Endocrine Effects of Selective Serotonin Reuptake Inhibitors (SSRIs) on Aquatic Organisms

Marsha C. Black & Emily D. Rogers
University of Georgia
Athens, Georgia, USA

Theodore B. Henry
University of Tennessee
Knoxville, TN

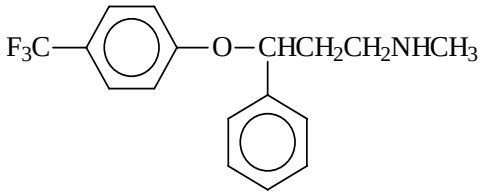
Outline

- SSRIs - MOA and clinical significance
- Presence in the environment
- Study objectives
- Results and Discussion
  - Acute toxicity (macroinvertebrate, fish)
  - Chronic effects (macroinvertebrate, fish, frog)
- Summary and conclusions
- Future research directions

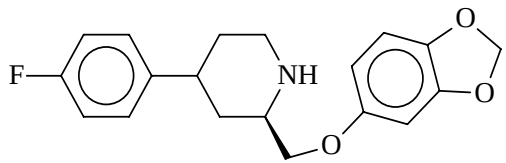
Selective Serotonin Reuptake Inhibitors (SSRIs)

- Treat clinical depression, obsessive-compulsive and panic disorders, PMS, etc.
- Clinical MOA: block serotonin reuptake
- Examples:
  - Fluoxetine (Prozac® and Sarafem®)
  - Sertraline (Zoloft®)
  - Citalopram (Celexa® and Lexapro®)
  - Fluvoxamine (Luvox®)
  - Paroxetine (Paxil®)

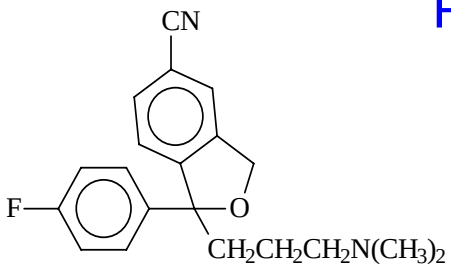
SSRI Structures



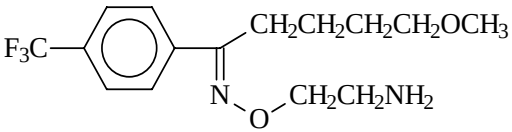
Fluoxetine (Prozac®)



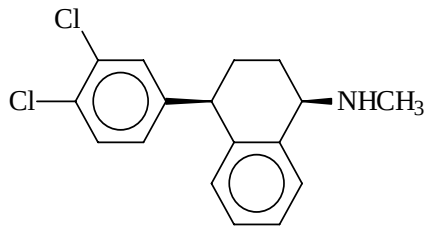
Paroxetine (Paxil®)



Citalopram (Celexa®)

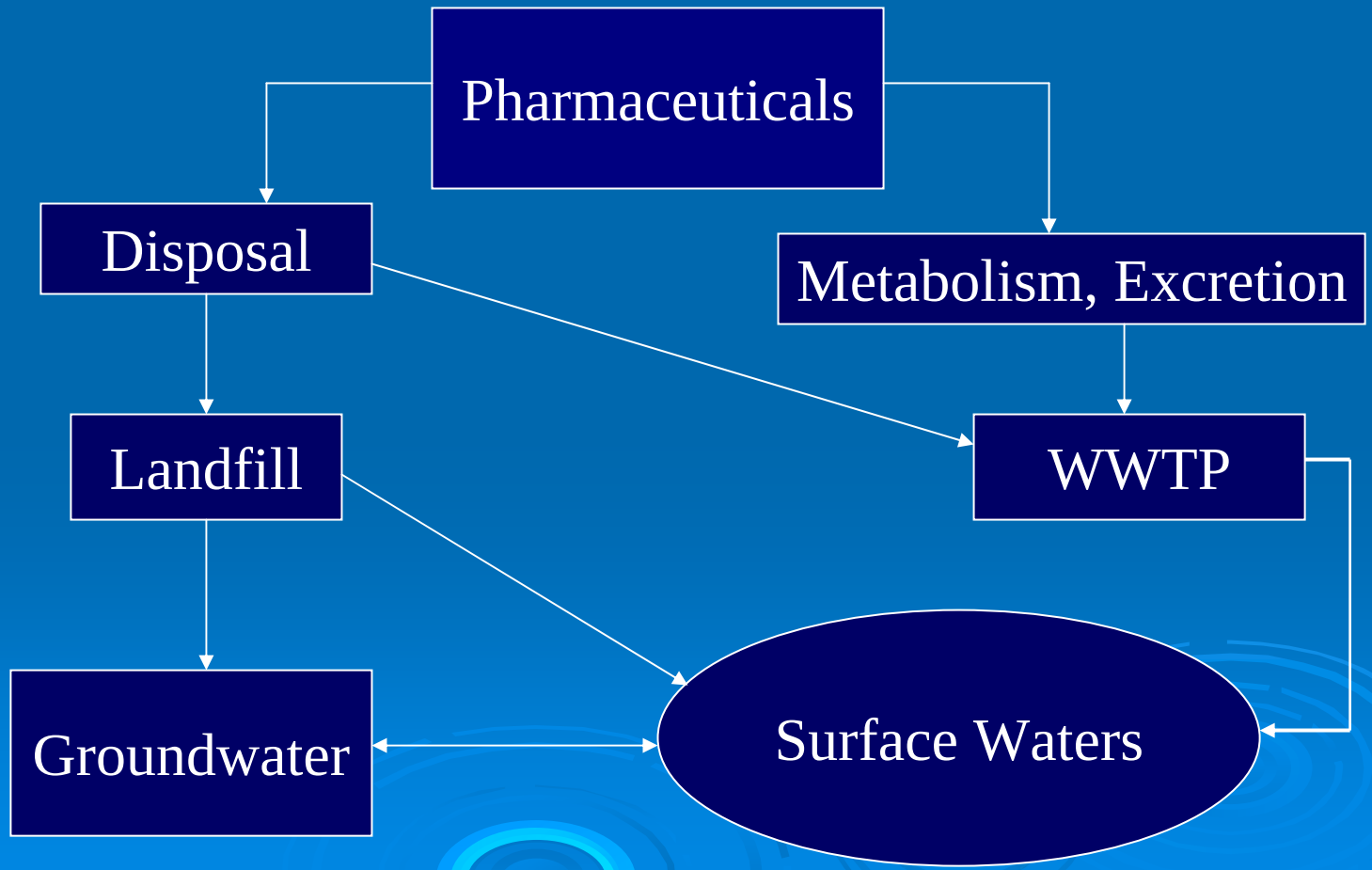


Fluvoxamine (Luvox®)



