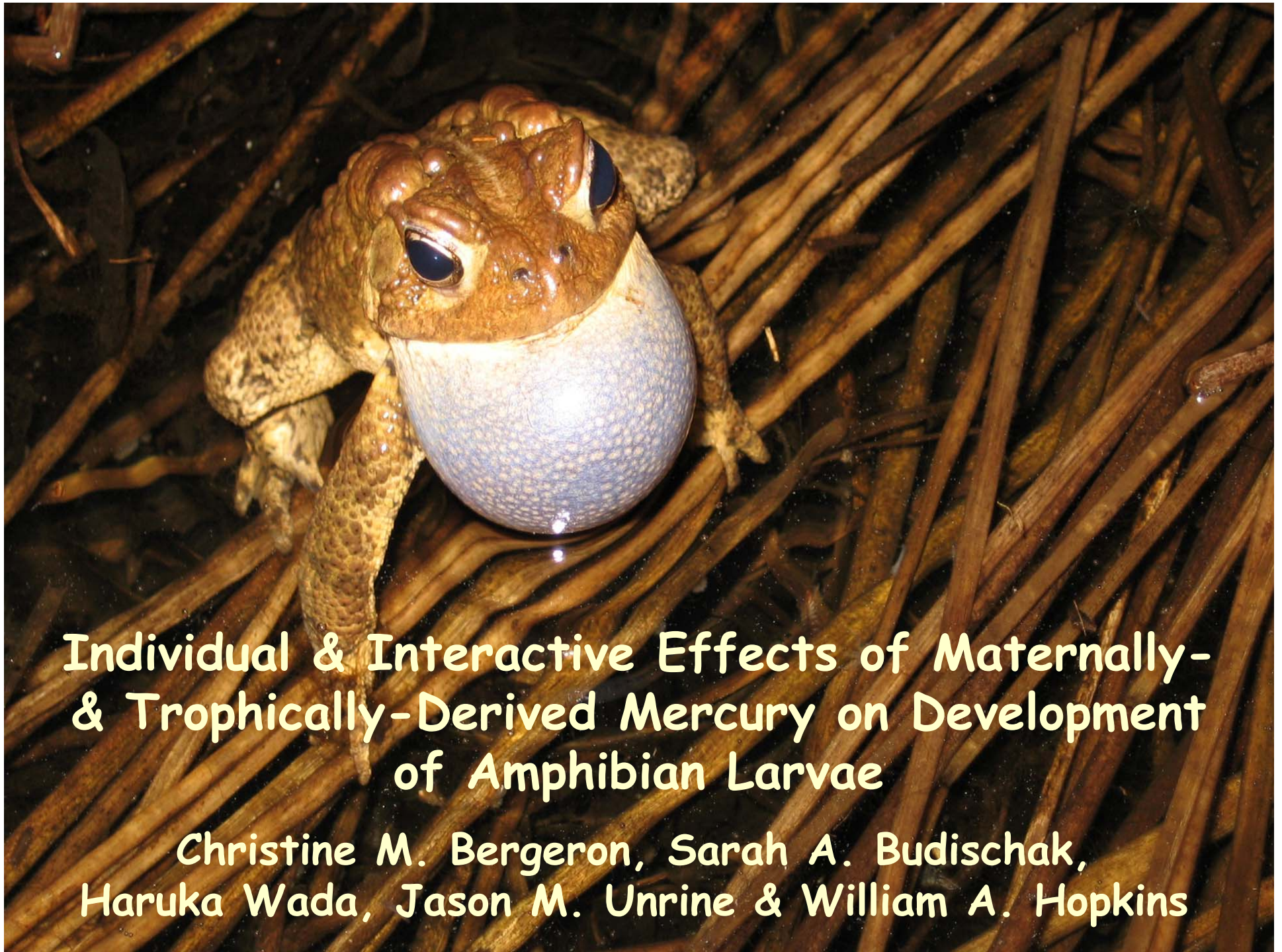




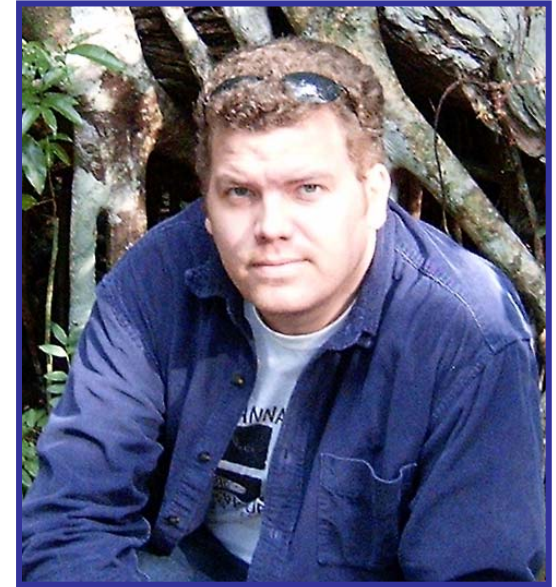
Individual & Interactive Effects of Maternally- & Trophically-Derived Mercury on Development of Amphibian Larvae

Christine M. Bergeron, Sarah A. Budischak,
Haruka Wada, Jason M. Unrine & William A. Hopkins

Team of "Mavericks"



Christine Bergeron



Jason Unrine, Ph.D.

John Burke, Sarah Budischak,
Haruka Wada, Ph.D.



2007 Synopsis

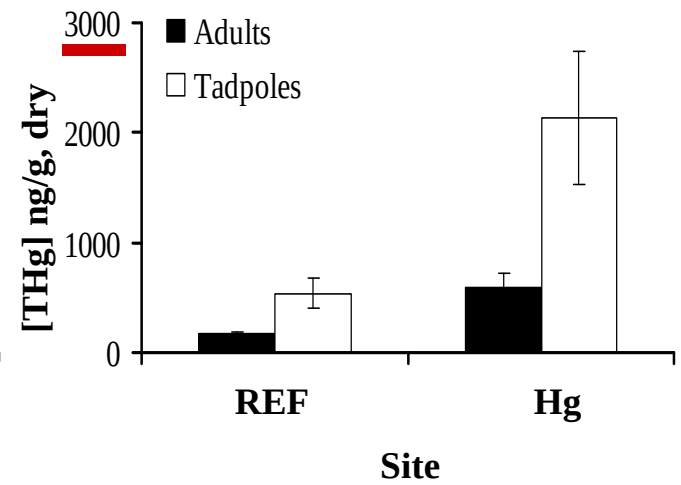
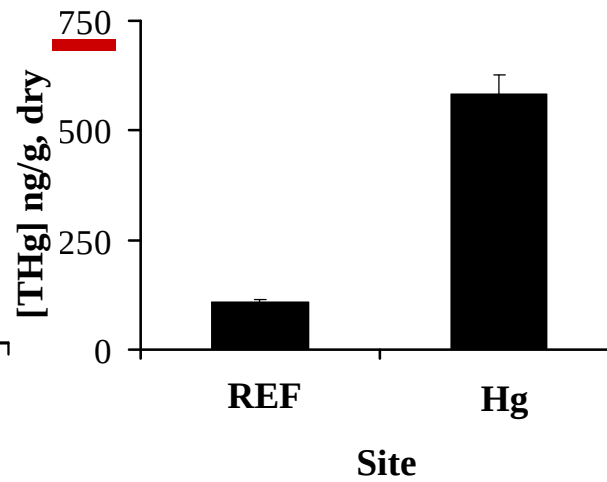
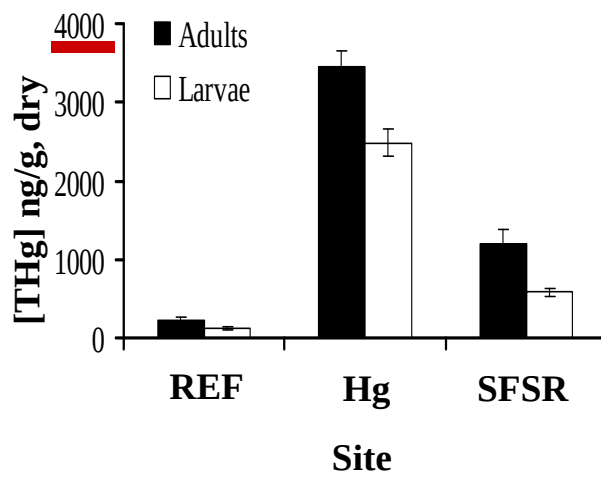
Eurycea bislineata
Two-lined Salamander



Plethodon cinereus
Red-Backed Salamander



Bufo americanus
American Toad



2007 Synopsis

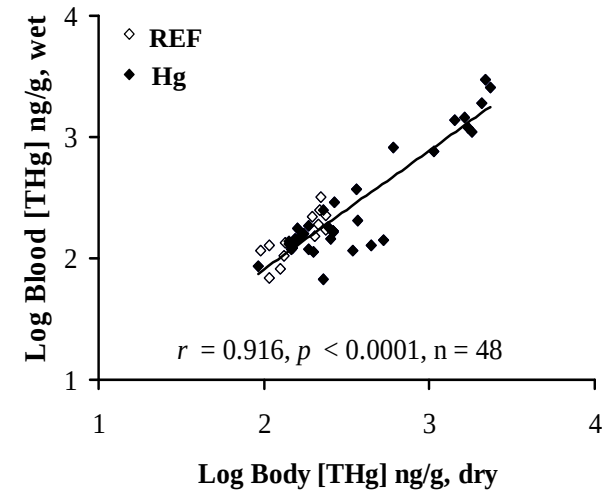
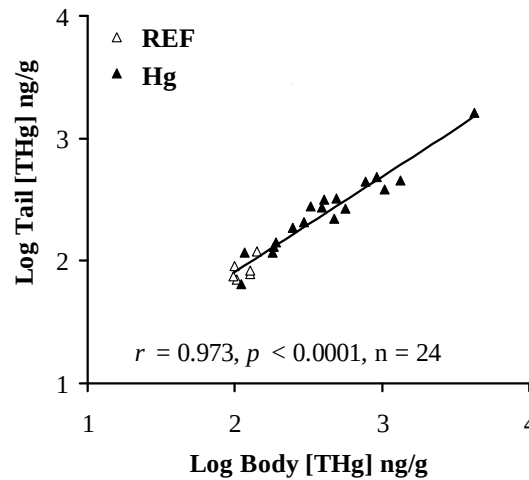
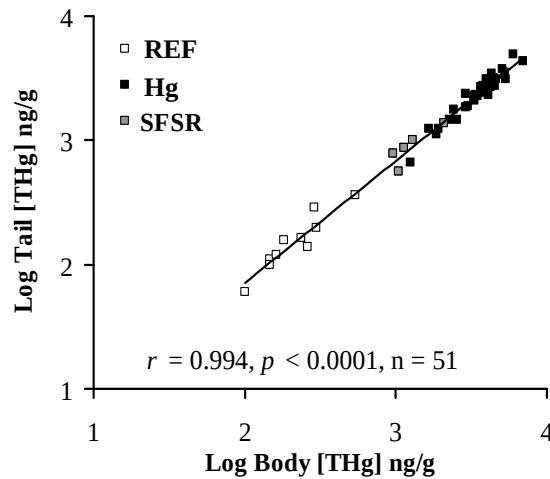
Eurycea bislineata
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Red-Backed Salamander



