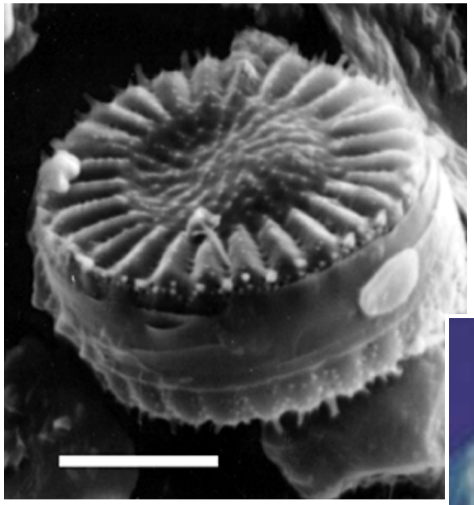
Science to Achieve Results (STAR):

Exposure Measurement Tools for Endocrine Disrupting Chemicals in Mixture

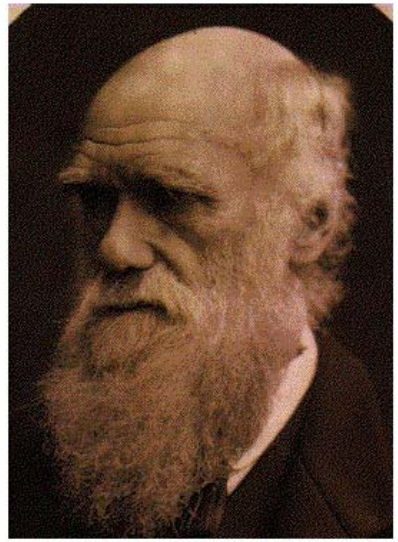
Heiko L. Schoenfuss[1], Larry B. Barber[2], David O. Norris[3], and Matthew L. Julius[1]

[1]St. Cloud State University, [2]US Geological Survey and [3]Colorado University



Primary producer

Grazer



Cyclotella meneghiniana

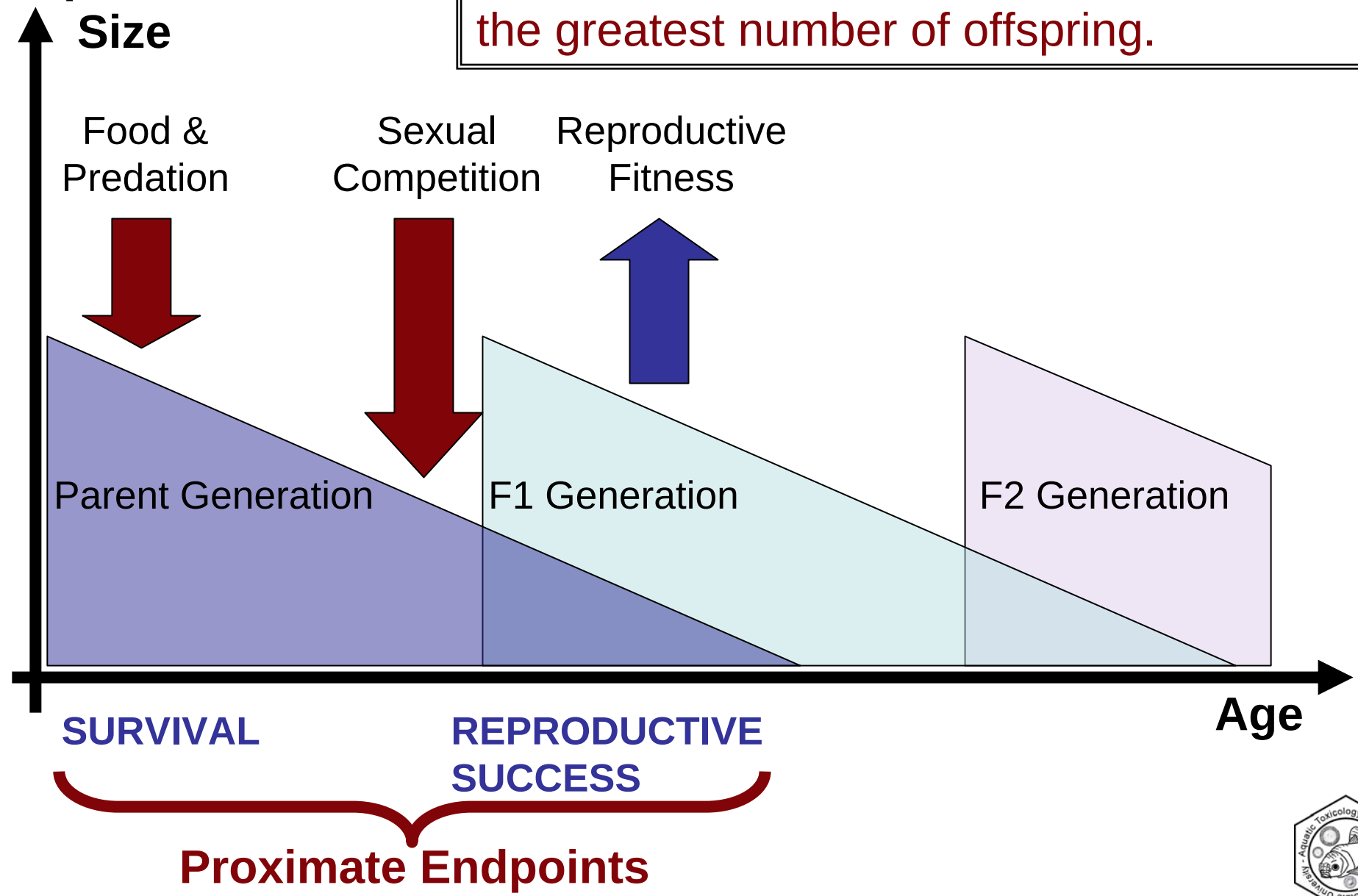
Daphnia sp.