Sertraline (Zoloft®)

Sources of Surface Water Contamination by Human Pharmaceuticals



SSRIs: Detection in the Environment

- Fluoxetine detected in surface waters
  - 0.012 ppb detected in USGS reconnaissance study (Kolpin et al. 2002)
  - 0.030-0.099 ppb in Canada (Metcalfe et al. 2003)
  - 0.031-0.076 ppb in Mississippi (Wook-Kwon and Armbrust, unpublished)
- Fluoxetine, sertraline and metabolites detected in fish tissues (Brooks et al., 2005)

Physicochemical Properties of SSRIs

(data from Wook-Kwon and Armbrust)

Compound	Log K_{OW}[a]	Log K_{OC}[b]	Photolysis $t_{½}$[c] (d)
Citalopram	1.39	5.63	39
Fluoxetine	1.22	4.65	122
Fluvoxamine	1.21	3.82	0.57; 29
Paroxetine	1.37	4.47	0.67
Sertraline	1.37	4.17	23

[a]Measured on salt form

[b]Average calculated from experiments with 5 different soils and sediments

[c] Average calculated from experiments with 2 different lake water samples

Why Worry?

- Pharmaceuticals are *designed* to have a therapeutic (=biological) effect
  - Effects on non-target organisms are mostly unknown
- Aquatic organisms are exposed throughout their lifetime
- Potential for multigenerational exposure
- Little is known about persistence, fate of drugs in the environment
- SSRIs known to promote spawning in mollusks

Overall Research Plan…

- Determine environmental fate of SSRIs
  - Techniques used for pesticide registration
  - Measure hydrolysis, photolysis, metabolism, etc.
- Measure parent and major degradation products
  - Wastewater effluent
  - Downstream receiving water
- Determine acute, chronic impacts to aquatic organisms
  - *Ceriodaphnia dubia* (macroinvertebrate)
  - *Gambusia affinis* (Western mosquito fish)
  - *Xenopus laevis* (frog)

Toxicity Tests

- Test organism: *Ceriodaphnia dubia*
- Acute toxicity (48 h)
  - Single compound exposures
  - Binary, quaternary mixture exposures
  - Mortality (LC50) as endpoint
- Chronic toxicity
  - 7 day mini-chronic test
  - Brood size, # broods as endpoints
- All tests followed US EPA protocols

Acute Toxicity (LC50) of SSRIs

SSRI	LC50 ppb[a]
Citalopram (Celexa®)	3180 (220)
Fluvoxamine (Luvox®)	1260 (830)
Paroxetine (Paxil®)	470 (60)
Fluoxetine (Prozac®)	590 (130)
Sertraline (Zoloft®)	140 (20)

[a]Mean (± SD) of 3 tests

Henry et al. 2004, *Environ Toxicol Chem* 23:2229-2233

Chronic Toxicity of SSRIs

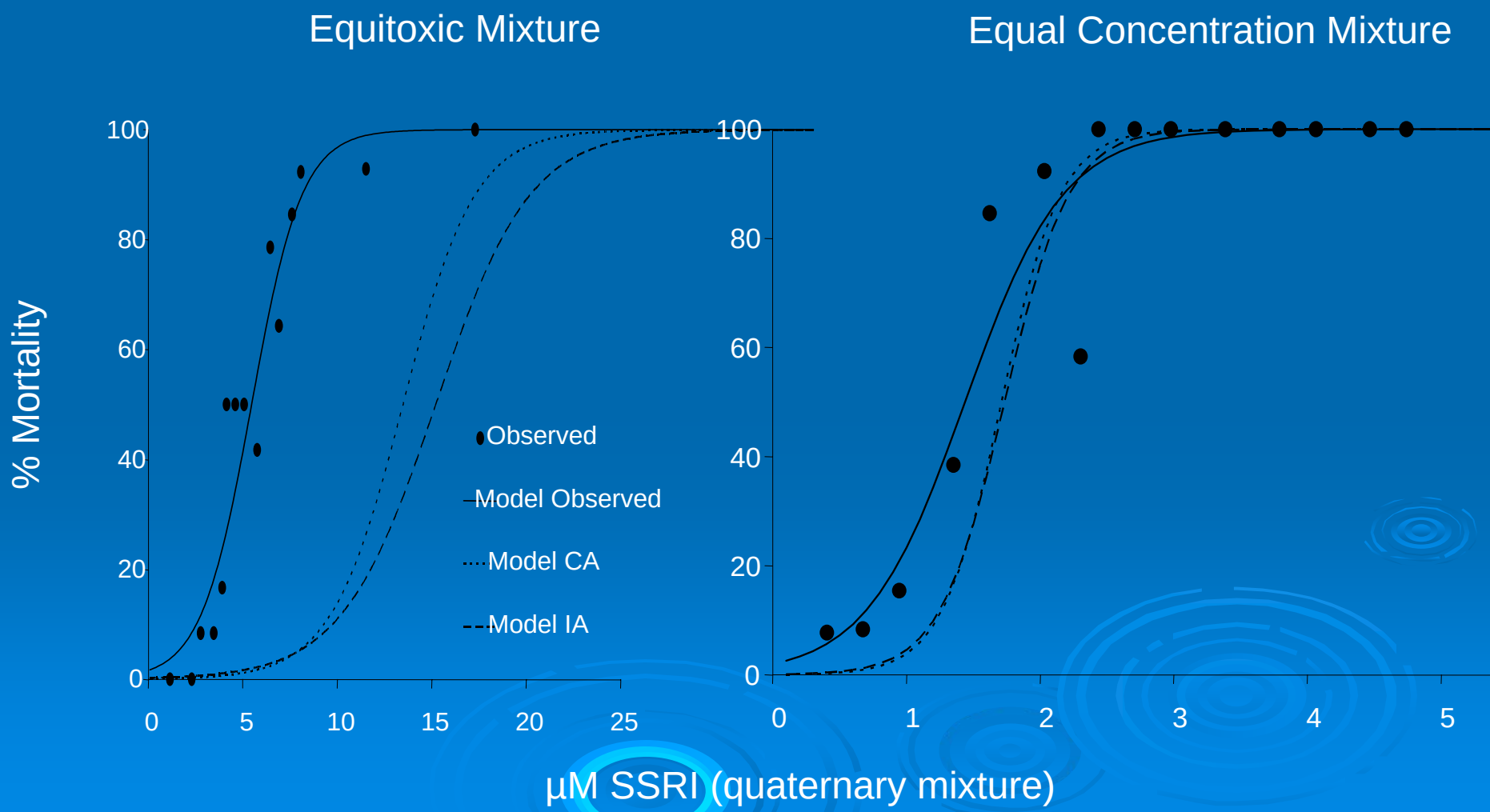
SSRI	NOEC[a] (ppb)	LOEC[a] (ppb)
Citalopram (Celexa®)	800	4000
Fluvoxamine (Luvox®)	366	1466[b]
Paroxetine (Paxil®)	220	440[b]
Fluoxetine (Prozac®)	89	447[b]
Sertraline (Zoloft®)	9	45