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American Toad

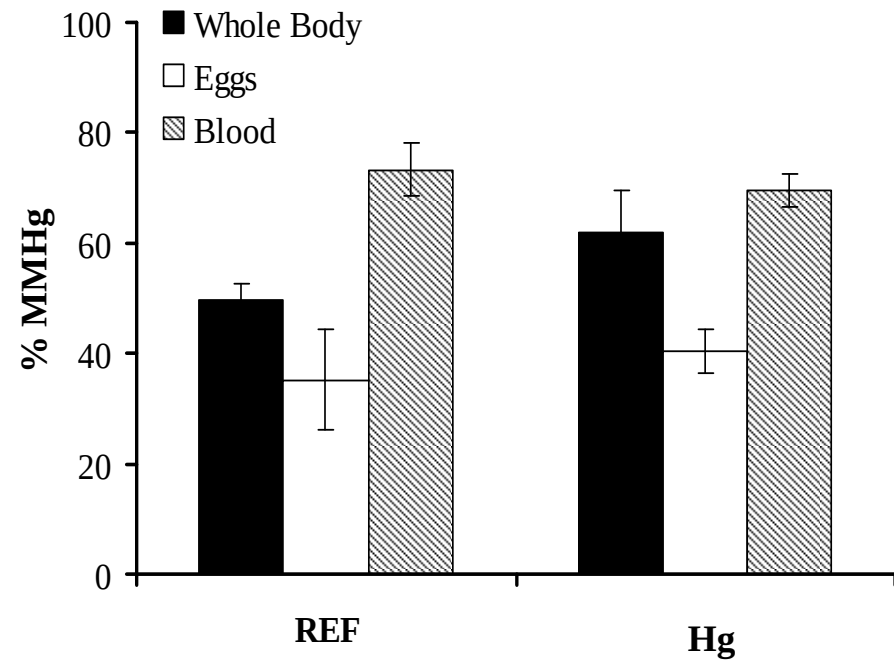
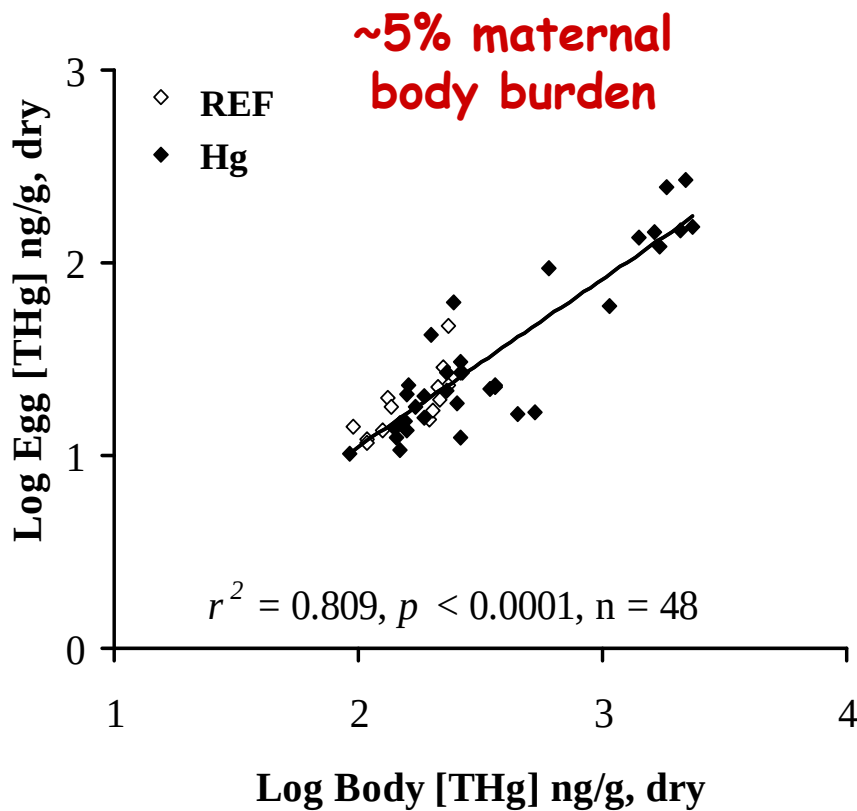




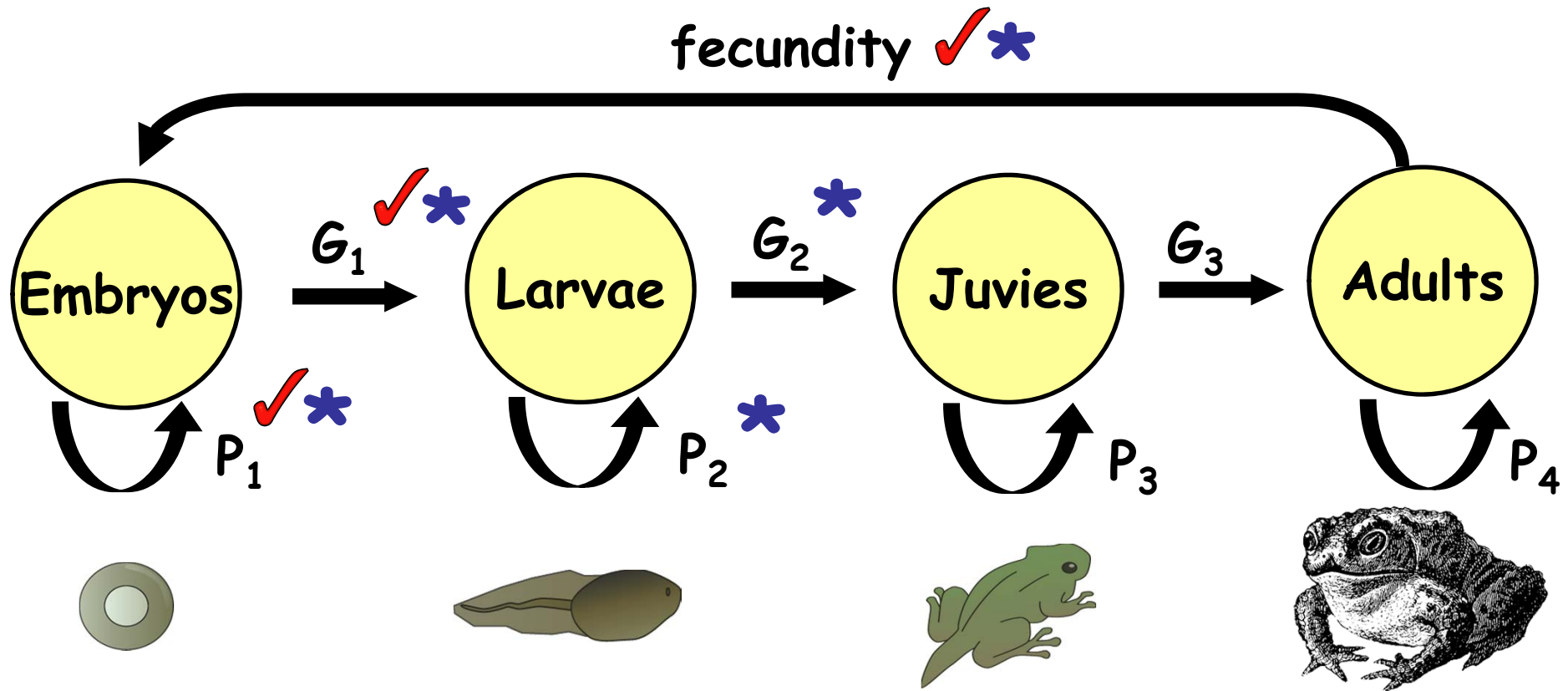
2007 Synopsis



B. americanus females maternally transfer Hg to their eggs



Ultimate Goal: Stage-based Population Model



G - probability of surviving to next stage
 P - probability of staying in the same stage