Pimephales promelas

Effects of Endocrine Disrupting Compounds on Aquatic Organisms

The Ultimate Endpoint

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

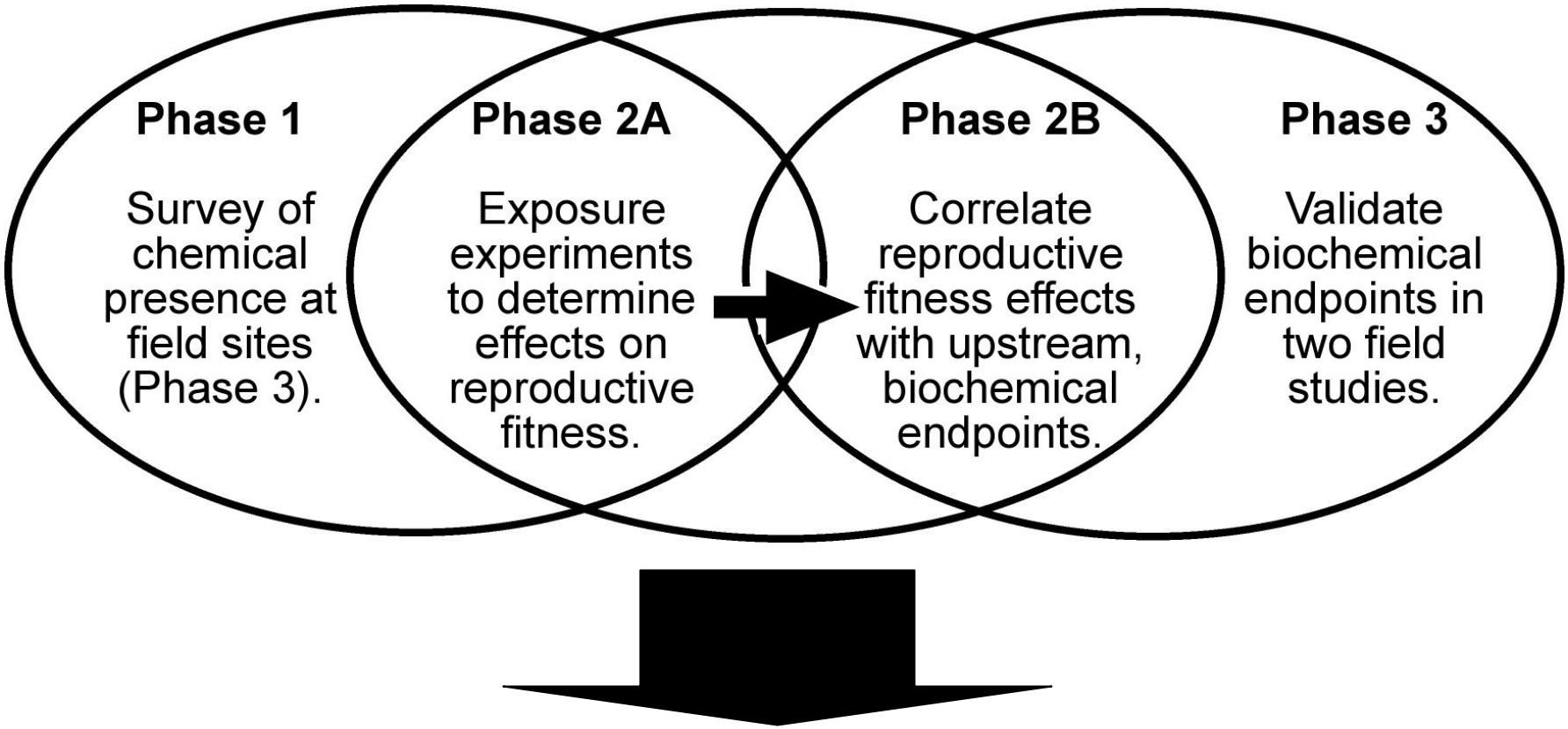


Project Goal

This project evaluates the hypothesis that mixtures of estrogenic compounds will have adverse effects on aquatic vertebrate reproductive fitness that can be rapidly assessed by quantifying alterations in the neuroendocrine system of fishes.

1. Assess synergistic interactions of mixtures.
2. Assemble broad, biologically relevant effects matrix.
3. Develop neuroendocrine endpoints for rapid assessment.