[a]Total number of neonates produced over 7-8 d

[b]Number of broods also significantly reduced

(Henry et al. 2004, *Environ Toxicol Chem* 23:2229-2233)

Mixture Toxicity
(In preparation, Henry and Black)



Acute Toxicity of Fluoxetine to Western Mosquitofish

- 7-d acute tests
- Endpoints:
  - Mortality (LC50)
  - Fish behavior

Western mosquitofish
Gambusia affinis

Acute Toxicity of Fluoxetine to Western Mosquitofish

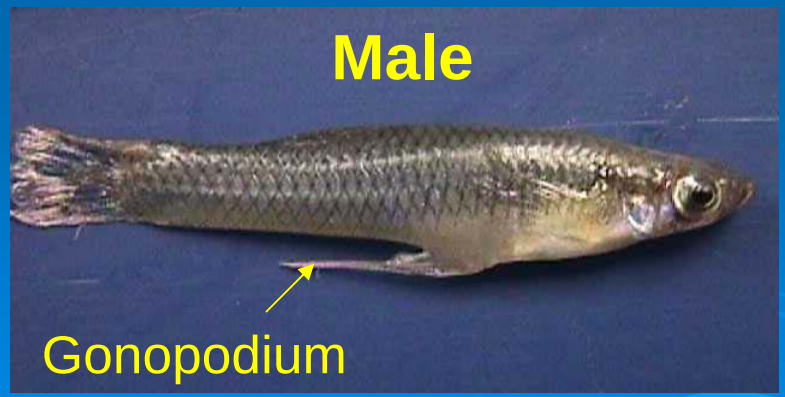
- Mortality
  - 7-day LC50 = 614 ppb
- Behavioral effects (0.6 and 6 ppb)
  - Uncoordinated swimming
  - Lethargy, lack of response to stimuli
  - Less aggression, interaction between individuals

