



**Snyderville Basin Water
Reclamation District**

Are Downstream Fish Under the Influence of Drugs?

Idaho Department of Environmental Quality

2015 Water Reuse Conference

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Acknowledgements

- Craig Ashcroft and Clint Rogers, Carollo Engineers
- Bryan Brooks and graduate students, Baylor University
- Utah Division of Wildlife Resources



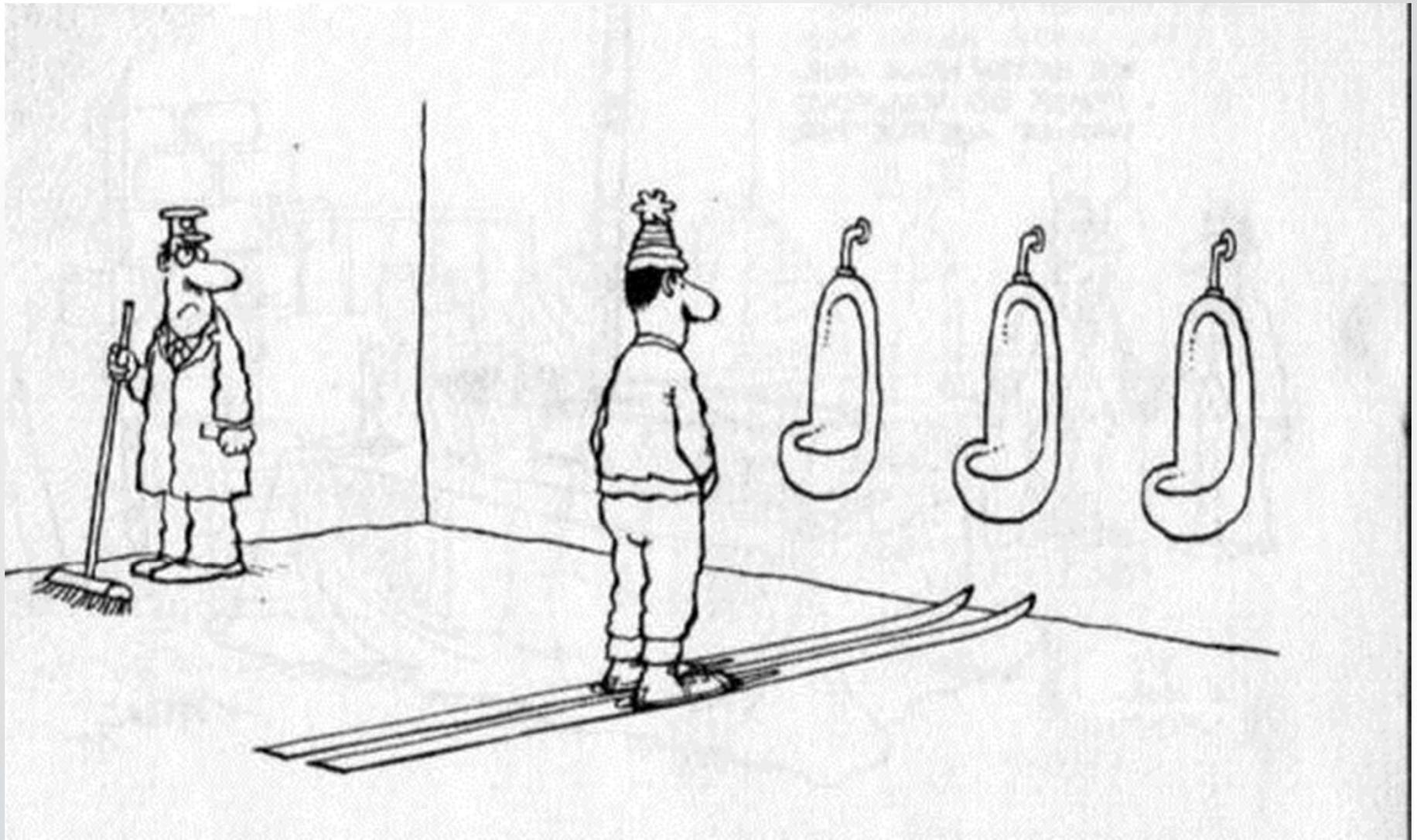






East Canyon Water Reclamation Facility

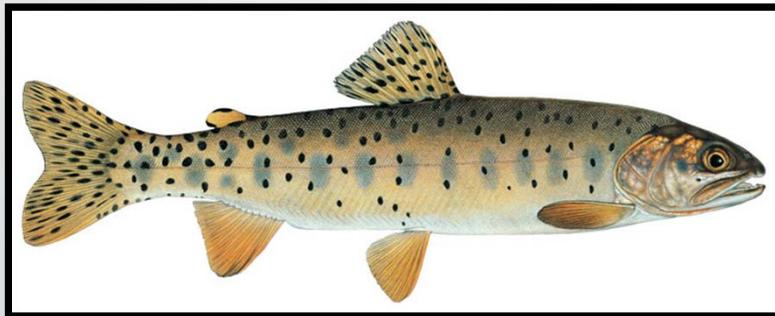




East Canyon Creek provides habitat for sensitive trout species and is a source of drinking water



Brown Trout
(*Salmo trutta*)



Bonneville Cutthroat
(*Oncorhynchus clarki*)



East Canyon Becomes an Effluent Dominated Stream in Summer Months



Year	7Q10
1988	16.0 cfs
1993	3.5 cfs
2003	1.8 cfs

Estrogenicity and the Environment

“The occurrence of feminized fish is associated with effluent discharges ... the incidence and severity is positively correlated with the proportion of treated sewage effluent in receiving waters.”

Gross – Sorokin et al. 2006. Env. Health Perspectives

We recognized that was potentially our situation in Park City!