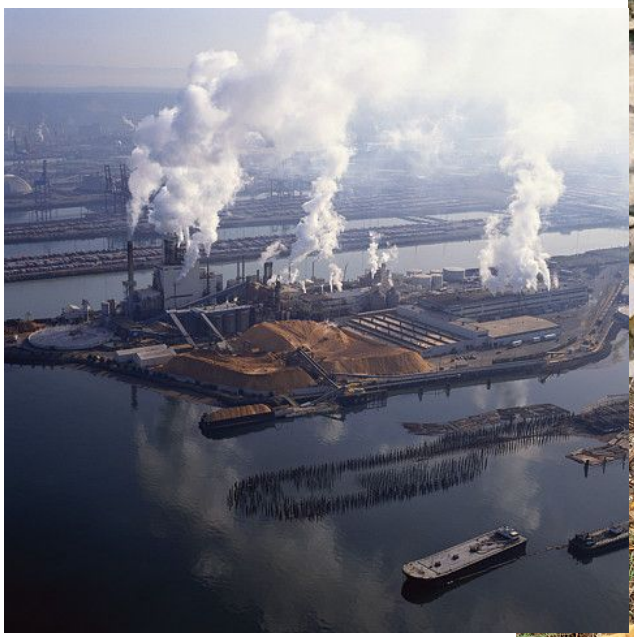
Approach



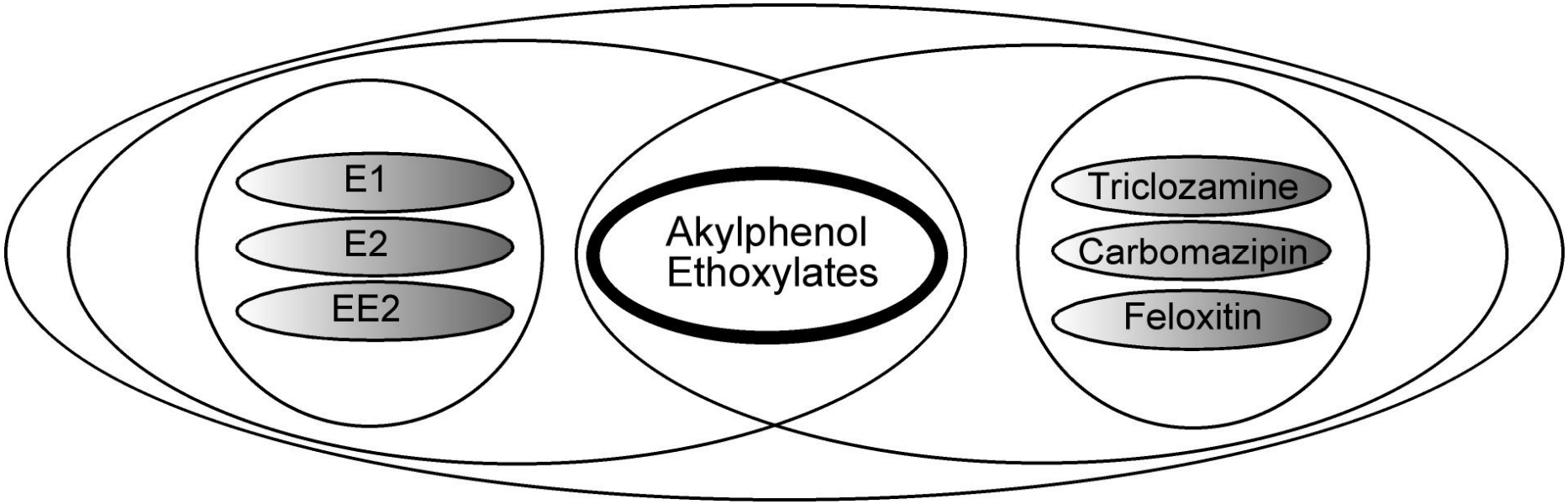
Outcome

Rapid & Biologically Relevant Neurochemical Assessment Tools Correlating with Reproductive Fitness

Chemical Mixtures



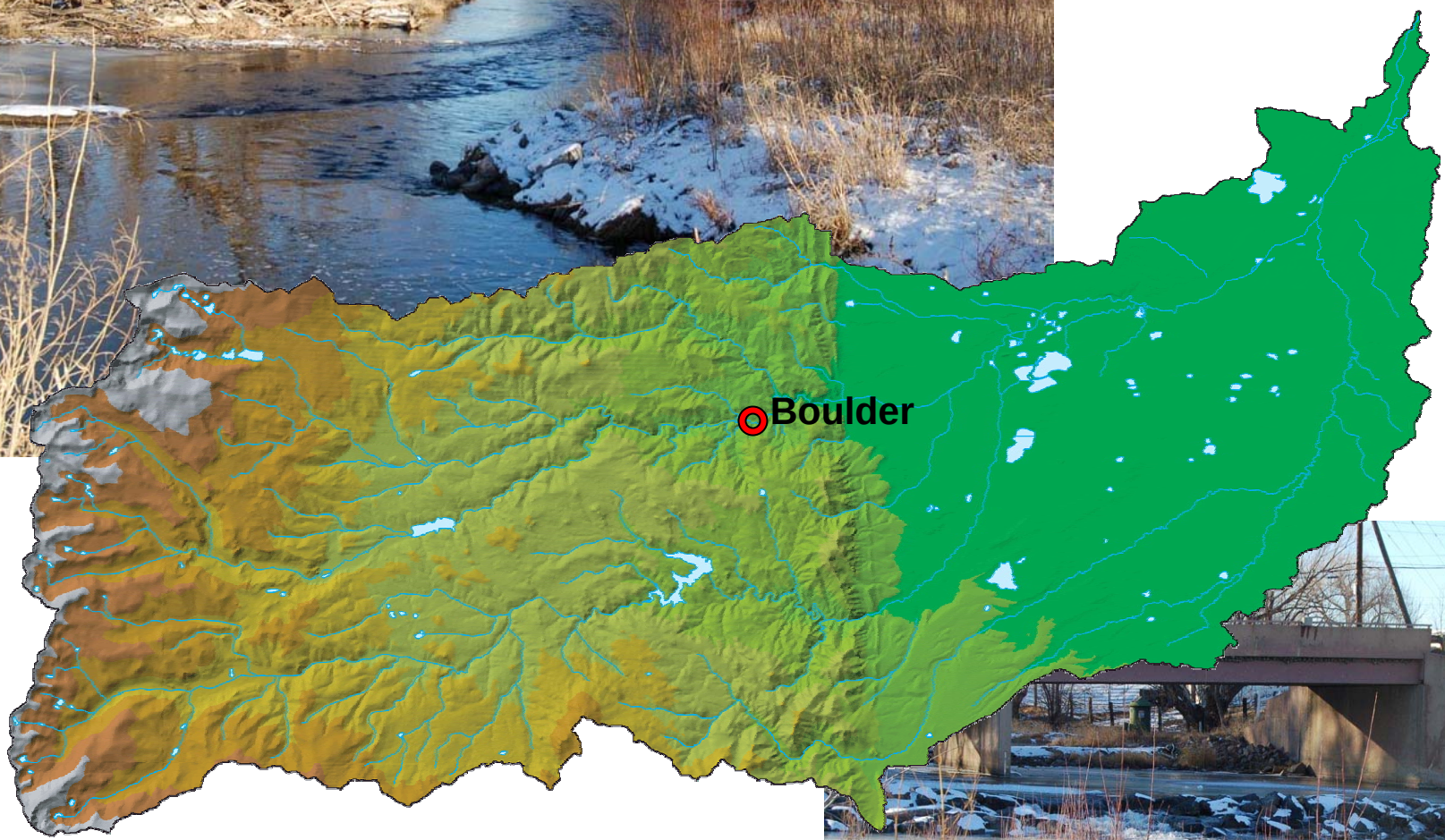
Endocrine Disrupting Compounds & Mixtures