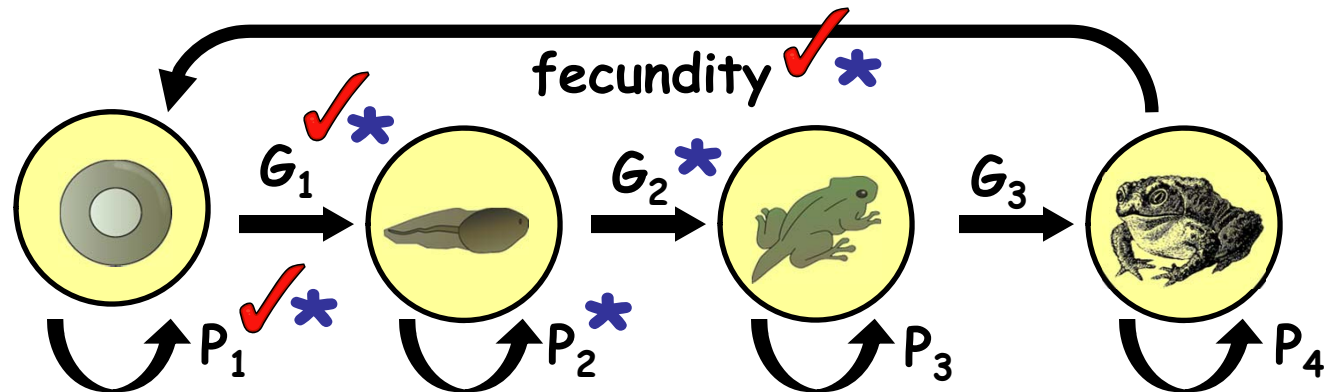


Spring 2007

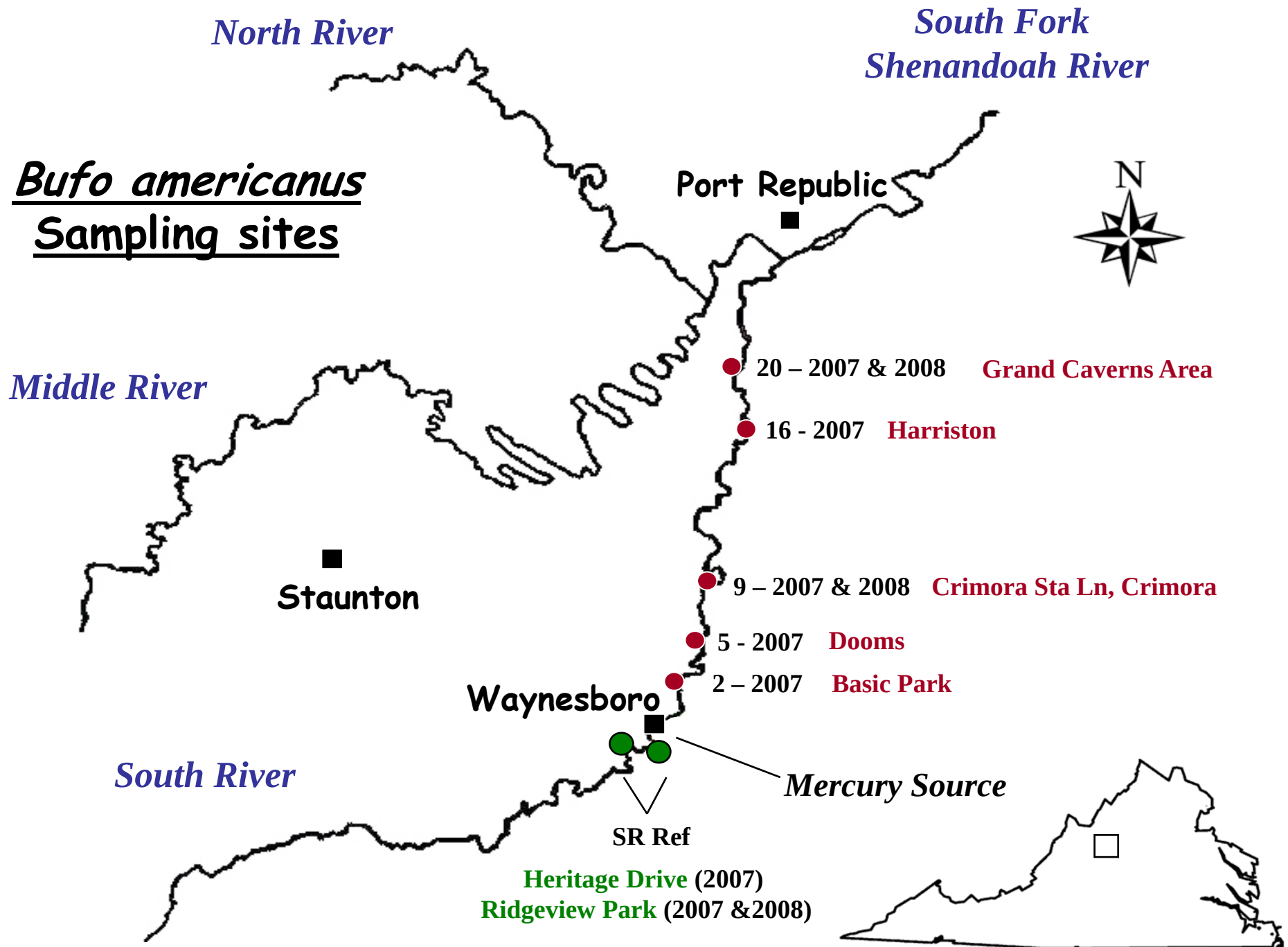


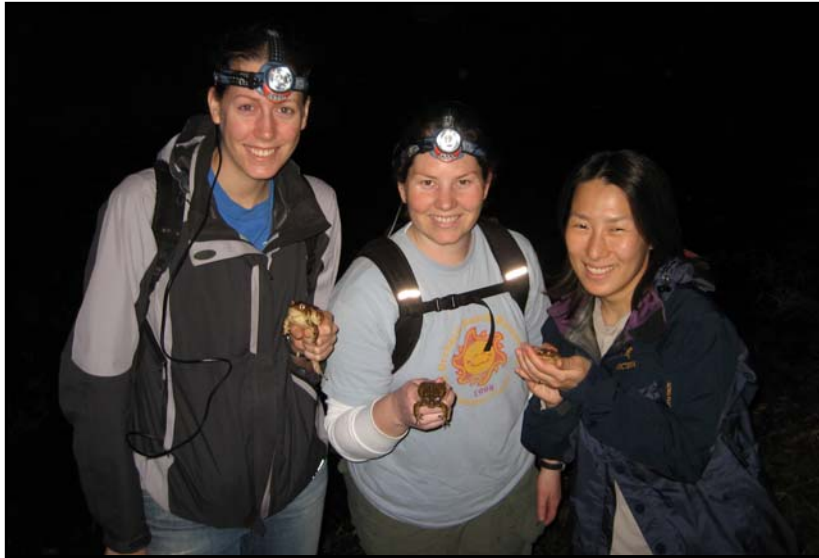
Spring 2008

2008 Objectives



- 1) Collect 2nd year embryonic development data
 - Relate female [Hg] to hatching success
 - Quantify malformation freq for 2007 & 2008
 - Quantify overall viability frequency
- 2) Determine whether "normal" hatchlings experience latent effects of maternal Hg exposure





In the field



Collect breeding pairs from reference and contaminated sites



Breeding pairs lay eggs in clean water

Egg masses are counted (548,035 eggs) and subsets allotted for:

- 1) Hg analysis
- 2) Developmental Bioassays (28,000 eggs/20,551 hatchlings)
- 3) hatchlings for exps (2,544 hatchlings)

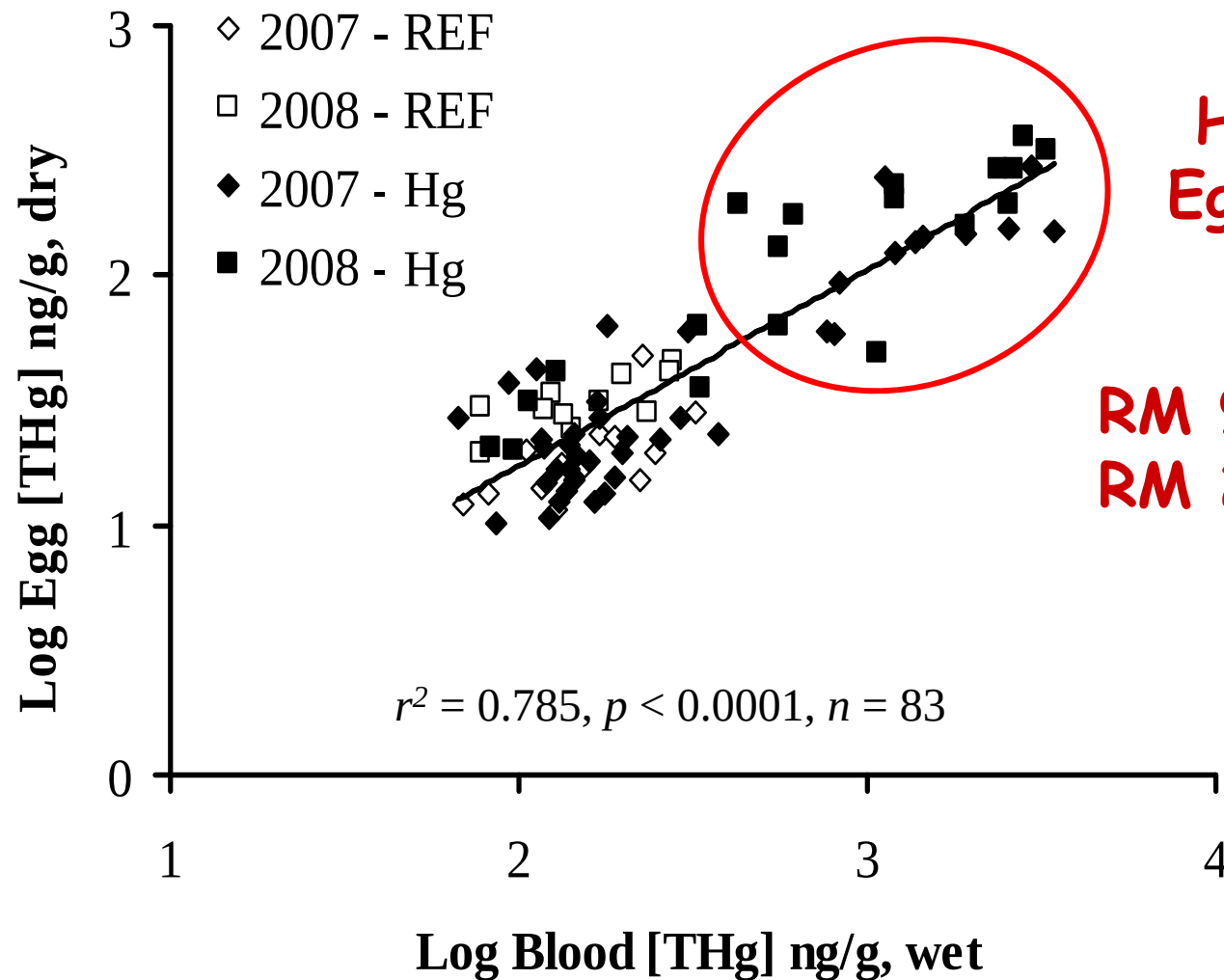
In the field



- Males are released immediately
- Females are processed (2007 & 2008) and released 24 hours after egg laying (2008)
 - measured and weighed
 - blood collected
 - individually marked (2008)