Source of Constituents of Emerging Concern (CEC) & Endocrine Disrupting Compounds (EDC)

Natural and Synthetic Hormones

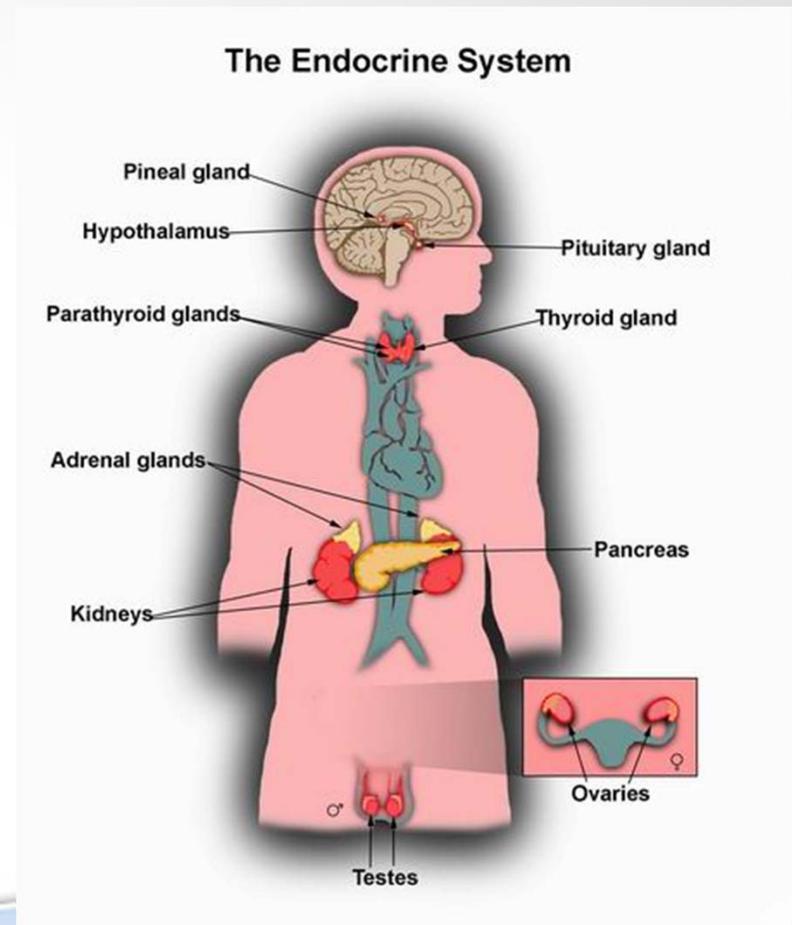
Pharmaceuticals and Personal Care Products



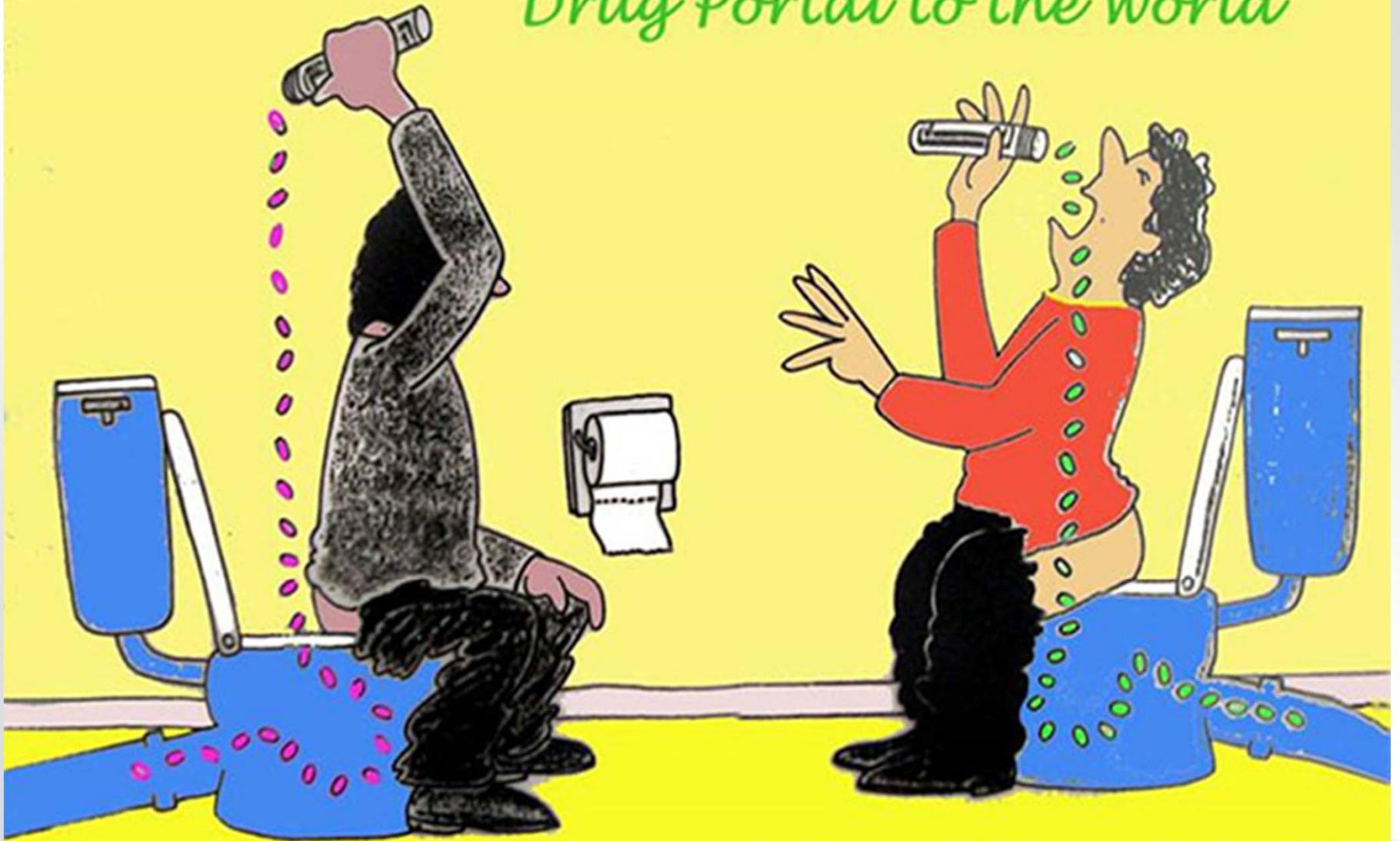
Treated wastewater is the primary pathway of CEC to our local streams

What Is The Endocrine System?

- The Endocrine System: System of glands each of which secrete a type of hormone.
- Compounds that block, mimic, stimulate or inhibit the endocrine system – Endocrine Disrupting Compounds (EDC's).