Criteria for Determining Exposure Concentrations:

- Compound must have been found in at least one of the analyzed effluents.
- Exposure concentrations should be within one order of magnitude of published effects concentrations (if available).
- Compounds will be tested at three concentrations bracketing measured effluent concentrations.

Boulder Creek Watershed
Colorado Front Range



Metropolitan Wastewater Treatment Plant
St. Paul, MN

Plant Comparison

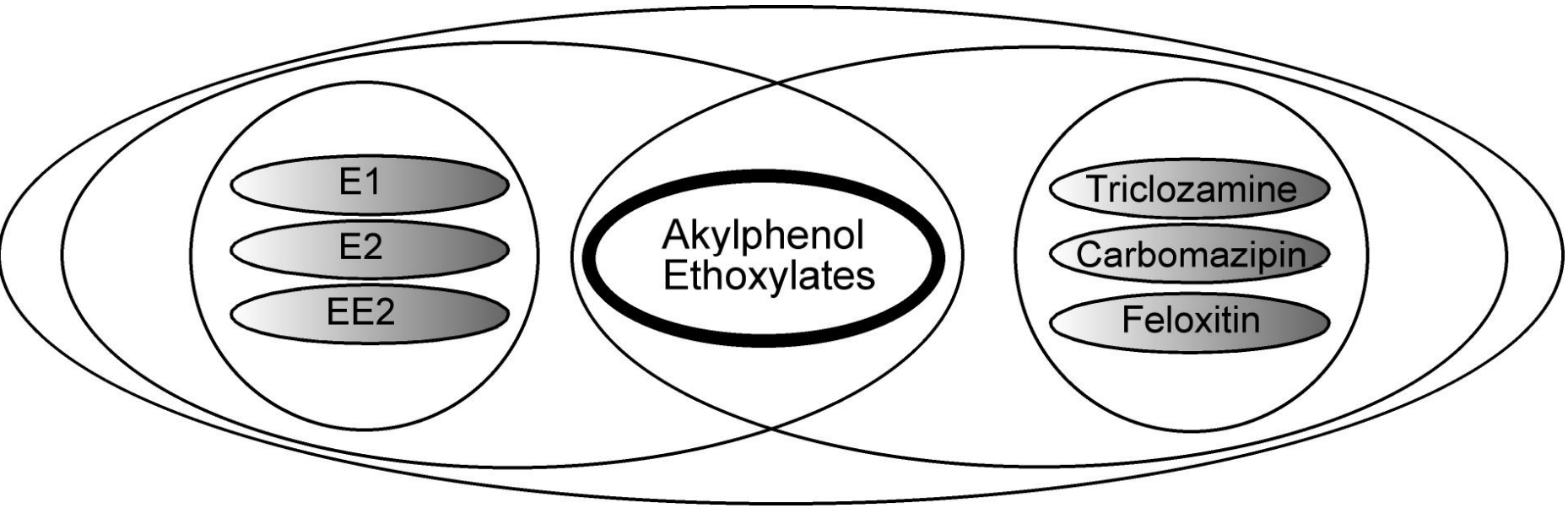
Boulder Creek:

- History of estrogenicity
- Mid-size pant with secondary treatment.
- Dam prevents fish upstream movement.
- Constitutes up to 75% flow or Boulder Creek.

Metro Plant, St. Paul

- History of estrogenicity
- Large plant with tertiary treatment.
- 500 m natural discharge channel with 100% effluent content.
- Constitutes less than 20% of Mississippi River flow.

Endocrine Disrupting Compounds & Mixtures



Endocrine Disrupting Compounds & Mixtures

I. Natural & Synthetic Estrogens:

- Estrone (E)
- Estradiol (E2)
- Ethynylestradiol (EE2)

- ➢ Well understood interactions with estrogen receptors.
- ➢ Action along hypothalamus-pituitary-gonadal axis is mechanistically apparent.
- ➢ Wealth of previous studies as benchmarks in lab & field.

Endocrine Disrupting Compounds & Mixtures

II. Pharmaceuticals & Personal Care Products:

- Fluoxetine - Antidepressant blocking reuptake of serotonin (SSRI).
- Carbamazepin - Antiepileptic acts in a receptor mediated manner by inhibiting neurotransmitters.
- Triclosan - Anti-microbic chlorophenol suspected of being hormonally active.