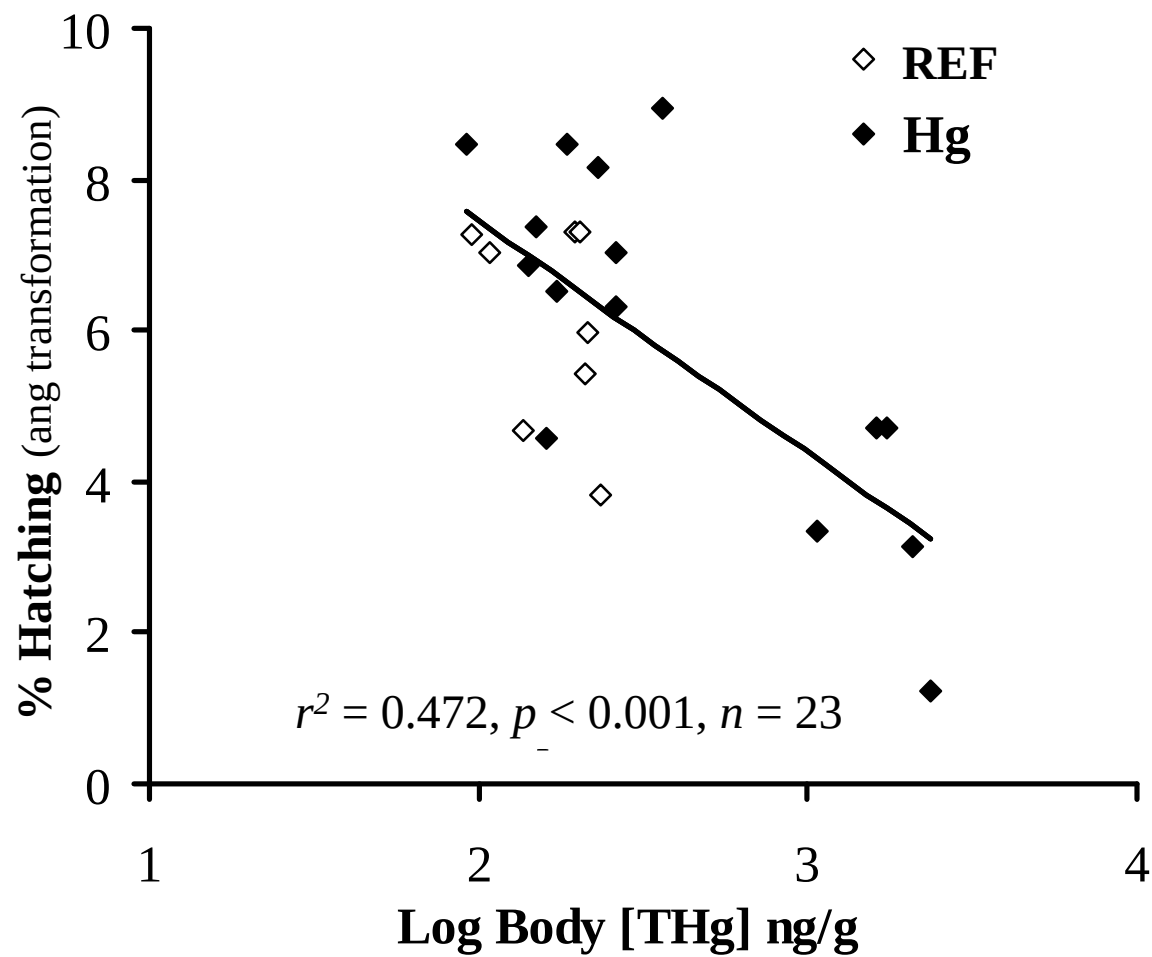
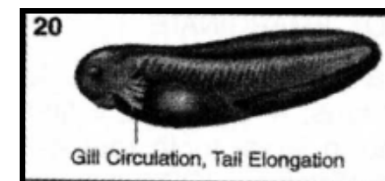
Egg relationships 2007 & 2008



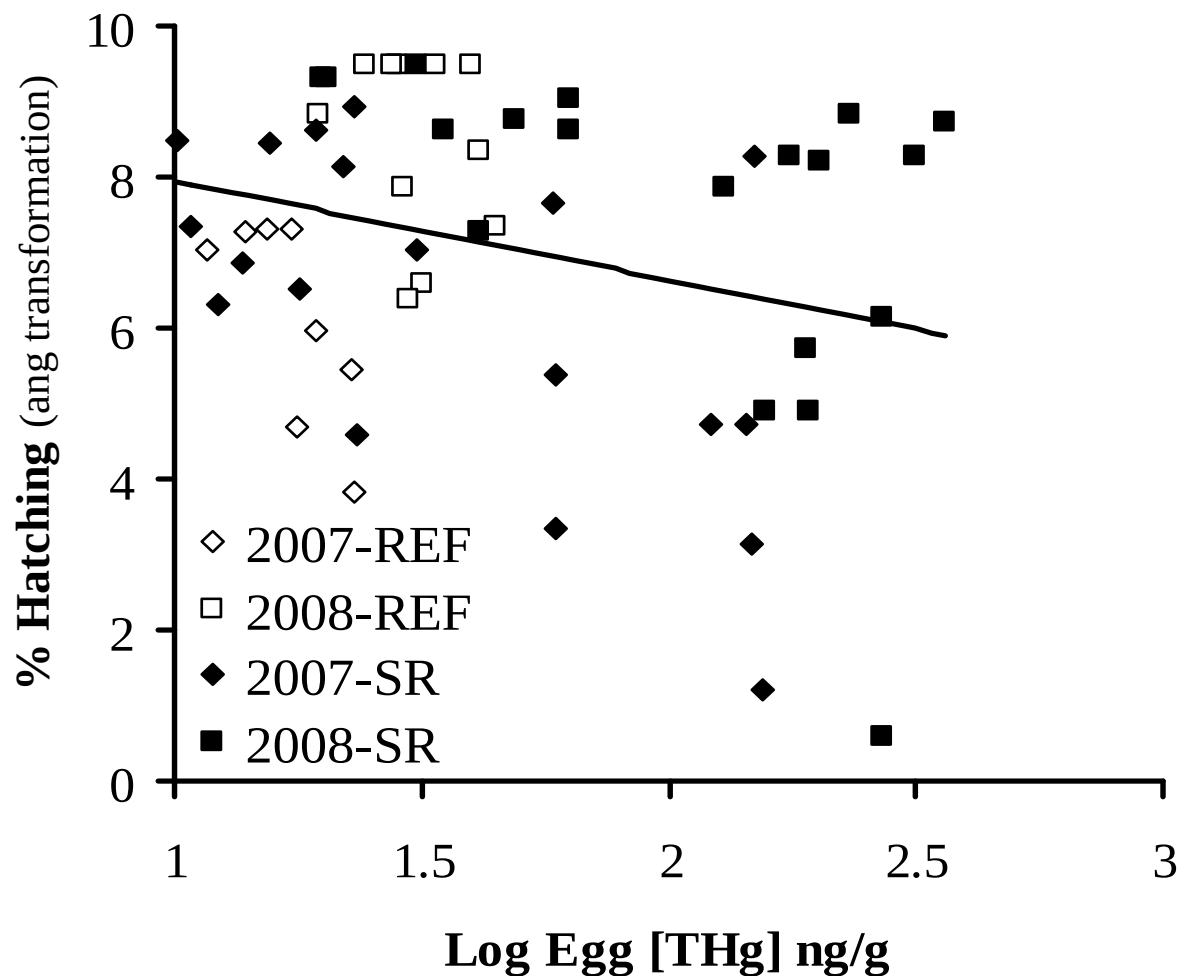
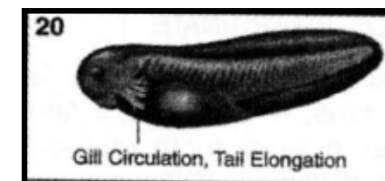
High [THg]
Eggs & Blood
from:

RM 9: Crimora
RM 20: Grand
Caverns
Area

Hatching Success 2007



Hatching Success 2007 & 2008



ANCOVA

Egg THg

$F_{1,53} = 19.27, p < 0.001$

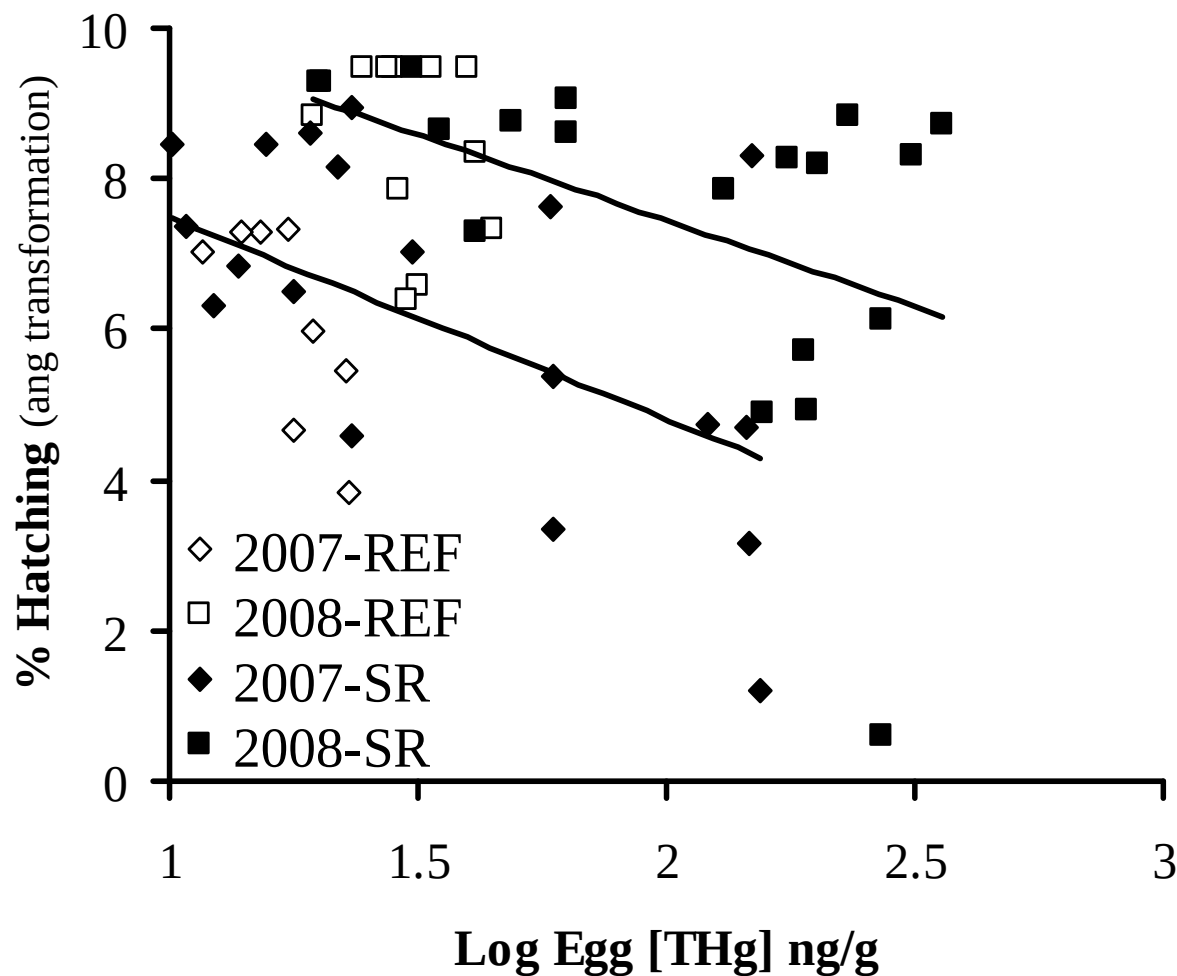
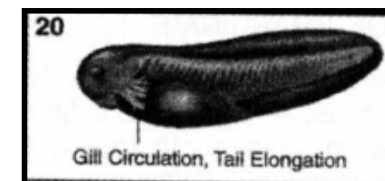
Year