Drug Portal to the World



adapted by Daughton from Ternes (April 2000)

Current Literature Describes Potential Effects of EDC Exposure for Male Fish

Estrogenicity (feminization)

G
R
E
A
T
E
R

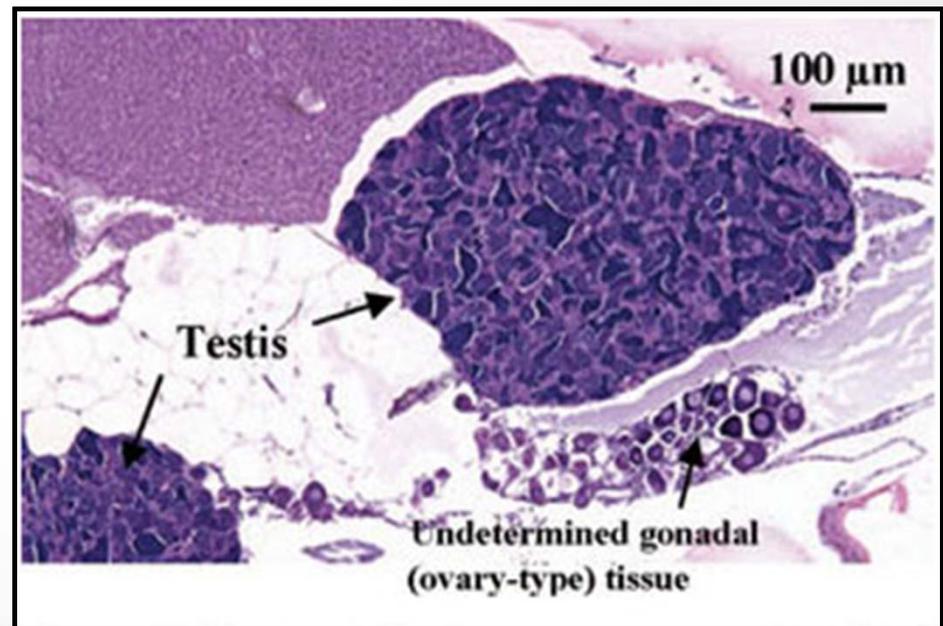
I
M
P
A
C
T

Vitellogenin induction in male fish

Intersex fish

Skewed sex ratios

Population collapse



(Nash et al, 2004)

Concentrations of Estrogens that begin to affect Male Fish

Inducement of vitellogenin production

- ≈ 5 ng/L 17β -estradiol
- estriol is 30 times less potent than above
- ≈ 3.2 ng/L for estrone
- ≥ 1 ng/L for 17α -ethinylestradiol

Inducement of intersex

- ≈ 10 ng/L for estrone, or 17β -estradiol
- estriol is 100 times less potent than above
- ≈ 4 ng/L for 17α -ethinylestradiol
- estrogenic substances are additive



White
sucker



Boulder Creek

Detection of Trace CECs Possible by Advances in Analytical Methods



Liquid Chromatography/Mass Spectrometer

Public Perception of CECs in Water is a Challenge for Water Reuse Professionals

AP: Drugs found in drinking water

Updated 36d ago | Comments 147 | Recommend 76

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By Jeff Donn, Martha Mendoza and Justin Pritchard, Associated Press

A vast array of pharmaceuticals — including antibiotics, anti-convulsants, mood stabilizers and sex hormones — have been found in the drinking water supplies of at least 41 million Americans, an Associated Press investigation shows.

To be sure, the concentrations of these pharmaceuticals are tiny, measured in quantities of parts per billion or trillion, far below the levels of a medical dose. Also, utilities insist their water is safe.



AP Associated Press

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Washington Post - Apr 14, 2008

[Feds Not Addressing Drugs in](#)

Senators rip EPA over lack of knowledge on drugs in water

By MARTHA MENDOZA - 17 hours ago

WASHINGTON (AP) — The Environmental Protection Agency was lambasted during a Senate hearing Tuesday for allowing the American public to learn that traces of pharmaceuticals are in much of the nation's drinking water from an Associated Press investigative series, not the federal government.

Intersex Fish Raises Pollution Concerns in US

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US: September 8, 2006

TODAY'S
ENVIRONMENT
NEWS

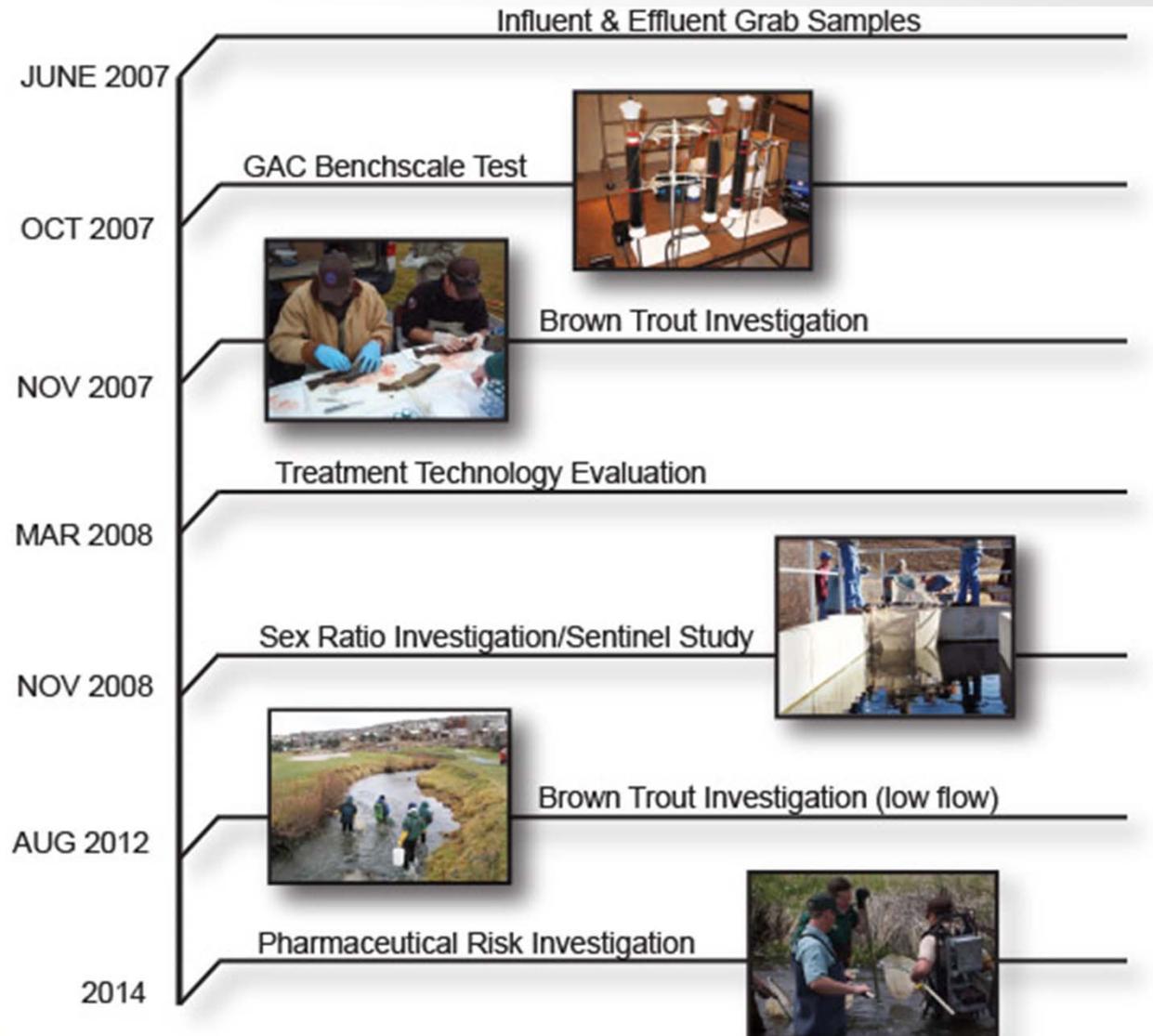
AUSTRALIA:
Coral Flourishing At
Bikini Atoll Atomic Test