- ➢ Have been documented in US surface waters.
- ➢ Are known to, or likely to interact with hypothalamus-pituitary-gonadal axis.

Endocrine Disrupting Compounds & Mixtures

III. Alkylphenols:

- Nonylphenol & Octylphenol - Parent compounds for surfactant mixtures.
- Nonylphenol/Octylphenol Carboxylates.
- Nonylphenol/Octylphenol Ethoxylates.

- ➢ Have been documented in many US surface waters.
- ➢ Exhibit estrogenic activity.
- ➢ Appear to facilitate the uptake of other compounds into biological organisms by increasing their solubility and bridging the phospholipids bilayer of the cell.

Endocrine Disrupting Compounds & Mixtures

IV. Mixtures:

- Alkylphenols - used and discharged as mixtures, occur always as mixtures, shorter chained NP/OP more potent, longer chained APs with greater surfactant qualities.
- Estrogens + Alkylphenols - commonly detected in same water samples, both estrogenic.
- Pharmaceuticals + Alkylphenols - different mode of action, likely that APs will facilitate uptake of pharmaceuticals by organism.
- Estrogens + Alkylphenols + Pharmaceuticals - a “biologically realistic” mixture representing the compounds common presence in effluents.

Mixture Concentrations

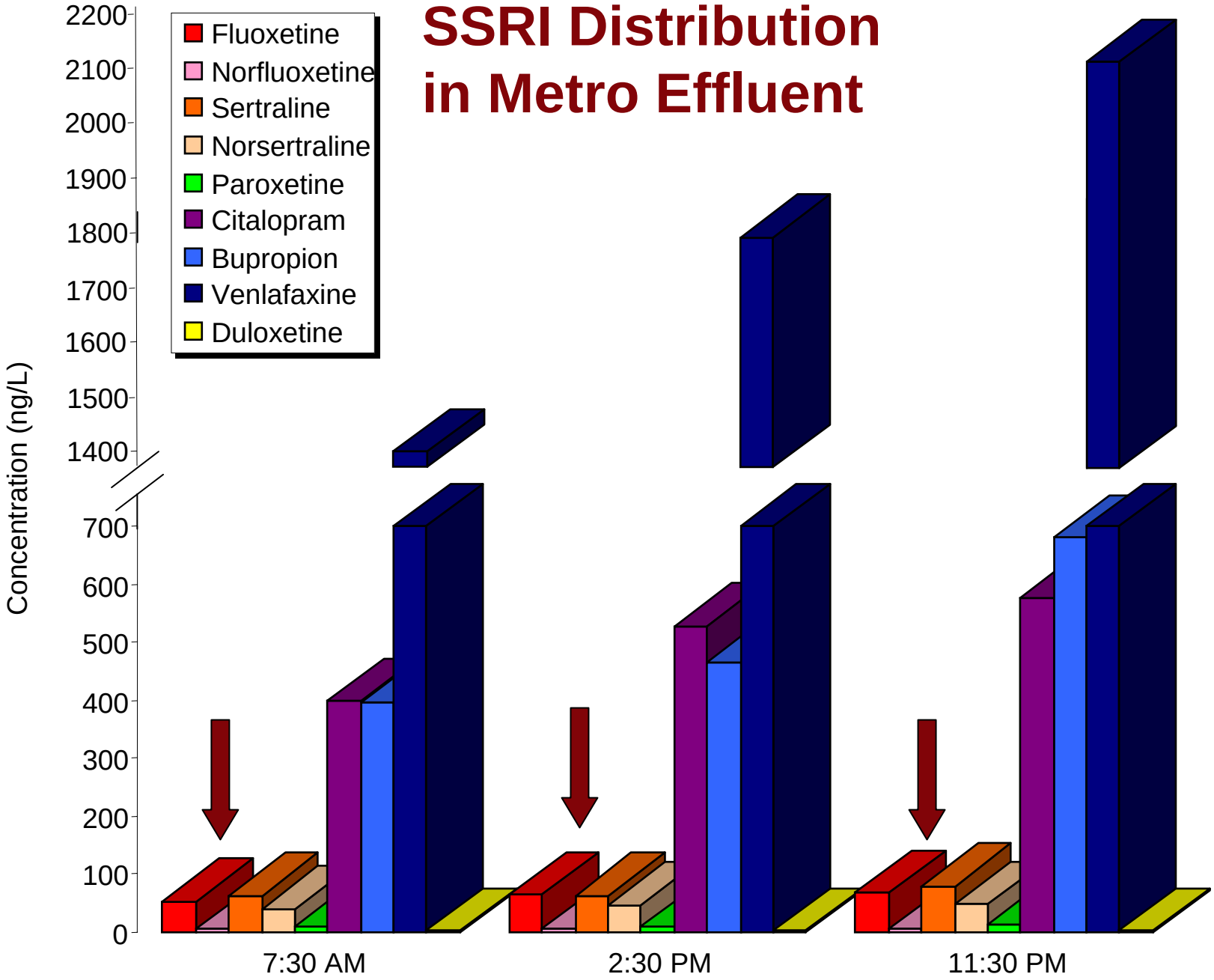
- Highest no-effect concentration for each compound used for mixtures.