$F_{1,53} = 0.93, p = 0.339$

Egg THg x Year

$F_{1,53} = 0.13, p = 0.720$

Hatching Success 2007 & 2008



ANCOVA

Egg THg

$F_{1,53} = 19.27, p < 0.001$

Year

$F_{1,53} = 0.93, p = 0.339$

Egg THg x Year

$F_{1,53} = 0.13, p = 0.720$

Effects of Dietary MMHg on Fish Reproduction



Fathead minnow
Pimephales promelas

(Hammerschmidt et al. '02, Drevnick & Sandheinrich '03, Sandheinrich & Miller '06)

- reduced spawning success
- delayed spawning
- suppressed reproductive behavior
- suppressed sex hormones

Lowest [MMHg] to produce
adverse reproductive effects

Whole body
treatment mean =
714 ng/g, dry



Bufo americanus

% MMHg = $53.3 \pm 2.3\%$

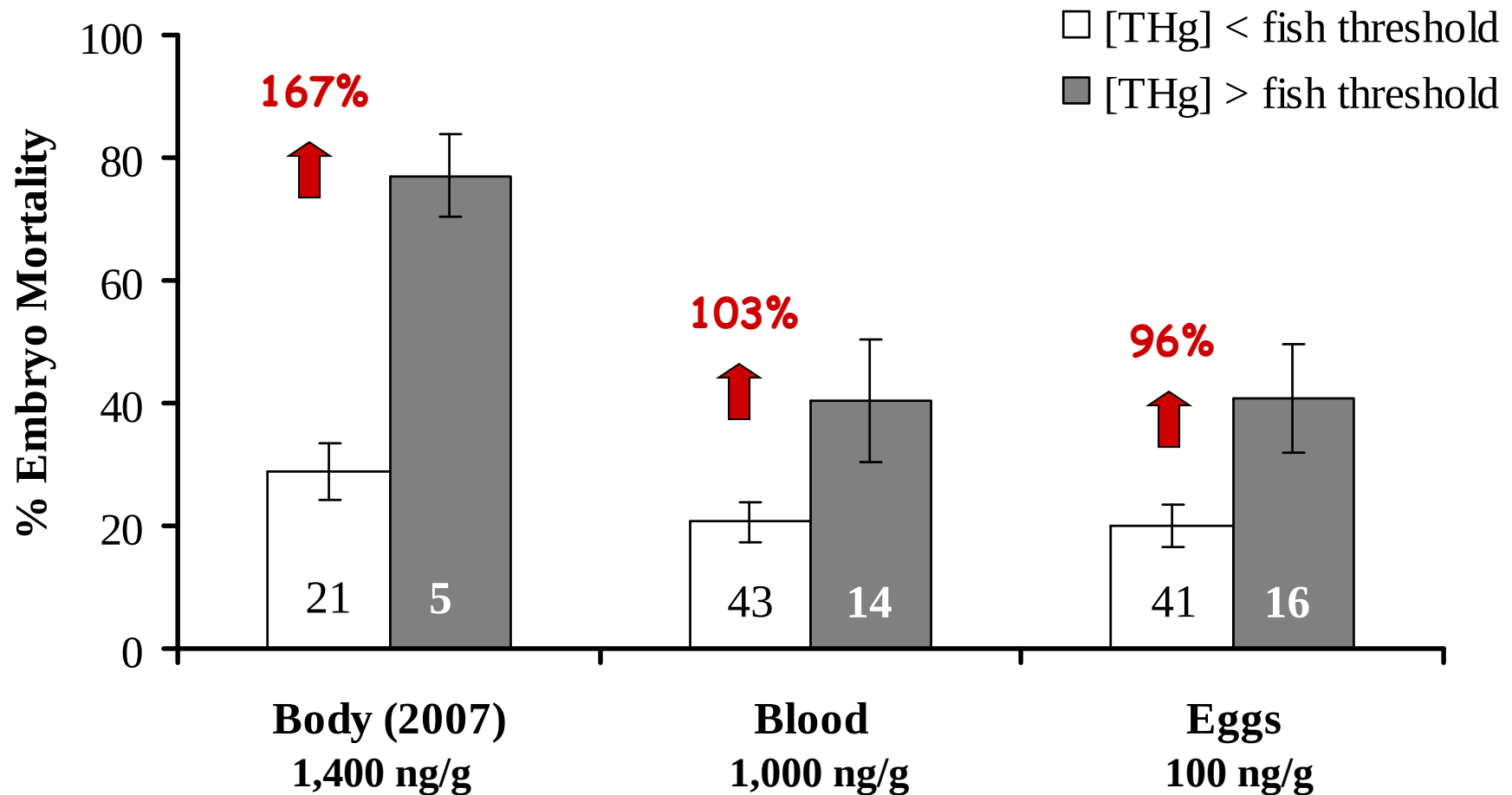
700 ng/g = 1,400 ng/g body THg, dry
body = 1,000 ng/g blood THg, wet
MMHg = 100 ng/g egg THg, dry

15, 29, 32% females >
Exceed body, blood, egg thresholds,
respectively (n = 40, 59, 59)



% Embryo Mortality

Qualitative Comparison to
Fish Literature



Determining Malformation Frequency

- 500 eggs per clutch allotted for hatching success (28,000 eggs total)



- Successful hatchlings (20,551) were preserved for blind malformation assessment



- Classified each individual as either "normal" or malformed based on gross morphology

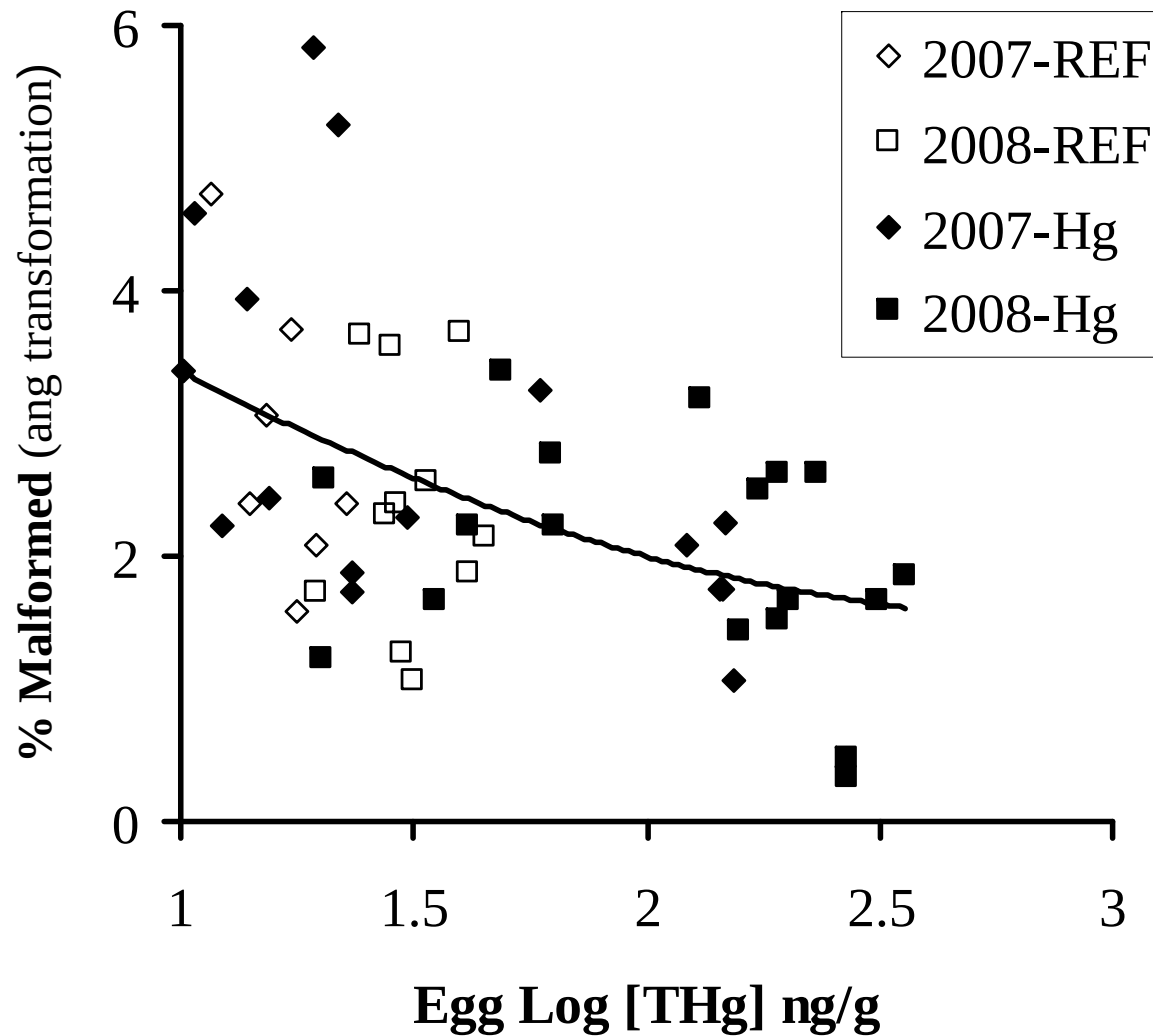


Example Malformations



"Normal"