Why Are Conducting This Research?

- We take the position that our job is to protect human health and the environment.
- We have spent millions of dollars protecting our local streams (tertiary treatment).
- Just because there are no state or federal requirements to study or address CEC, does not mean we should 'stick our head in the sand'.

Timeline of Research Efforts



EDCs Detected at Low Concentrations in SBWRD Effluent

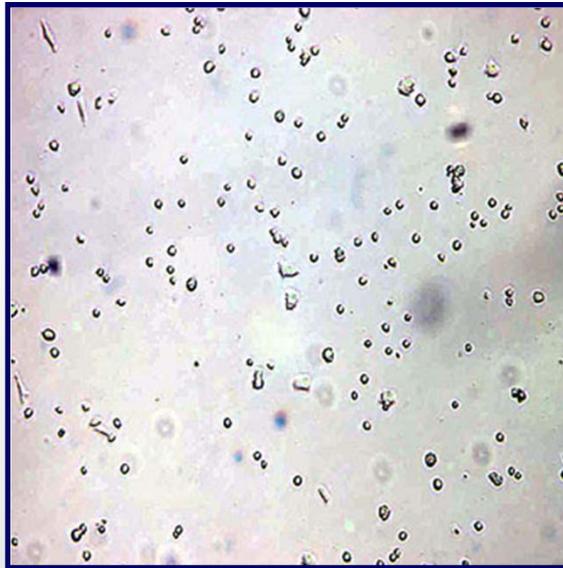


JUNE 2007

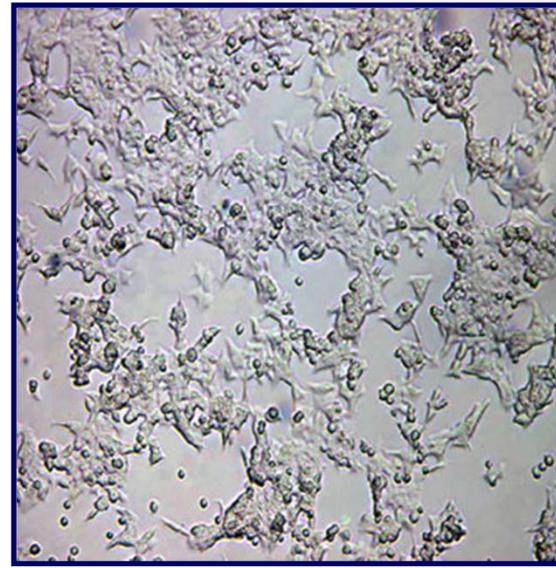
SBWRD Collects Influent & Effluent Grab Samples

Estrogen activity measured by E-screen bioassay

Breast cancer cell line with
growth response to estrogen



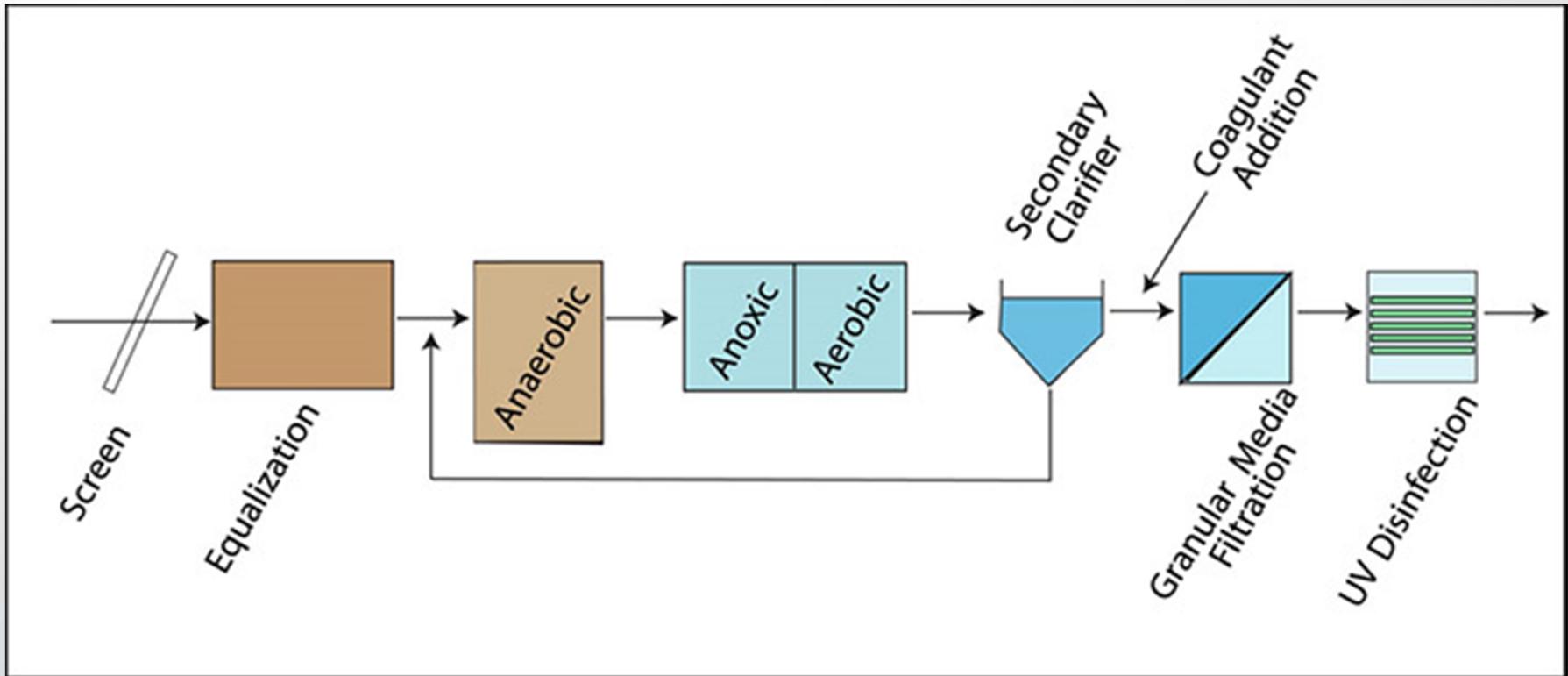
Negative Control



Positive Control

Reported as estradiol equivalents
(MRL = 0.030 ppt)