Phase 1 - Preliminary Results

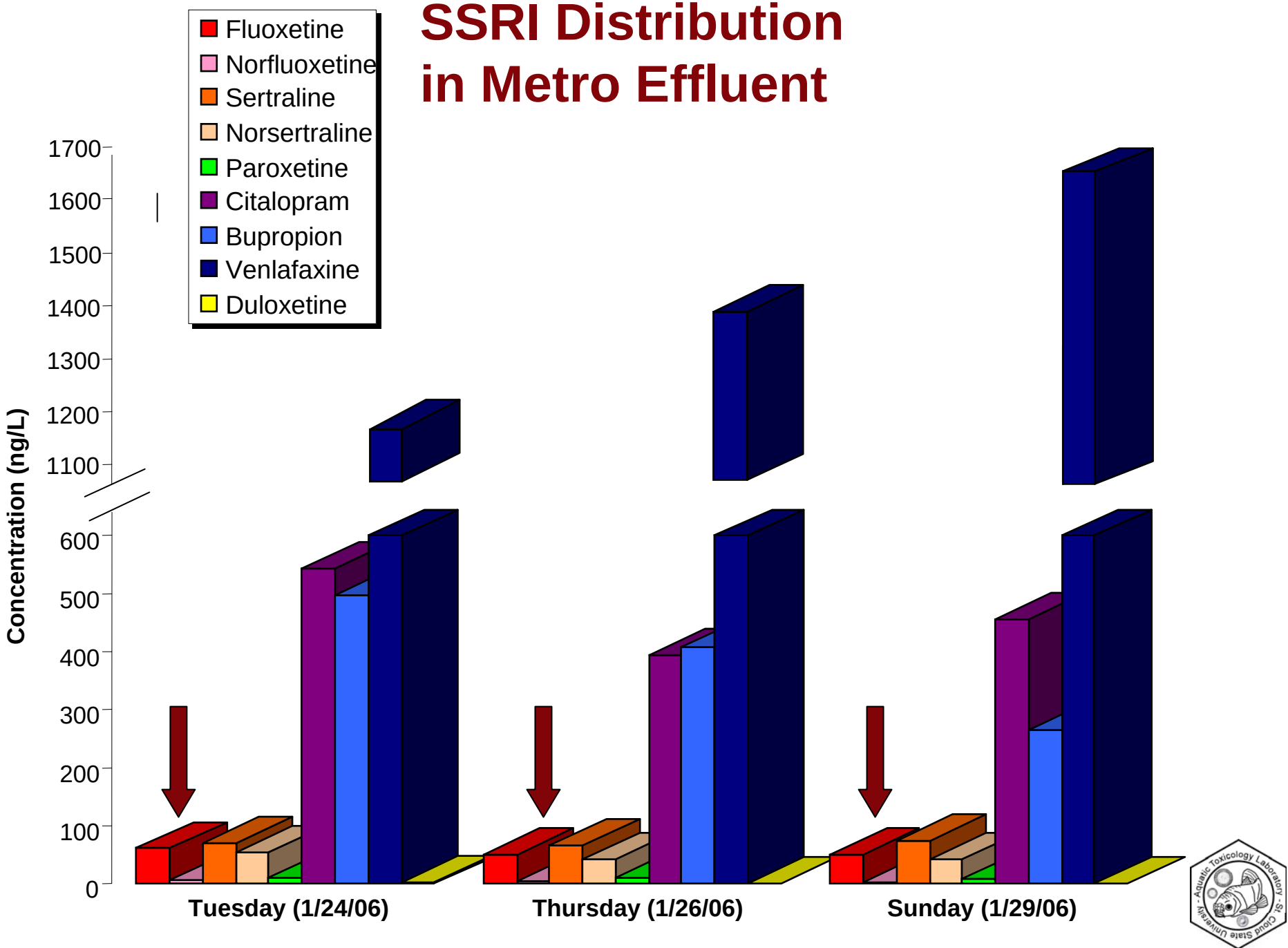
Analysis of Metro and Boulder Creek Treated Wastewater Effluent [ng/L]

	Metro Effluent	Boulder Effluent
Estrone (E)	nd	5 - 50
Estradiol (E2)	nd - 7.3	2 - 5
Ethynylestradiol (EE2)		0.8 - 1.5
Nonylphenol (NP)	333 - 13,000	
Octylphenol (OP)	50 - 933	In
NP1EC	8,756 - 21,364	Progress
NP2EC	17,530 - 35,512	
NP1EO	684 - 1,002	
NP2EO	342 - 731	
Fluoxetine	51 - 83	nd - 48
Carbamazepin	823 - 1360	nd - 58
Triclosan	82 - 318	nd - 150