Malformation Frequency 2007 & 2008



ANCOVA

Egg THg

$F_{1,48} = 10.16, p = 0.003$

Year

$F_{1,48} = 2.39, p = 0.129$

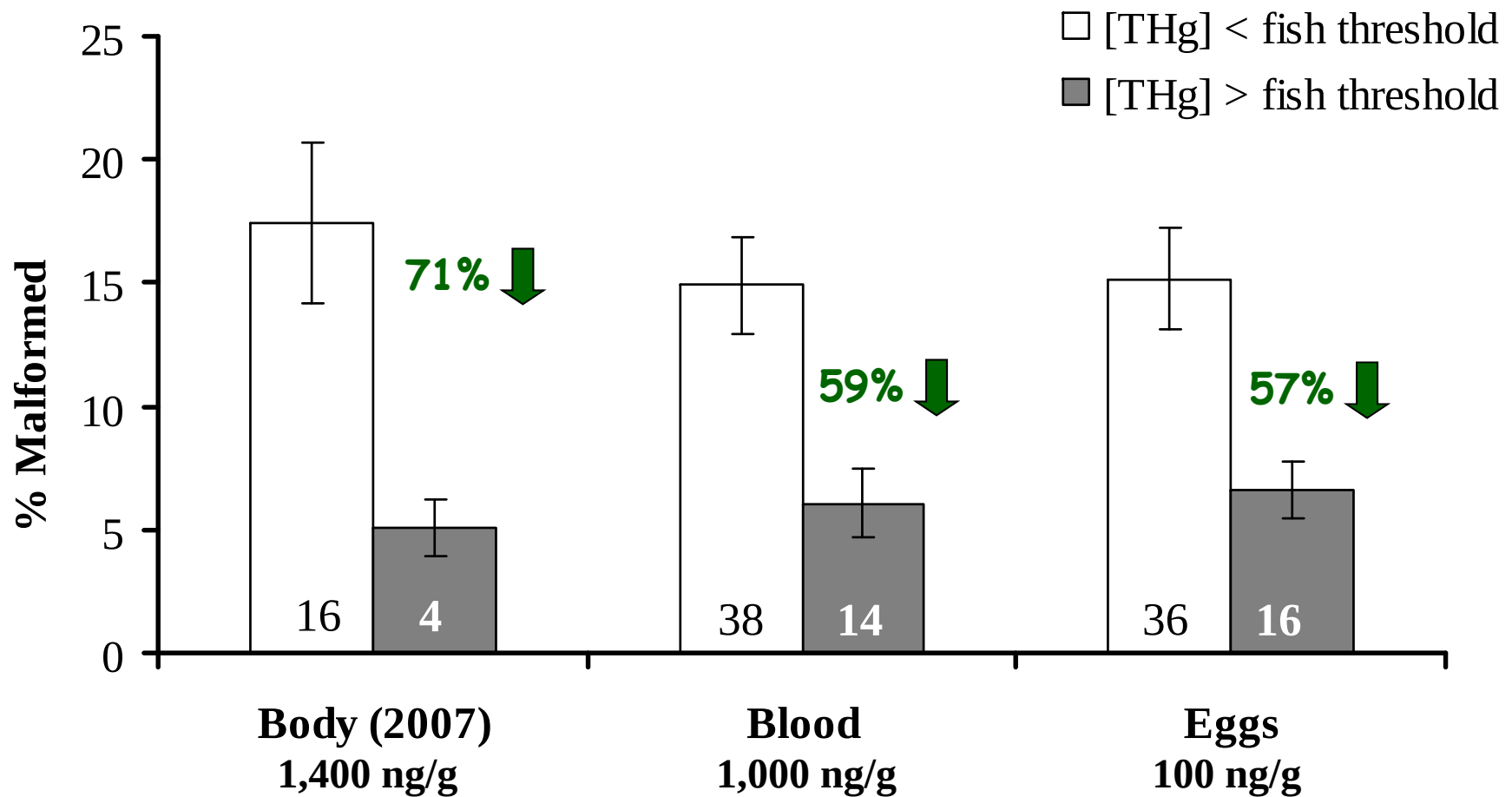
Egg THg x Year

$F_{1,48} = 0.170, p = 0.198$



Malformation Frequency

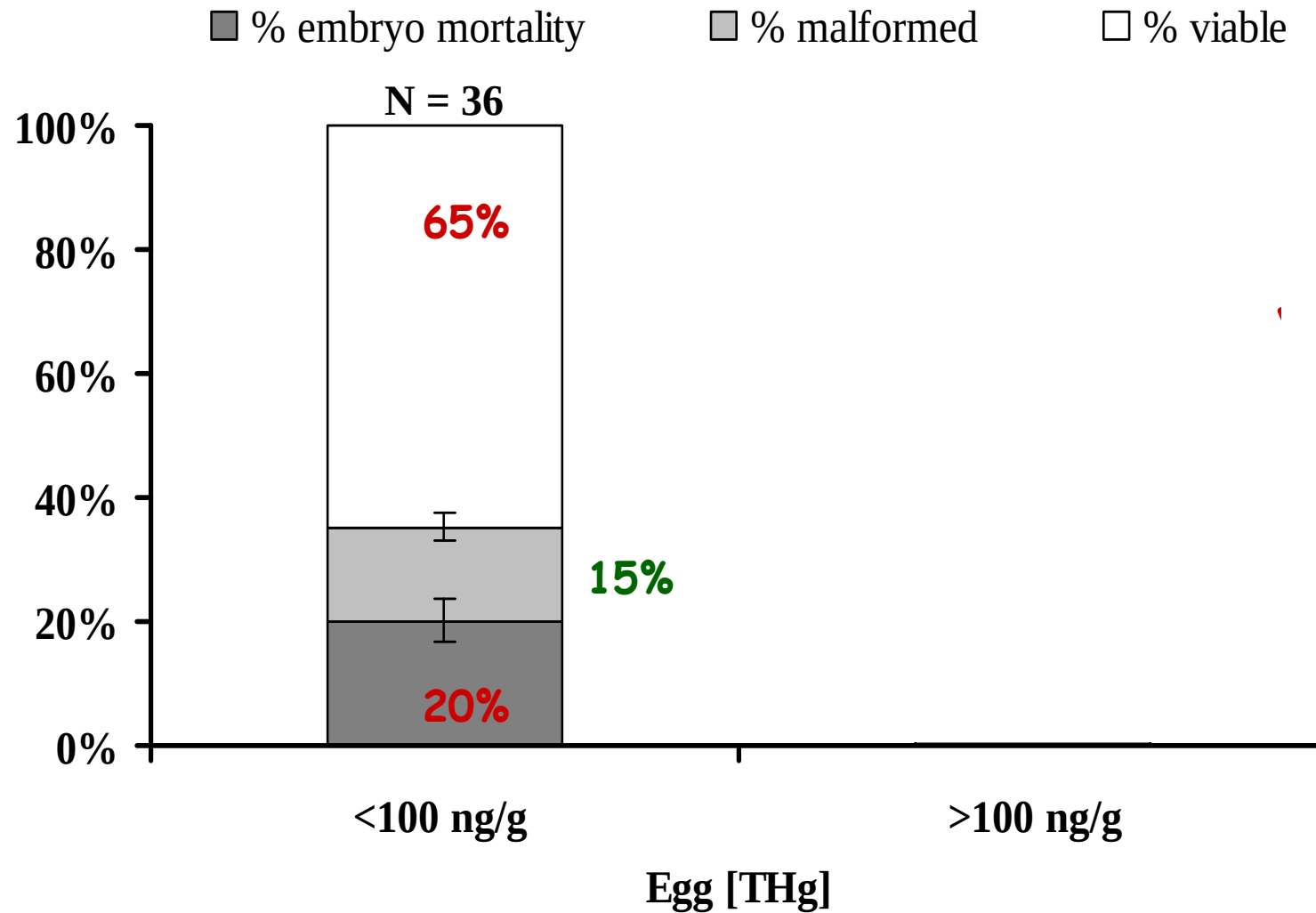
Qualitative Comparison to Fish Literature