Despite advanced treatment, CEC detected in the effluent to East Canyon Creek



Summary of Effluent Test Results

Constituent	Samples	Detection Frequency	AVG (ng/L)	MRL (ng/L)
E-Screen Bioassay	18	100%	0.69	0.03
Cotinine	15	100%	29	1.0
Sulfamethoxazole	24	100%	846	1.0
Gemfibrozil	19	95%	85	1.0
Trimethoprim	22	95%	73	1.0
Carbamazepine	34	94%	81	5.0
Triclosan	20	90%	36	5.0
Ibuprofen	19	89%	29	1.0
Caffeine	24	88%	23	3.0
Fluoxetine	24	88%	50	1.0
Diazepam	17	47%	87	1.0

¹Result shown is a single detection (not an average)

Summary of Effluent Test Results

Constituent	Samples	Detection Frequency	AVG (ng/L)	MRL (ng/L)
Ethinyl Estradiol- 17 α	34	29%	5.5	1.0
Estrone	34	26%	47	1.0
Estradiol	34	18%	1.6	1.0
Acetaminophen	24	13%	2.9	1.0
Iopromide	20	n/a	12 ¹	5.0
Testosterone	28	n/a	1.2 ¹	1.0
DEET	1	n/a	437 ¹	25
TDCPP	1	n/a	222 ¹	25
Tris (2-chloroethyl) phosphate	1	n/a	166 ¹	25

¹Result shown is a single detection (not an average)

Treatment Technology Evaluation

GAC Filtration



Ozone/Peroxide



UV/Peroxide



NF/RO not considered due to cost and concentrate disposal constraints. Capital costs as high as \$2.50 per gallon to remove CECs.

Efforts to Identify Impacts of EDC on Local Fish Populations



Sentinel Study

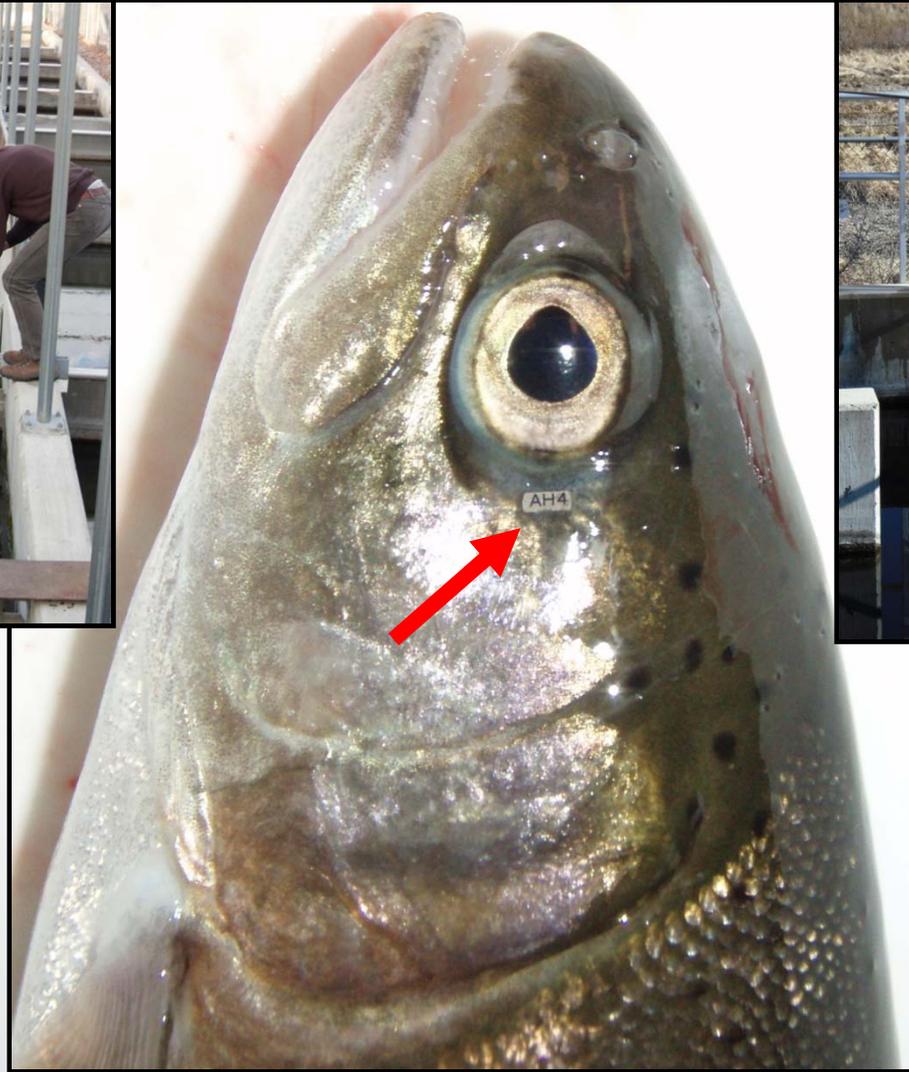
Study Objectives

- Determine if EDC concentrations are high enough to induce vitellogenesis by exposing male Rainbow Trout to 100% treated wastewater.