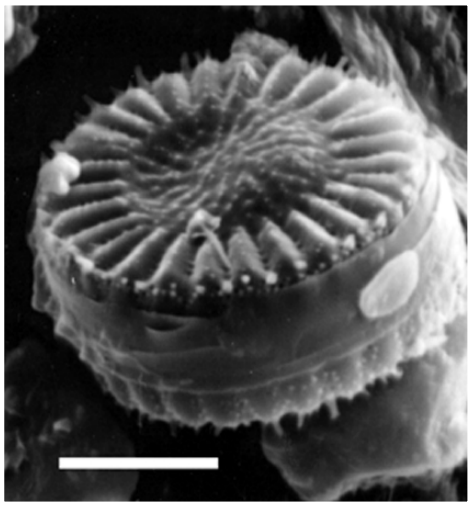
SSRI Distribution in Metro Effluent



SSRI Distribution in Metro Effluent



Phase 2A - Exposure Experiments



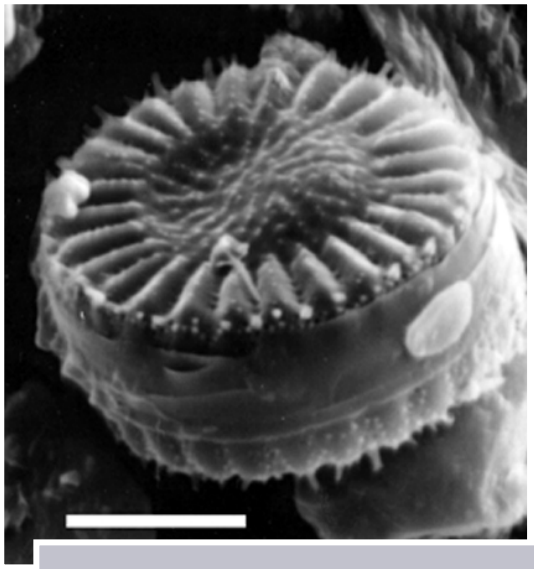
Locomotion (escape & swimming)
Male nest holding ability
Female fecundity
Offspring survival

Chlorophyll A :
Lipid Ratio

Food Quality

Juvenile Fathead Feeding
Performance

Survival & Reproductive Ability



Primary producer

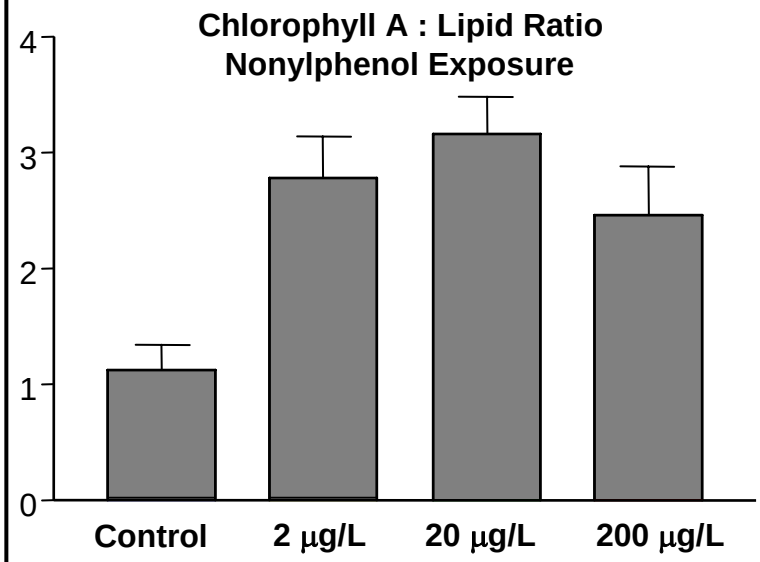
Cyclotella meneghiniana

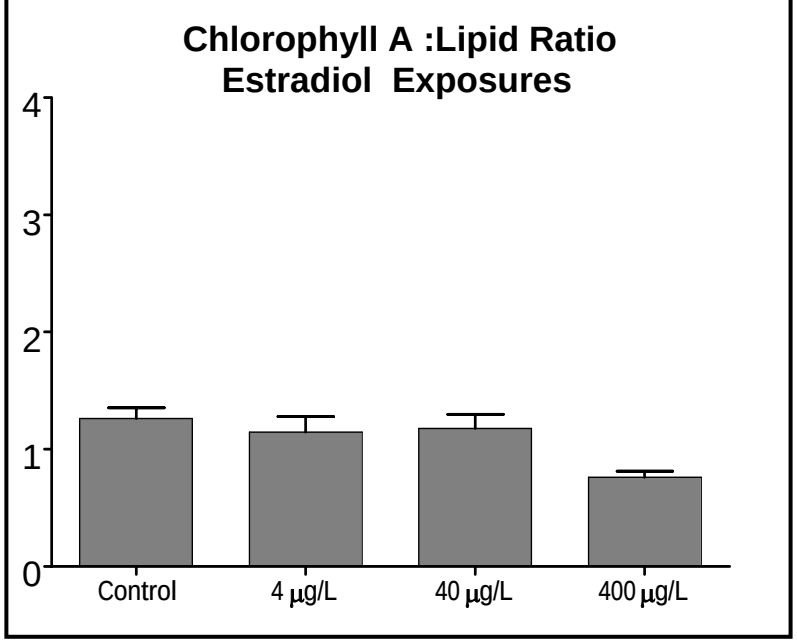
Experimental Design - Diatoms

- Expose pure culture of diatoms in static exposure to compound or mixture for 10 days in sterile culture flask.

- In regular intervals sample diatom culture for:
  - Cell count
  - Lipid content
  - Chlorophyll content

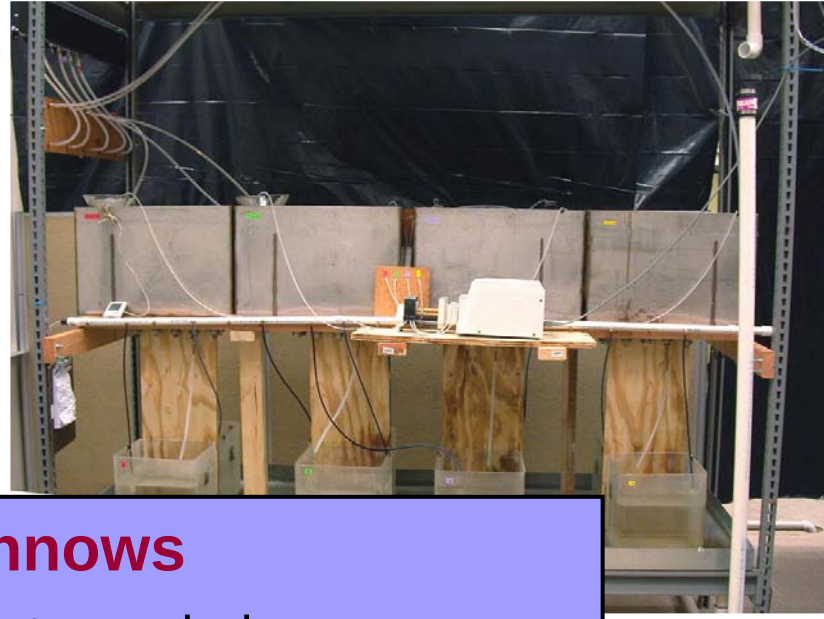
Phase 2A - Preliminary Results





- An increased ratio indicates a reduced nutritional value of the diatoms within the trophic cascade.
- E2 affects diatom food value very differently than nonylphenol.