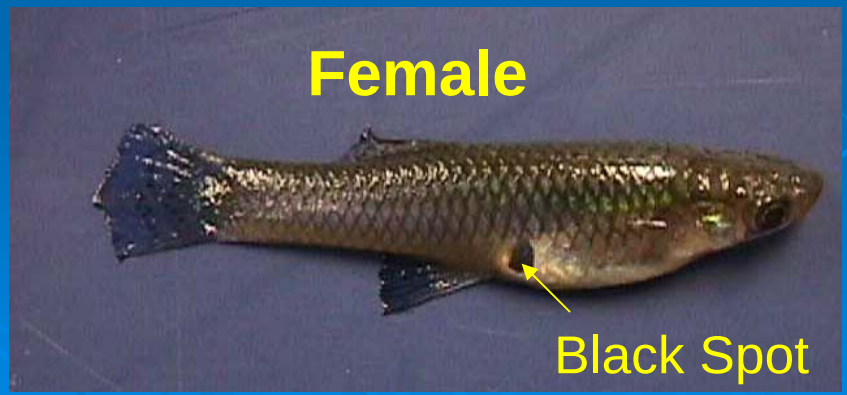
Chronic Exposures in Outdoor Mesocosms

- 110-L plastic tanks
- 50 fish/tank
- 85-d exposure
- Water change 1x/wk

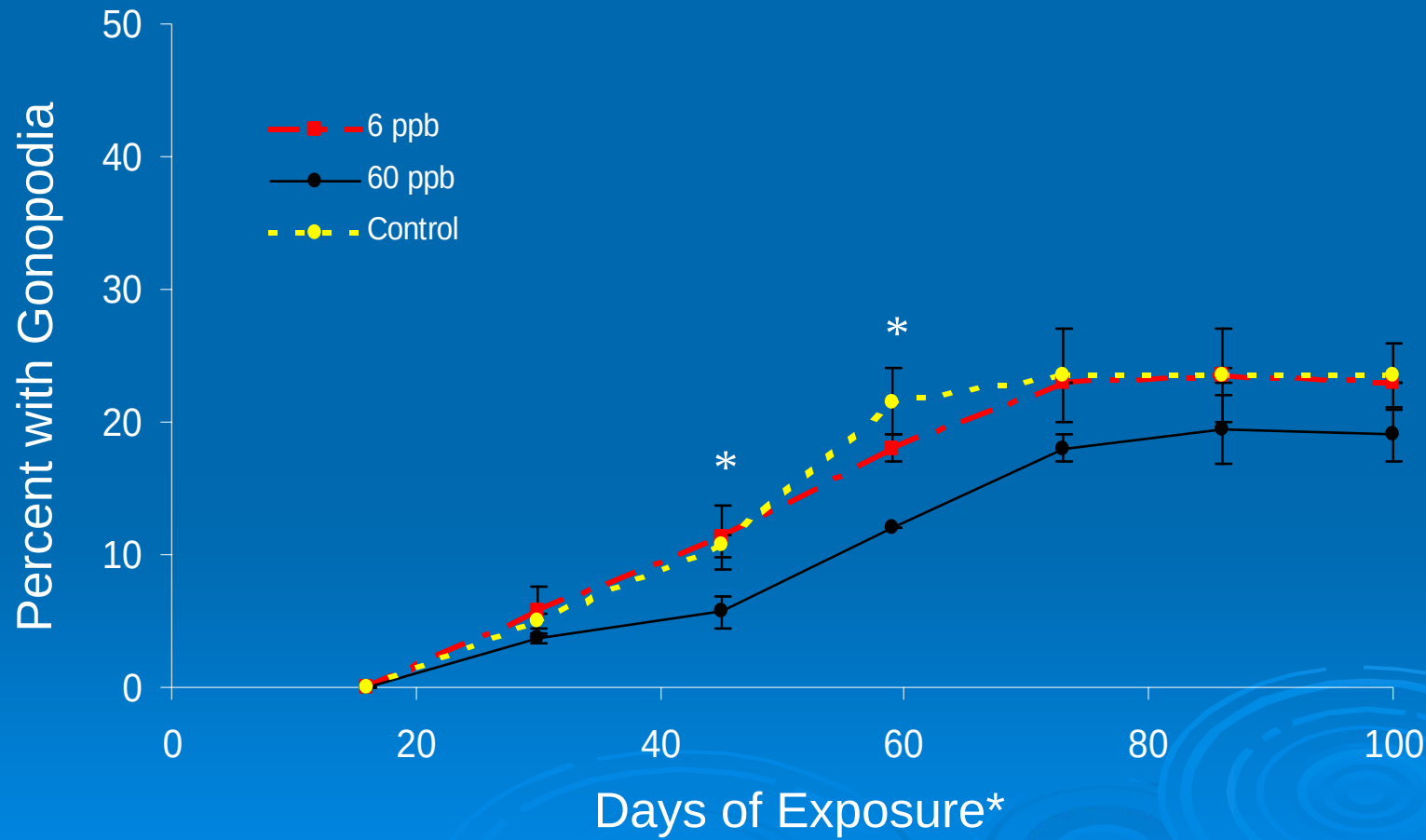
Chronic Tests (140 d) with Mosquitofish

- Time to reproductive maturity
  - Fully developed gonopodium (males)
  - Formation of black spot (females)
- Histological effects on gonads?