Sentinel Study Methods



Holding pen
at fish
hatchery



Holding pen
in effluent
aeration
basin

Sentinel Study Methods



Blood Sample (0.5mL) Collected From Each Fish

Vitellogenin (Vtg) is a Biomarker of Estrogen Exposure in Male Fish



Egg yolk protein

Vtg not normally found in male fish

Vtg detected after exposures less than 1.0 ng/L of EDC

Sentinel Study Results

(Rainbow Trout)

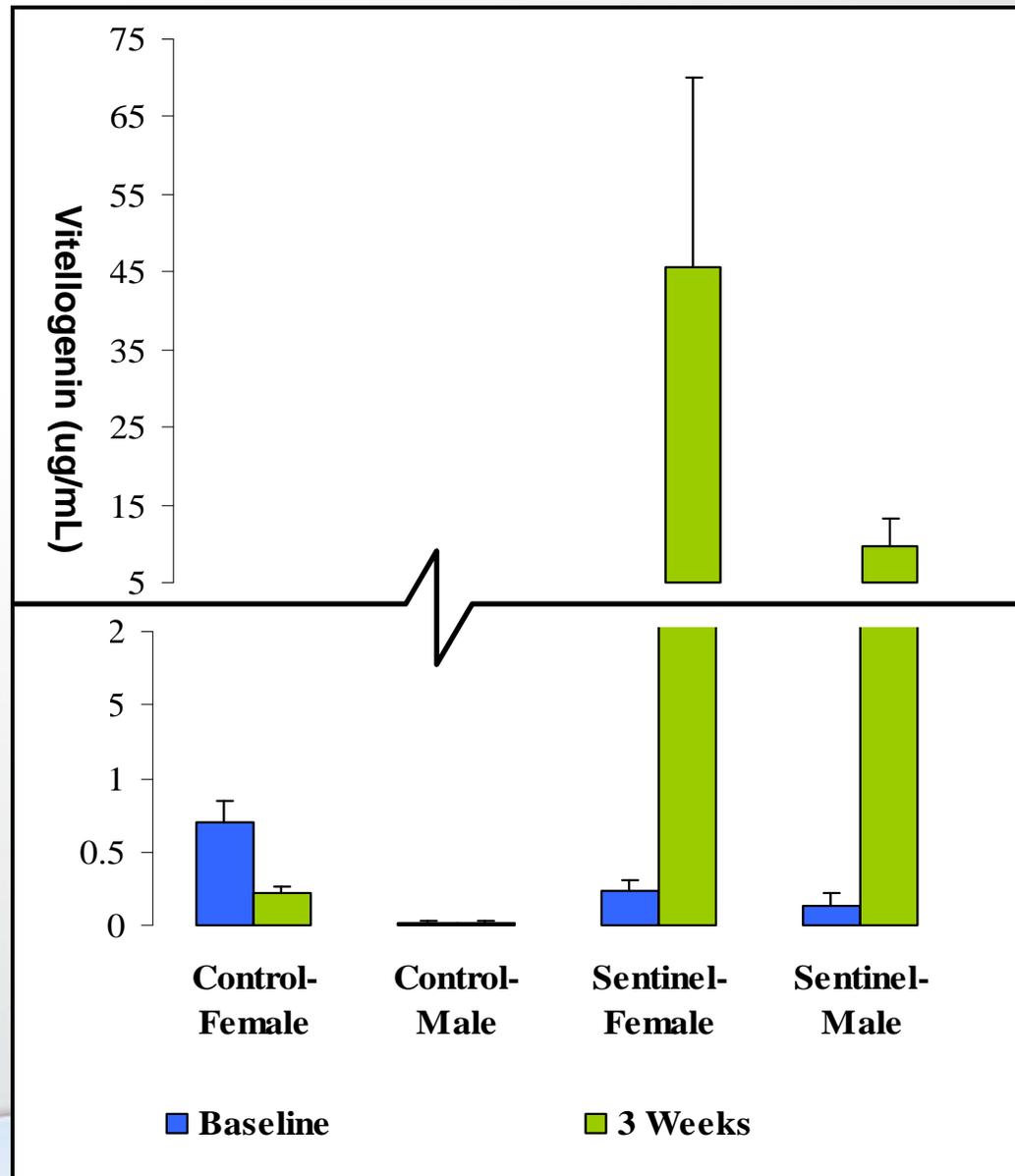
	Control		ECWRF	
	Baseline	3 weeks	Baseline	3 weeks
Vitellogenin (ng/ml)	0.343 ± 0.09	0.110 ± 0.03	0.136 ± 0.05	

Sentinel Study Results

(Rainbow Trout)

	Control		ECWRF	
	Baseline	3 weeks	Baseline	3 weeks
Vitellogenin (ng/ml)	0.343 ± 0.09	0.110 ± 0.03	0.136 ± 0.05	26.049 ± 9.02

Vitellogenin Increased for both Male and Female Sentinel Fish



Efforts to Identify Impacts of EDC on Local Fish Populations



Sex Ratio
Investigation

Study Objectives

- Determine if downstream Brown Trout are being feminized.
- Evaluate fish tissues.

Sex Ratio Investigation Methods



Gender Determination: Palpitation or Necropsy

4:1 Dilution of Effluent in East Canyon During Sex Ratio Study



Upstream Flow
75%

+



Effluent Flow
25%

=



Downstream Flow
100%

Excess Number of Female Fish Not Seen in the Downstream Population

Description	Number	Percentage
Total Fish Captured	71	
Male Fish	33	46.5%
Female Fish	38	53.5%

Compounds Found in Sentinel and Stream Fish Tissues*

Common Name	Pharmaceutical Name	Fillet Downstream	Liver Downstream	Fillet Upstream	Sentinel Fillet	Sentinel Liver
Caffeine	Caffeine	ND	1.2	ND	ND	2.4
Benadryl	Diphenhydramine	0.295	2.85	ND	0.187	6.77
Hypertension	Propranolol	ND	0.90	ND	ND	1.2
Blood pressure control	Diltiazem	ND	0.205	ND	0.03	0.46
Tegretol	Carbamazepine	ND	ND	ND	0.49	1.0
Antidepressant	Paroxetine	ND	ND	ND	0.66	8.8
Prozac (metabolite)	Norfluoxetine	ND	20.0	ND	ND	81.7
Prozac	Fluoxetine	ND	18.5	ND	ND	61.0
Zoloft (metabolite)	Desmethylsertraline	ND	140.0	ND	10.2	533.3
Zoloft	Sertraline	ND	ND	ND	ND	92.0
Valium	Diazepam	ND	ND	ND	2.6	9.0
Cholesterol control	Gemfibrozil	ND	ND	ND	ND	22.3

*Results are presented as an average of all positive test results, Results in units of nanograms/gram

Developing an Understanding of Spatio-temporal Bioaccumulation of Pharmaceuticals by Aquatic Life in East Canyon Creek, Utah

Study Objectives:

The *overarching objective* of this research is to determine if effluent from the East Canyon Water Reclamation Facility is causing or has the potential to cause permanent or long term negative impacts to native fish populations and aquatic life.