Pimephales promelas



Experimental Design - Fathead Minnows

- Expose male fathead minnows for 28 days to graded concentrations of compound in flow-through exposure system.
- Allow males to compete with control males for access to spawning opportunities for seven days following exposure.
- After 35 days sample all males for:
  - Vitellogenin
  - Relative gonad and liver size, secondary sexual characters
  - Reproductive ability (nest holding, fecundity, offspring survival)
  - Locomotor ability (escape velocity & station holding ability).
  - Neuroendocrine endpoints

Competitive Spawning Trials

Well Water

Well Water & NP

28 day exposure

7 day trial

+ 2 females & nest site

Phase 2B - Linking Survival & Reproduction to Upstream Neuroendocrine Endpoints

Experiment	Species	Reproductive Endpoints	Neurochemical Endpoints
SURVIVAL			
(i) Feeding			
(i.i) Food quality	Diatom *Cyclotella meneghiniana*	Chlorophyll A : Lipid Density	n/a
(i.ii) Feeding performance	FM juveniles	# ingested diatoms	neurotransmitter
(ii) Locomotion			
(ii.i) Escape	FM juveniles	Reaction time Velocity of escape	abundance,
(ii.ii) Station holding	FM adults	Water flow velocity	distribution, and
REPRODUCTIVE ABILITY			metabolism
(i) Male nest protection	FM males	Nest holding day	
(ii) Female fecundity	FM females	# of eggs	
(iii) Offspring survival	FM larvae	# surviving offspring	

Neuroendocrine Endpoints

1. Characterize sexual dimorphism in the neuroanatomical distribution of enzymes and receptors responsible for expression of steroid-dependent behaviors.

2. Identify changes in the distribution of these enzymes and receptors following exposure to WWTP contaminants singly or in combination.

3. Identify minimum exposure duration for inducing changes in neurobehavioral endpoints.

Neuroendocrine Endpoints

I. Neurotransmitter Biosynthesis

- Tyrosine-hydroxylase is the rate limiting enzyme in the biosynthesis of norepinephrine and dopamine.
- The distribution of tyrosine-hydroxylase immunoreactive neurons in the preoptic brain is sexually dimorphic and responds to circulating concentrations of reproductive steroids.
- We hypothesize that endocrine compound exposures will alter tyrosine-hydroxylase immunoreactivity in the preoptic region of the brain.
- This change should be apparent after several weeks of exposure (“baseline change”).

Neuroendocrine Endpoints

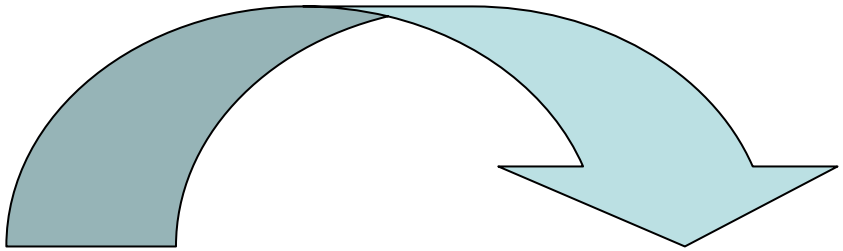
II. Neurotransmitter Analysis

- The turnover of the monoamines dopamine, norepinephrine, serotonine to their respective catabolites will be quantified in several regions of the brain.
- We hypothesize that changes in monoamine turnover rate will become apparent after exposure and after stimulation during the competitive reproductive assays.
- This change should be apparent after several weeks of exposure and should be dramatic following the competitive reproductive assay (“stimulation capacity”).

Neuroendocrine Endpoints

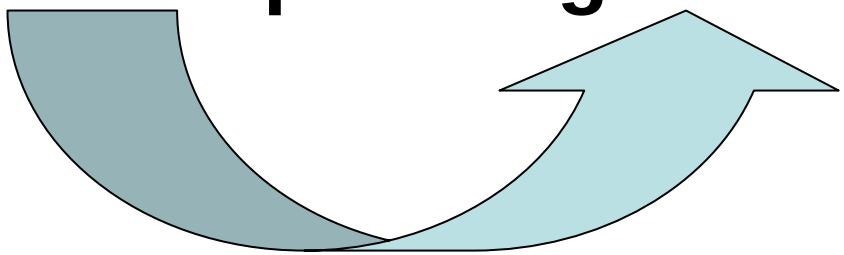
Distribution & abundance of tyrosine-hydroxylase immunoreactive neurons

"Baseline Alteration"



Fish Exposure

Competitive Spawning



Monoamine turnover quantification

"Stimulation Capacity"

Phase 3 - Field Validation of Neuroendocrine Endpoints in Exposure Experiments at Both Field Sites

Boulder, CO

St. Paul, MN


This research is funded by
U.S. EPA - Science To Achieve Results (STAR) Program
Grant #
R832741-01-0