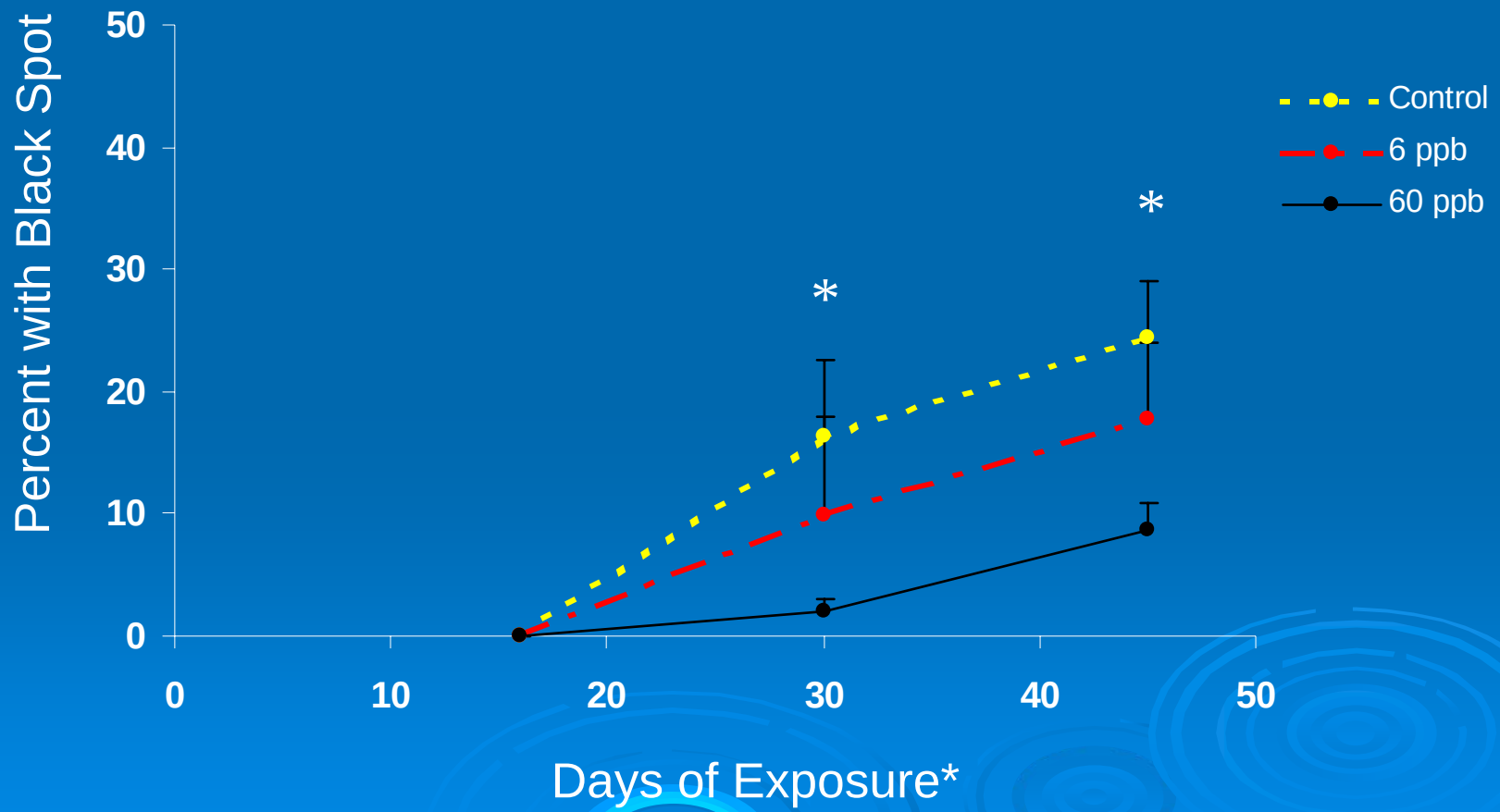


Effect of Fluoxetine on Male Sexual Development



*Fish were 39-d old at t=0

Effect of Fluoxetine on Female Sexual Development



*Fish were 39-d old at t=0

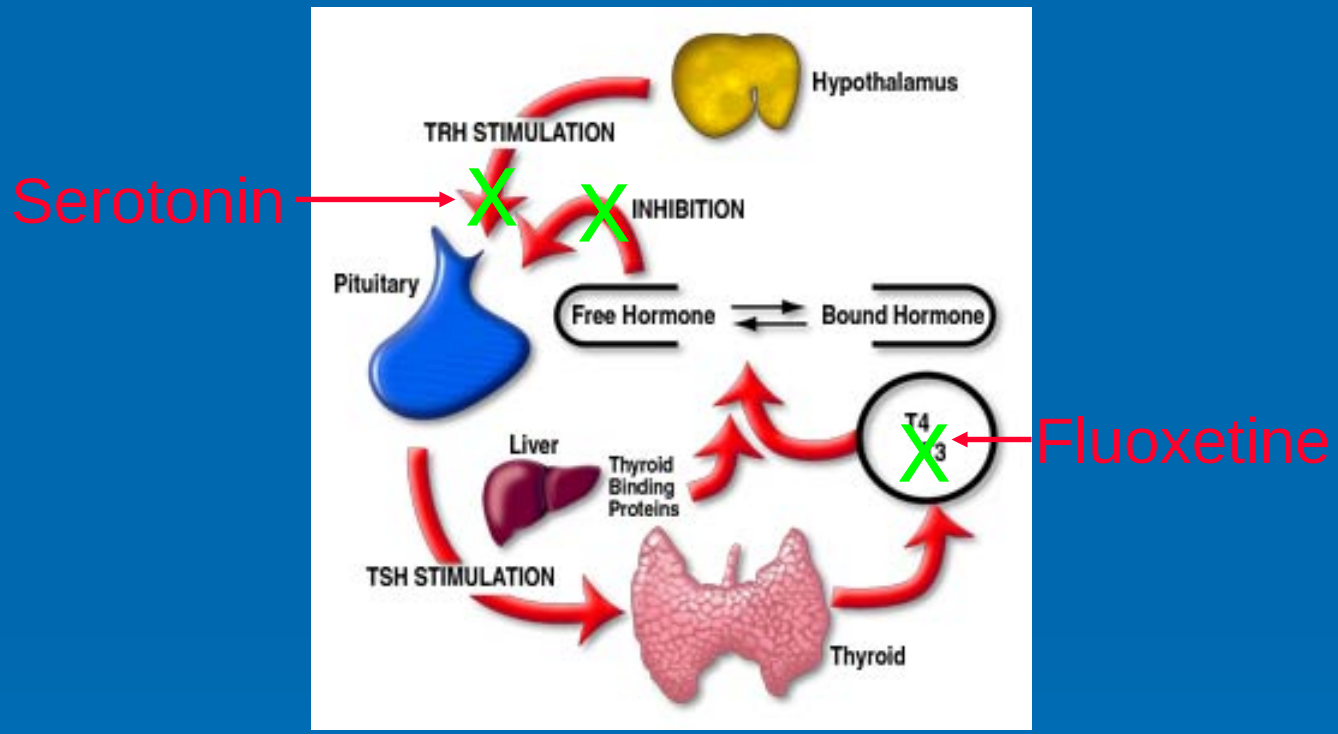
Research with the African Clawed Frog (*Xenopus laevis*)

- Easy to breed in the lab
  - Inject with HCG
- Tadpole to frog in 60-70 d
- Many measurable endpoints
  - Mortality
  - Developmental malformations
  - Time to metamorphosis

Why Study Frogs?

- Thyroid hormones (T_3,T_4) cue metamorphosis
- Tadpoles with no thyroid – metamorphosis inhibited
- Exposure to chemicals that reduce circulating T_3 will delay or inhibit metamorphosis

Regulation of Thyroid Axis in Mammals



www.dpcweb.com/images/medicalconditions/thyroid/thyroid%20illustration.jpg

- Serotonin inhibits the release of TRH from the hypothalamus in rats
  - Mitsuma et al. 1983; Mitsuma et al. 1996
- Fluoxetine reduces circulating T3 and T4; increases TSH
  - Golstein et al., 1983