Study Objectives

1. To determine the seasonal and longitudinal levels of targeted CECs in East Canyon Creek;
2. To determine the accumulation and trophic transfer of CECs in East Canyon Creek; and
3. To predict risks of CEC exposure to trout residing in East Canyon Creek.

Target CEC Analyte Selection

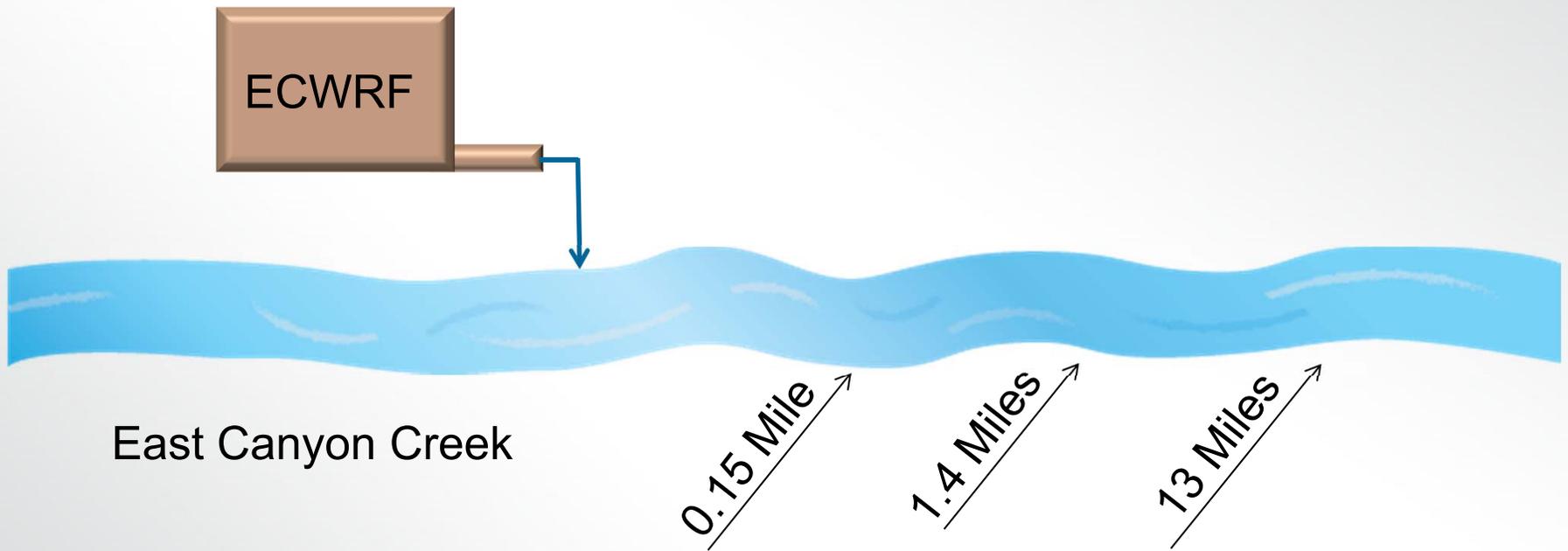
Class:

Acetaminophen
Amitriptyline
Amlodipine
Aripiprazole
Benzoyllecgonine
Buprenorphine
Caffeine
Carbamazepine
Desmethylsertraline
Diclofenac
Diltiazem
Diphenhydramine
Duloxetine
Fluoxetine
Methylphenidate
Norfluoxetine
Promethazine
Sertraline
Sucralose

Use:

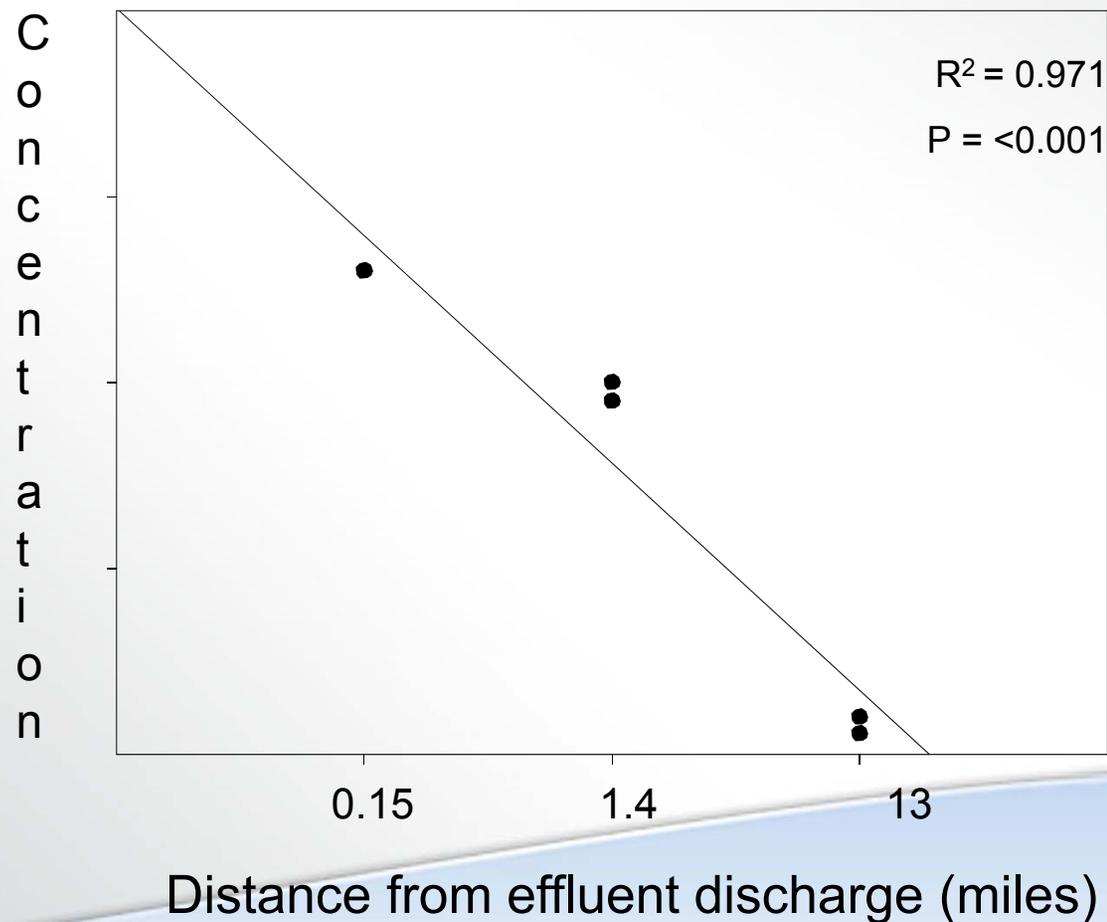
Analgesic
Antidepressant
Anti-hypertensive
Anti-psychotic
Cocaine metabolite
Narcotic
Stimulant
Anti-seizure
Sertraline metabolite
Anti-inflammatory
Anti-hypertensive
Antihistamine
Antidepressant
Antidepressant
Psychostimulant
Fluoxetine metabolite
Antihistamine
Antidepressant
Sweetener