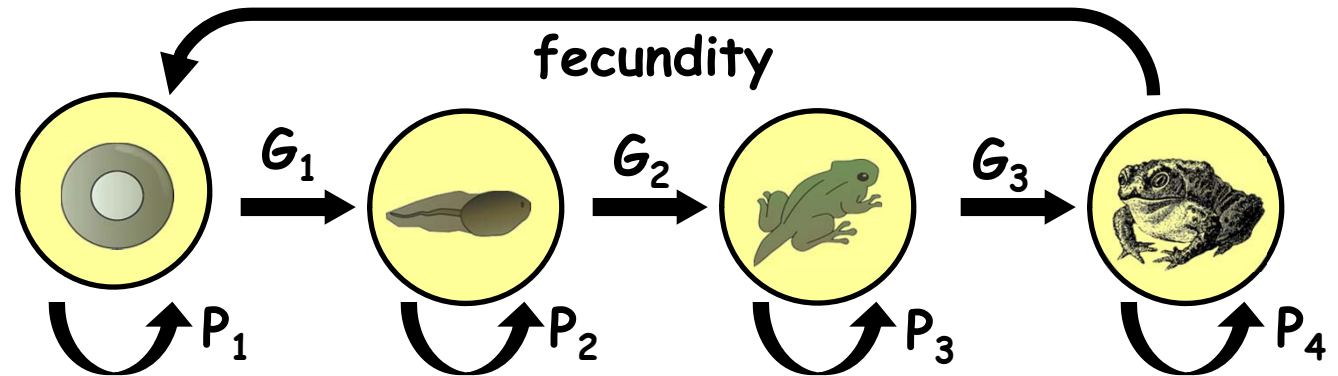


Synthesis

Qualitative Comparison to Fish Literature



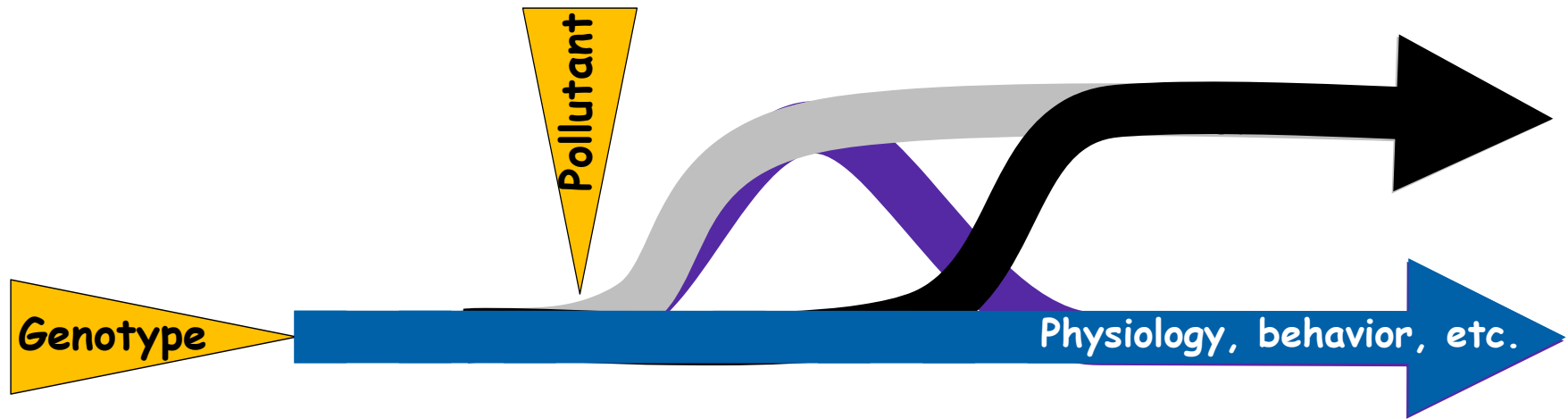
2008 Objectives



- 1) Collect 2nd year embryonic development data
 - Relate female [Hg] to hatching success
 - Quantify malformation freq for 2007 & 2008
 - Quantify overall viability frequency

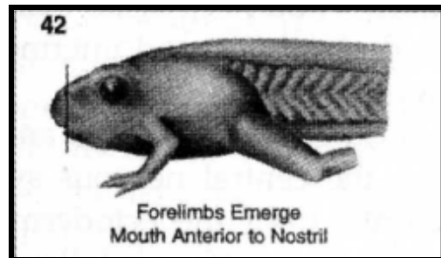
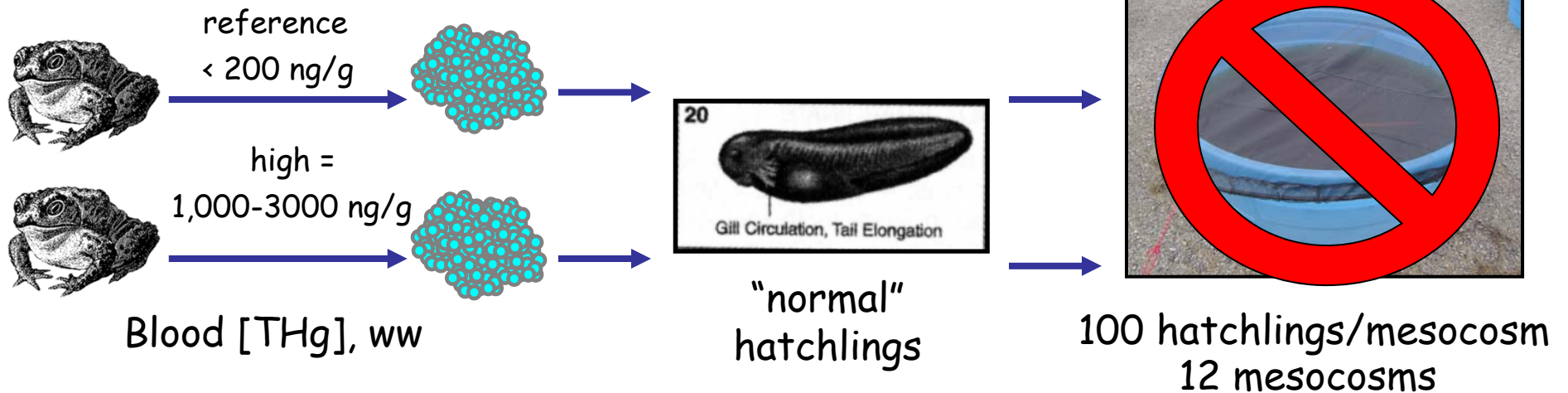
- 2) Determine whether "normal" hatchlings experience latent effects of maternal Hg exposure

Types of Effects From Acute Exposure



LONG-TERM EFFECT

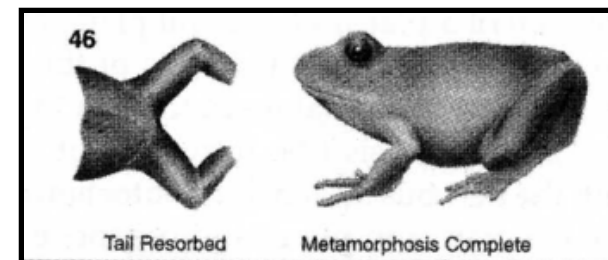
Mesocosms



Removed from
mesocosms

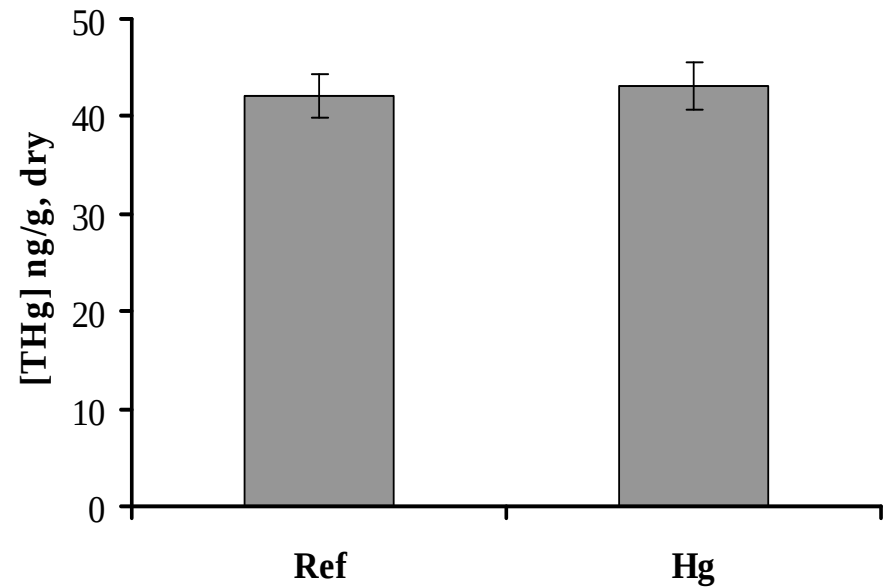


Held individually until
metamorphosis



Survival,
growth,
time/size
at meta
determined

[THg] of “normal” metamorphs



N = 6 replicate mesocosms per treatment
Each replicate = homogenate of 14 metamorphs

**[THg] in reference tadpoles (stages 28-32) from the South River =
536 ± 136 ng/g**

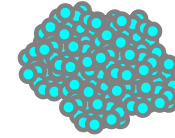
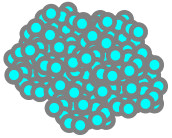
Mesocosms

Parameters:

- Body Size (svl)
- Body condition (mass/svl³)
- Days to complete metamorphosis
- % metamorphic success
- Days to complete metamorphic climax



Estimating Recruitment



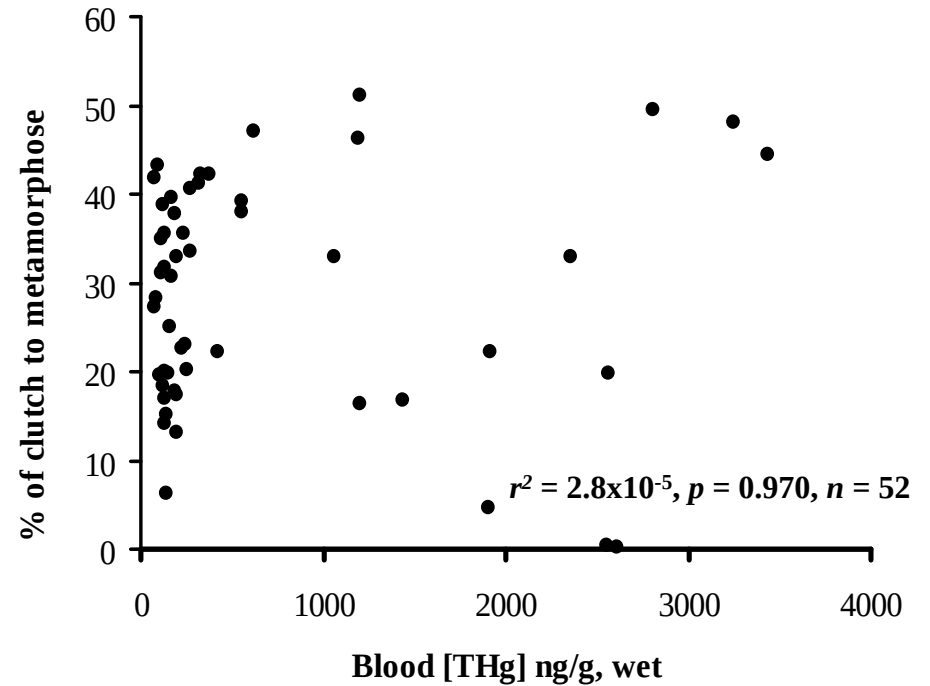
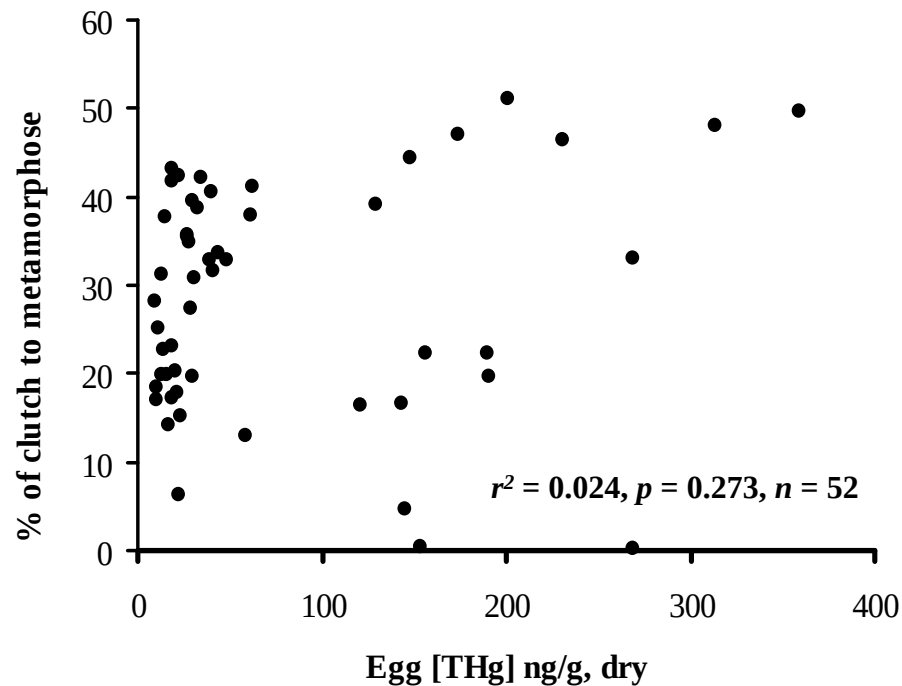
% of
Clutch
to
Meta-
morphose