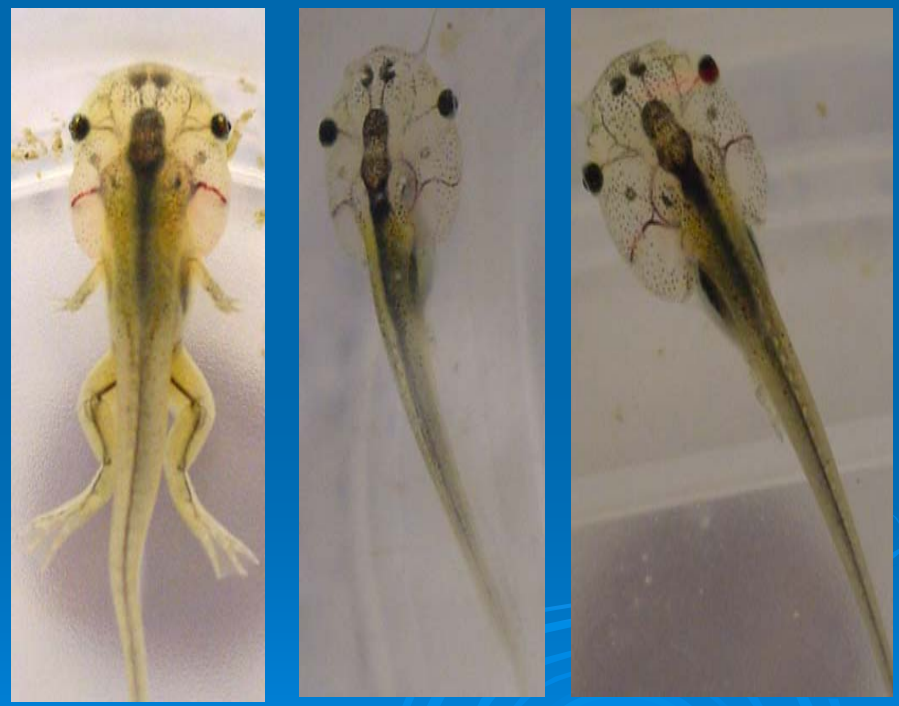
Does Fluoxetine Inhibit Frog Metamorphosis?

- Expose tadpoles from hatch until metamorphosis
  - Fluoxetine (FL): 0.059, 0.295, 2.95, 29.5 ppb (measured)
  - Ammonium perchlorate (AP): 10 ppb
  - Control (clean exposure water)
- Observe daily for limb development until metamorphosis is complete

Effects of Chronic Exposure to Fluoxetine (Xenopus)

- Developmental delays
  - Forelimb formation
  - Tail resorbtion
- Increased time to metamorphosis
- Mortality