Seasonal and Spatial Investigation of CEC Bioaccumulation at East Canyon Creek



Sampling Events: Spring, Summer & Fall

A regression of water concentrations of Benadryl at each sampling site (0.15, 1.44, 13 miles) along the sampling distance during, August



Objective 1 Results: Seasonal and Longitudinal Levels of Targeted CECs in East Canyon Creek

- Target CECs consistently higher in effluent discharge.
- Significant decreases of target CECs with distance from discharge was consistently observed.
- Sucralose, caffeine, and the cocaine metabolite observed in the upstream control study sites.

Fish, Invertebrate, & Periphyton Collection

Periphyton, benthic macroinvertebrate, Brown Trout and Mottled Sculpin were collected to measure the accumulation and trophic transfer of CECs.



Example of the Trophic Levels in East Canyon Creek



Caddis Fly Larvie

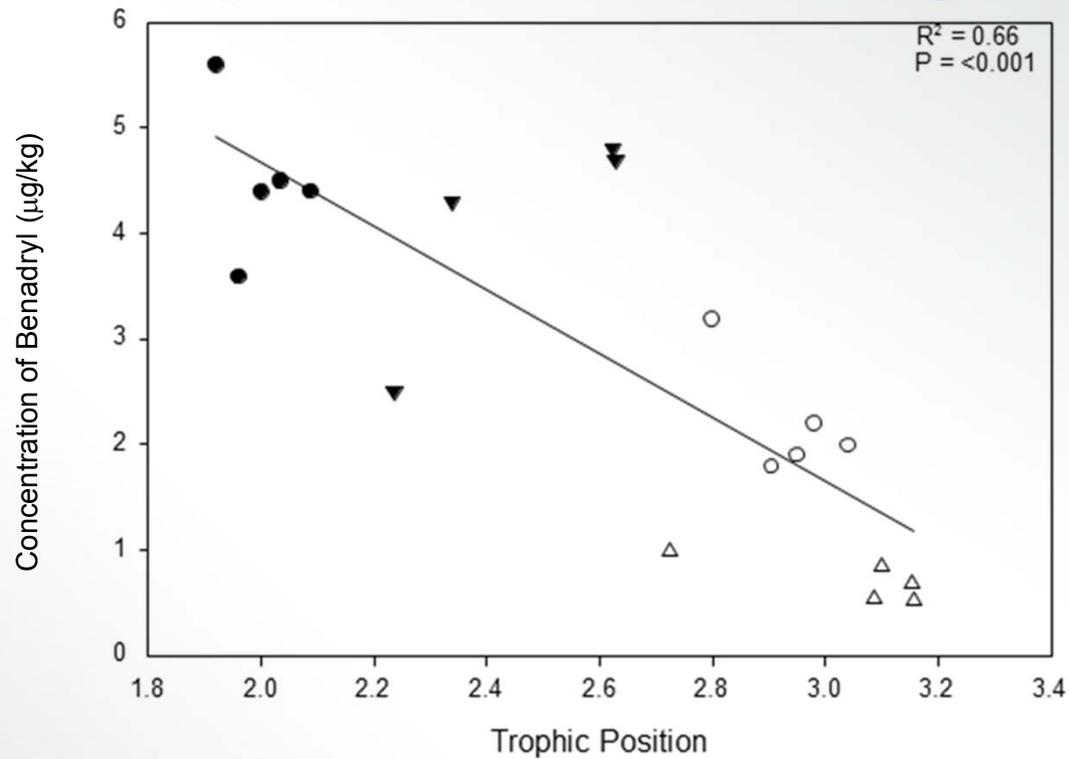


Mottled Sculpin



Brown Trout

Trophic Transfer: August



Benadryl as a Function of Trophic Position during August at the 1.44 mile site

- Caddisflies
- Mottled Sculpin
- ▼ Snails
- △ Brown Trout

Objective 2 Results: Accumulation and Trophic Transfer of CECs in East Canyon Creek

- It was found that certain CEC bioaccumulated in periphyton.
- It was found that certain CEC bioaccumulated in caddisflies.
- It was found that certain CEC bioaccumulated in Mottled Sculpin.
- It was found that certain CEC bioaccumulated in Brown Trout.
- Trophic magnification (biomagnification) was not observed for any CEC sampled. Preliminary data for trophic transfer points towards uptake be driven by respiration across the gill and not diet.

Objective 3 Results: Predicted Risks of CEC Exposure to Trout Residing in East Canyon Creek

- Fish plasma modeling had mixed results. Certain compounds could be modeled, others could not.

What Have We Learned:

- Effluent (100%) steroids concentrations are high enough to induce vitellogenesis in male Rainbow Trout.
- Targeted pharmaceuticals do not readily biomagnify from one trophic level to another.
- Targeted pharmaceuticals bioaccumulate from water concentrations.
- Modeling CEC concentrations in organism tissue from CEC water concentrations has not been successful.

Take Away Thoughts:

- CEC can have an impact on downstream ecosystems.
- Public perception must be taken into consideration when dealing with a reuse project (will my son be turned into a girl!).
- CEC can be removed from wastewater (\$\$\$).
- Not all of the questions can be answered at this time!
 - ✓ As an example, we do not know what the therapeutic dose is for fish for any of these compounds!

Where Do We Go From Here?

- In my opinion, we will need to start using a battery of assays to determine if reuse water is suitable for various uses.
- Assay examples include:
 - E-Screen bioassay
 - Steroidogenesis (Human Cell Line – H295R) Assay



Questions?



For more info visit: <http://www.sbwrdd.org>

Presentation Summary

- Background Information
- Why are we concerned with CEC
- SBWRD's research to date
- What we know, what we do not know