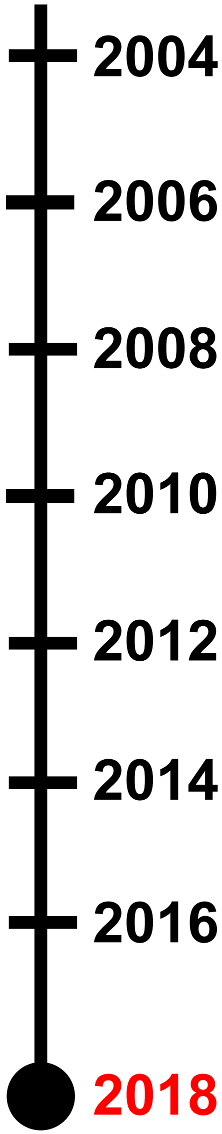
STUDY SUMMARY

- **Endocrine Active Compounds are ubiquitous in Chicago Area Waterways.**
- **Disinfection reduces EAC loads in on-site measurements and yields transformation products in bench experiments.**
- **Effluent disinfection reduces feminizing effects in male fathead minnows but not (yet) in sunfish.**
- **Cellular changes occur in testis (altered gametogenesis) and liver (altered vacuolization).**
- **Chemical transformation & biological effect are disinfection (UV/ chlorination) specific.**

NSF

provisional data – do not cite



Where to go from here?

- **Disinfection (especially UV) does reduce EAC loads and feminizing effects in exposed fishes.**
- **To soon to observe longer-term improvements to receiving waters in the Chicago Area Waterways.**
- **Further infrastructure upgrades (i.e., Stickney WRP) may accelerate environmental improvements.**
- **Understanding other pathways for EACs to waterways is needed (i.e., storm water).**
- **Impacts on biota will vary by species and habitat – identifying sensitive species and habitats is critical.**
- **Further improvements in water quality and ecosystem services are likely and worth continued monitoring.**

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Acknowledgments

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2004
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Dr. Thomas Granato
Dr. David Lordi

MWRDGC M&O Department

O'Brien WRP
Sandra Matual, Aruch Poonspaya

Calumet WRP
Pat Connelly, Brian Perkovich, Reed Dring

Stickney WRP
Trades that built MELTII including sheet metal fabricators, carpenters, electricians, and plumbers

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Thank You !

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