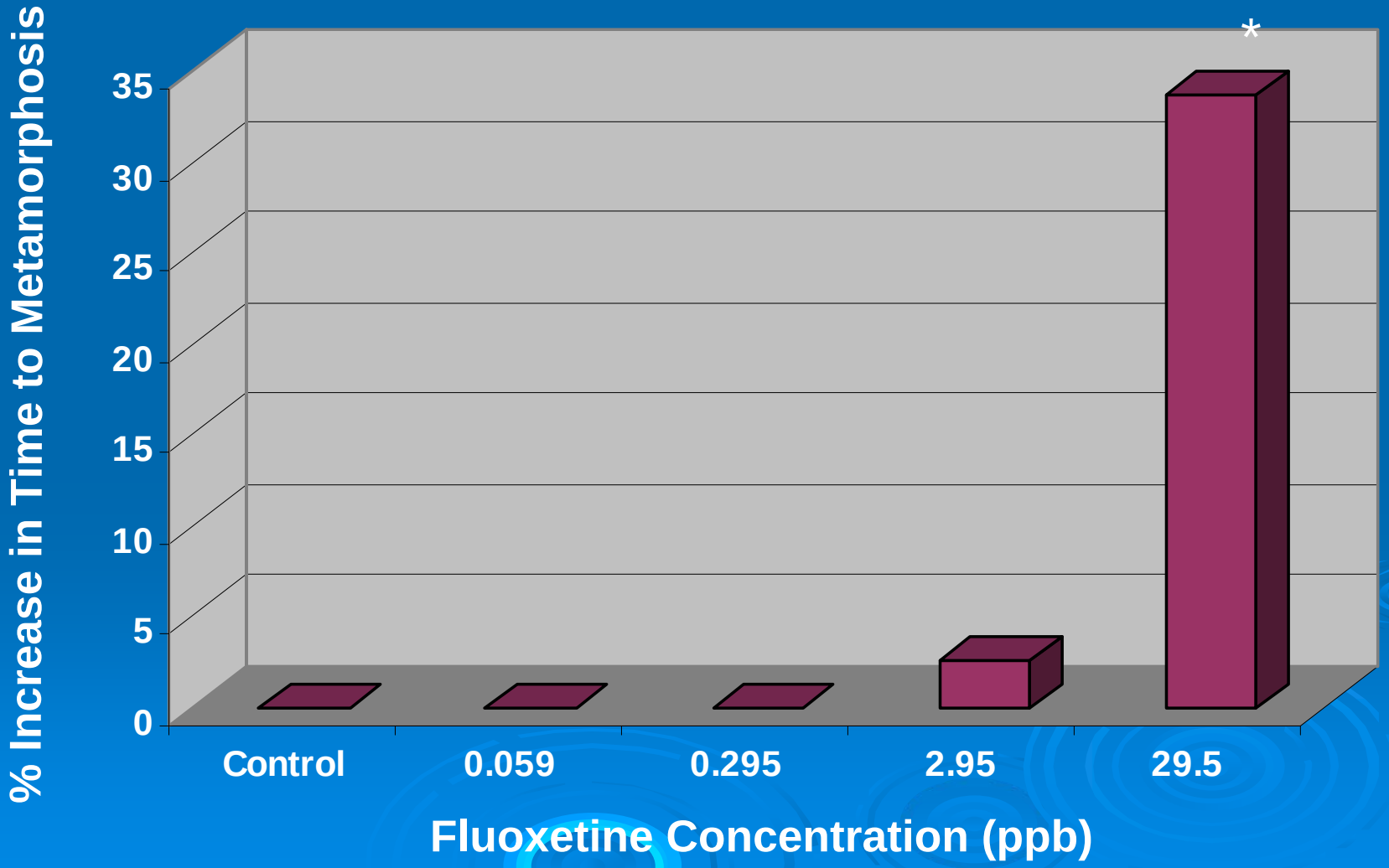
Tadpoles at 57 d*



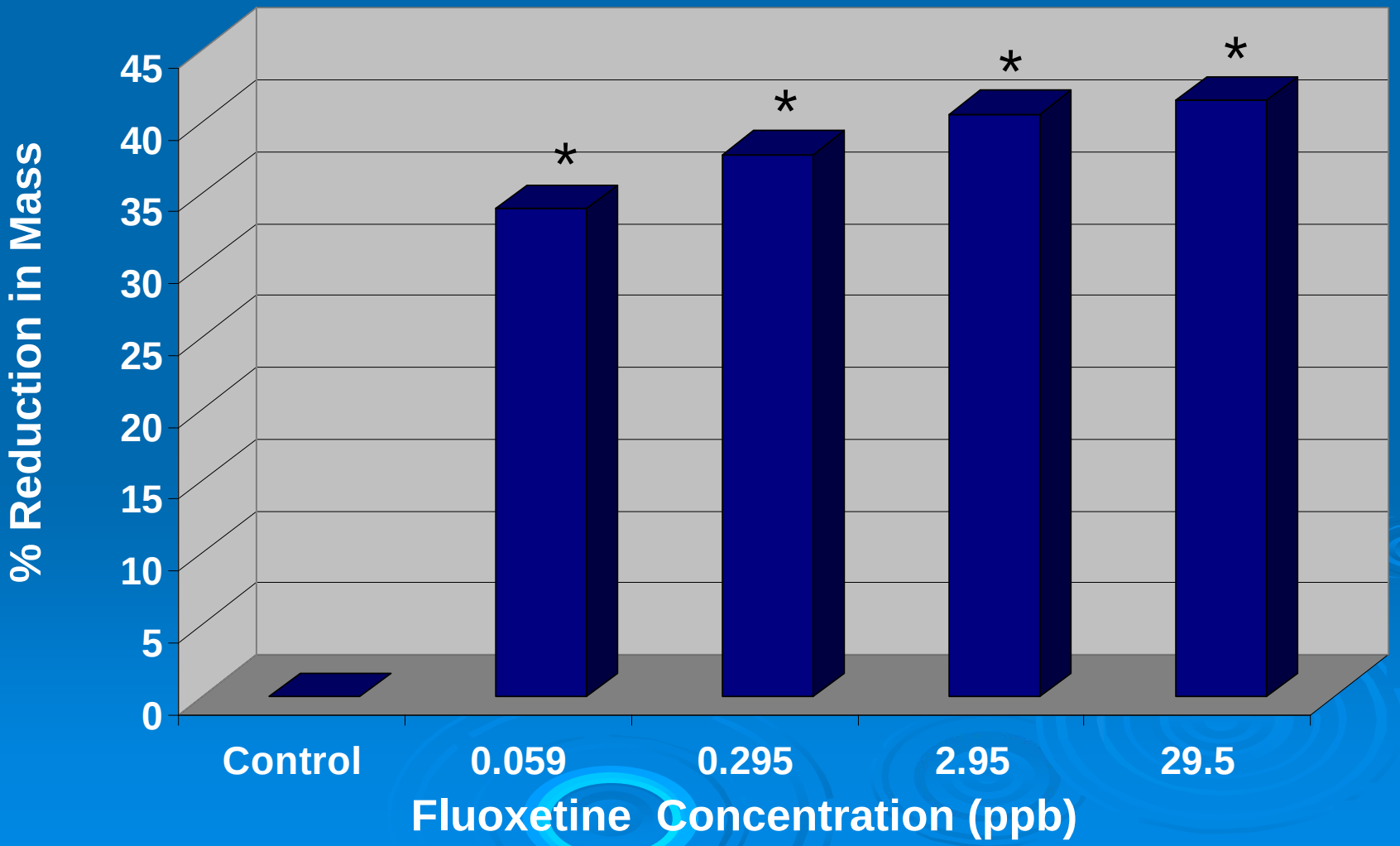
Control 38 ppb FL 9.5 ppb AP

*Data from range-finder experiment. Similar effects at 29.5 ppb in 2nd experiment.

Effect of Chronic Exposure to Fluoxetine on Time to Metamorphosis

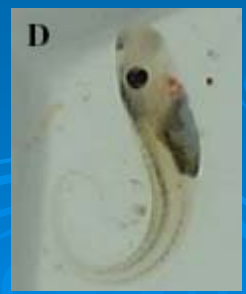


Effect of Chronic Exposure to Fluoxetine on Mass at Metamorphosis

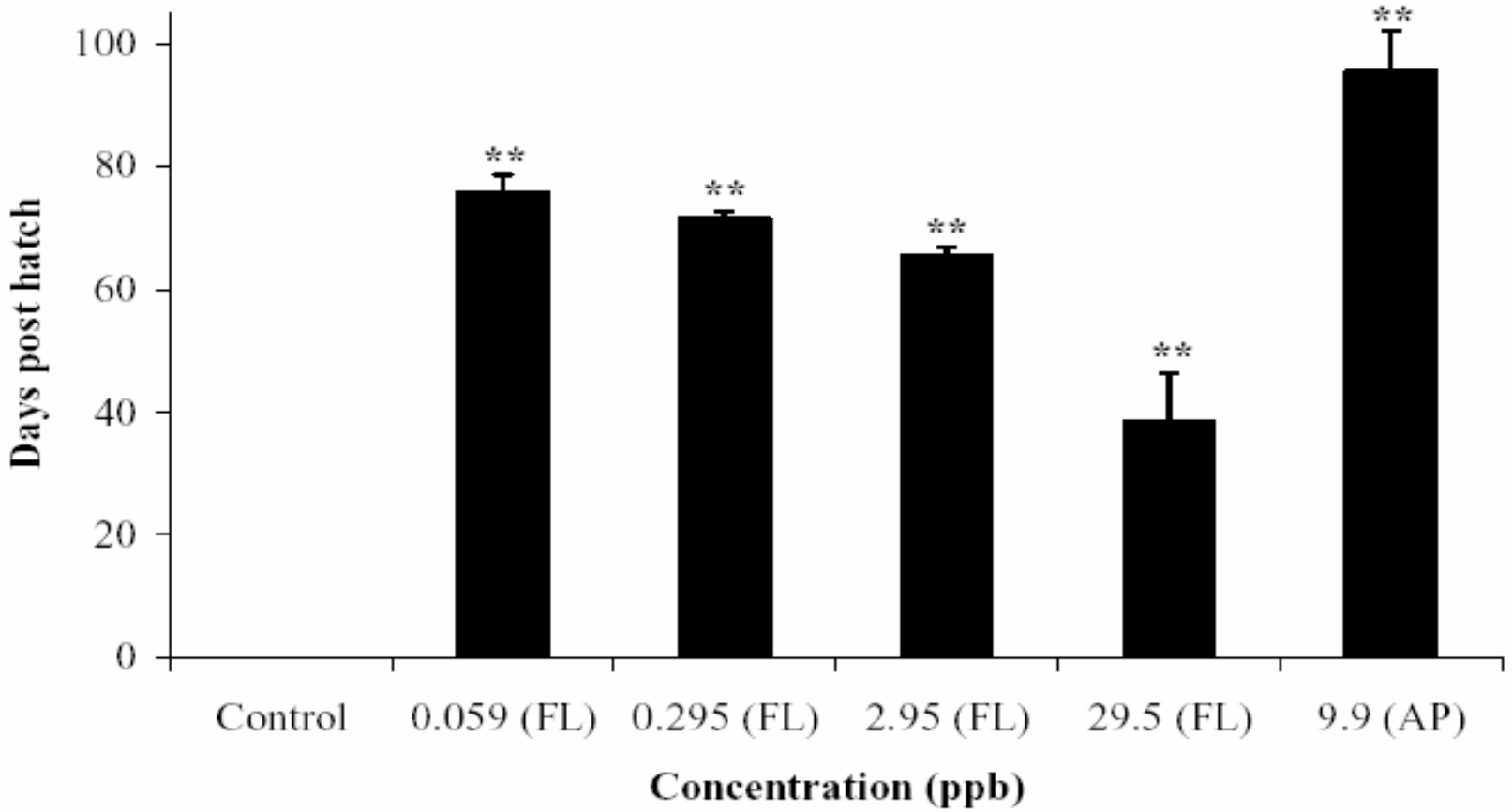


Effects of Chronic Exposure to Fluoxetine (Exp. 2)

- Limb malformations
  - Primary rotation of hindlimbs
  - Micromelia of forelimbs
  - Dorsal flexure of the tail



Time to Onset of Malformations



Conclusions (so far…)

- SSRIs are acutely toxic to *Ceriodaphnia* and mosquitofish
- Fluoxetine affects fish behavior
- Fluoxetine delays sexual development in fish
- Fluoxetine delays development and metamorphosis in frogs

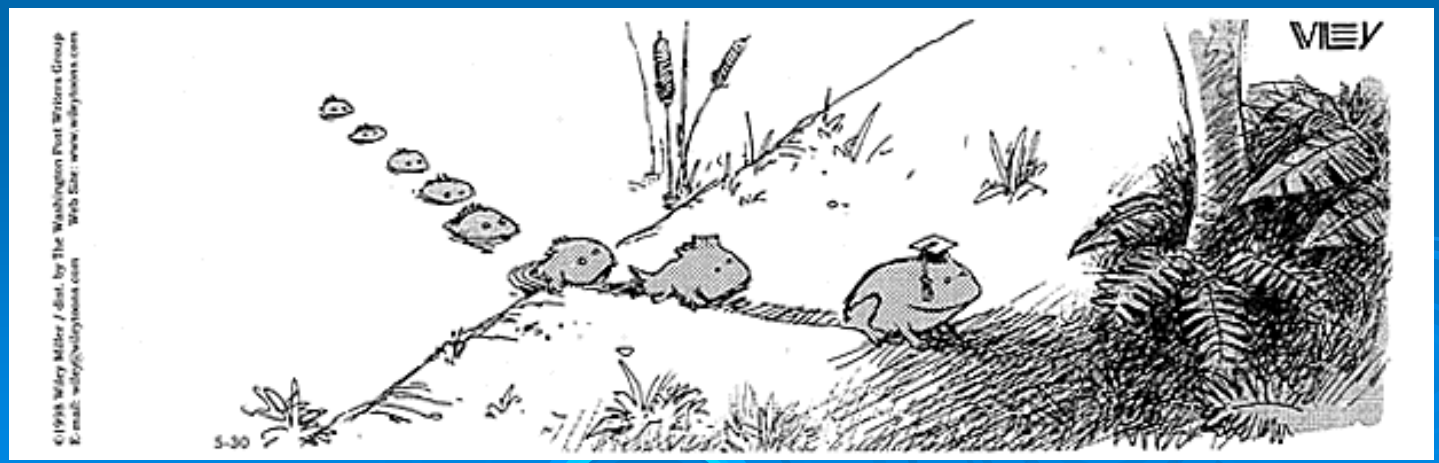
None of these effects observed at environmentally-relevant concentrations.

Conclusions (cont'd)

- Reduced mass and limb malformations observed with chronic exposure to FL
  - Both effects occured at environmentally relevant concentrations
  - Mass reductions confirmed in 2 experiments
  - Malformation data not yet confirmed
    - Lower temperature in experiment 2 (19ºC)
    - Increased exposure duration, TTM
    - Increased susceptibility of Exp. 2 frogs to developmental disorders?

Implications of the Research

- Delayed development (fish, frogs)
  - ↑ Predation, dessication (frogs), population decline?
- Reduced mass and limb malformations (frogs)
  - ↑ Predation, ↓ reproductive success, population decline?



Future Research Questions Generated by Research

- Conduct additional FL exposure with *Xenopus*
- Validate apparent impact of FL on the thyroid axis by measuring TH, TSH during frog development (with/without FL)
- Do other SSRIs have similar effects on frog development and growth?
- What is the toxicity of mixtures of SSRIs in the amphibian model?
- What are environmentally-relevant SSRI concentrations?

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