Clutch size
(Range:
2,410 - 13,689)

% Viability
(Based on 500
Egg subset)

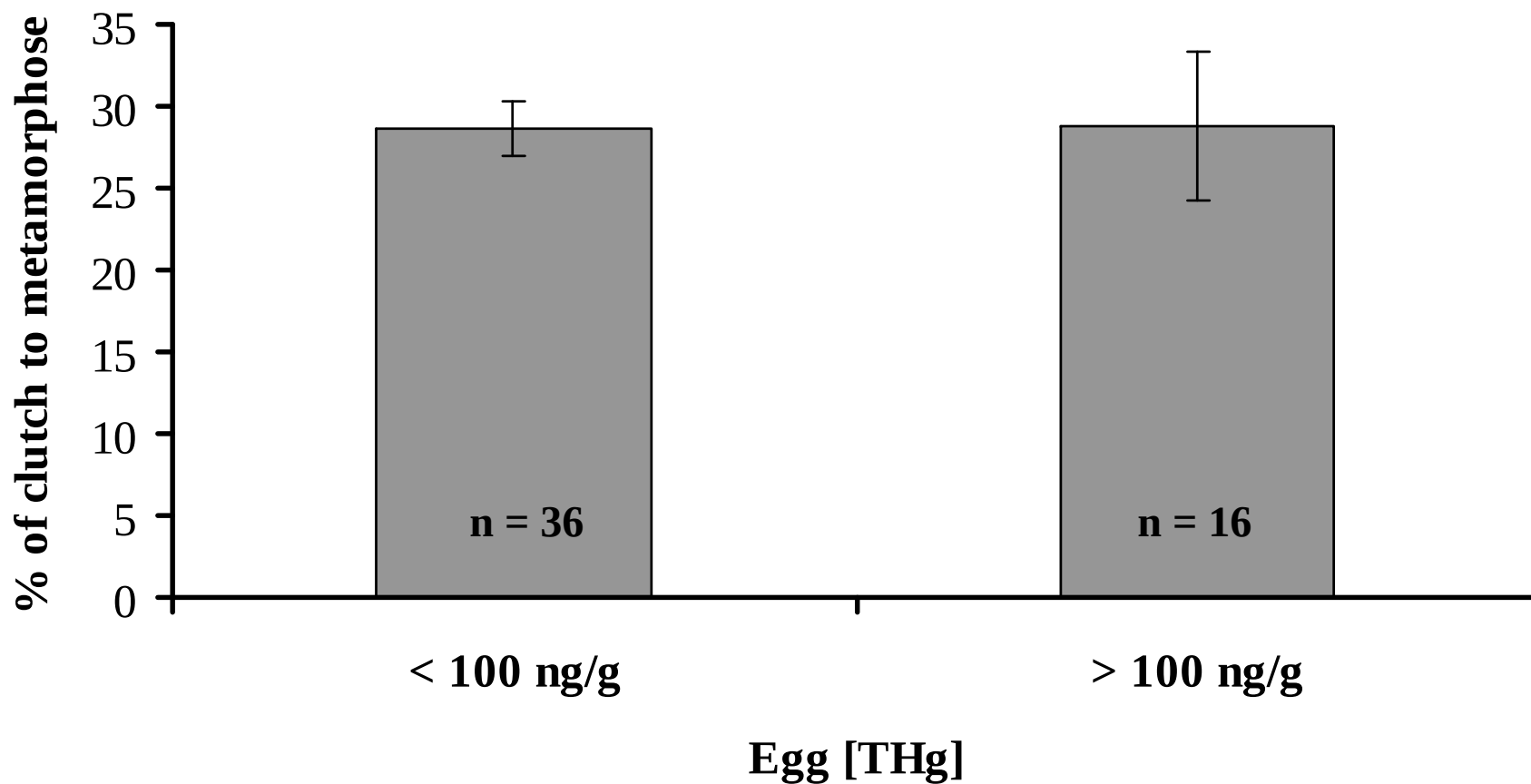
**% Metamorphic
Success**
(based on mesocosm
results)

**Clutch
size**





Estimating Recruitment

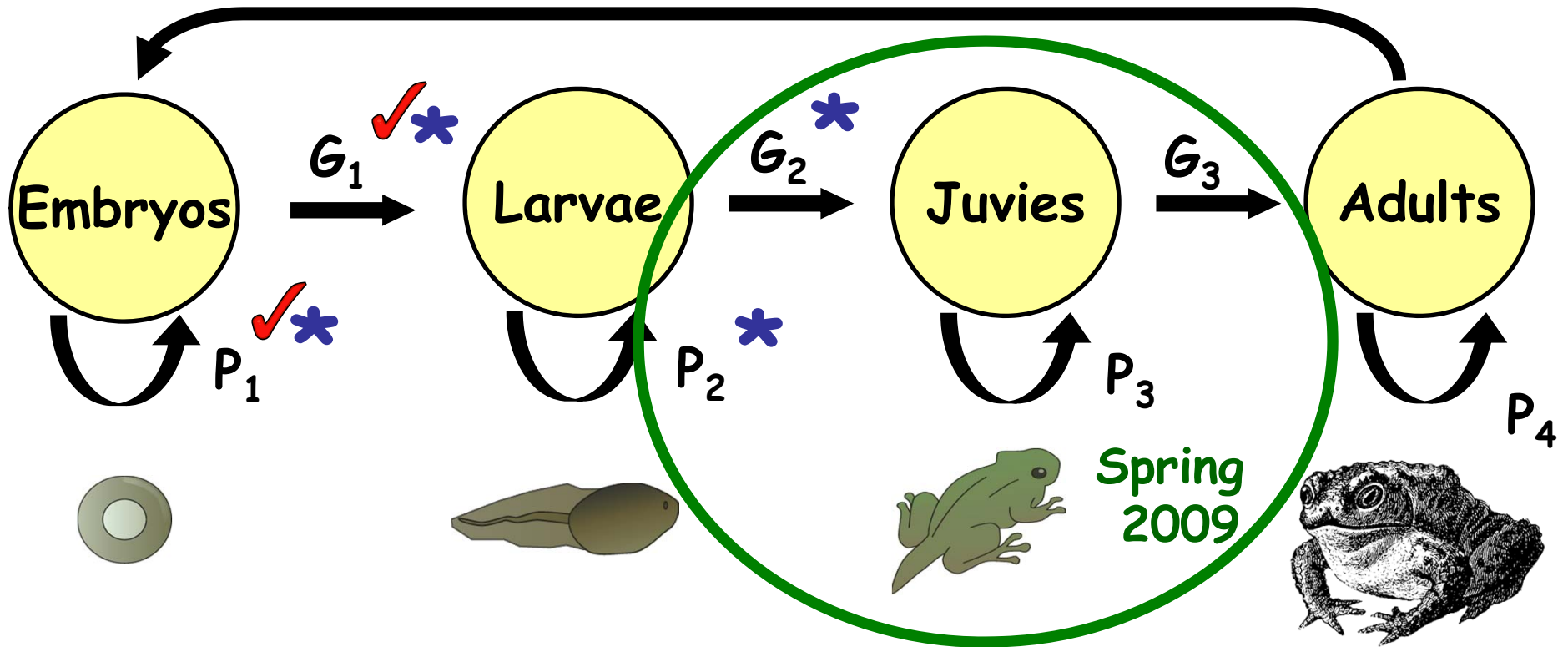


Conclusions

- Female *B. americanus* transfer Hg to their eggs
- Hatchling viability is negatively correlated with egg [Hg]
 - correlation driven by hatching success, not malformation rate
- 19% reduction in viability when using reproductive effects threshold from fish literature
- No deleterious latent effects of maternal Hg exposure observed in larvae (opposite appeared true!)
- Overall recruitment did not differ when using reproductive effects threshold from fish literature

Path Forward - 2009

fecundity ✓*



G - probability of surviving to next stage
 P - probability of staying in the same stage

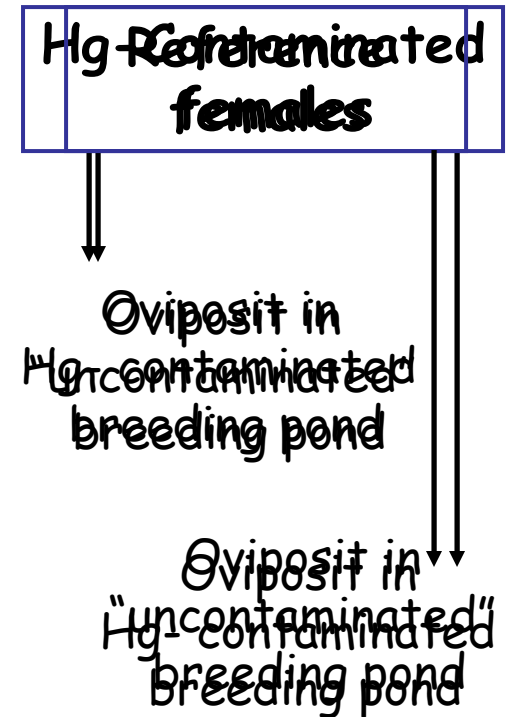
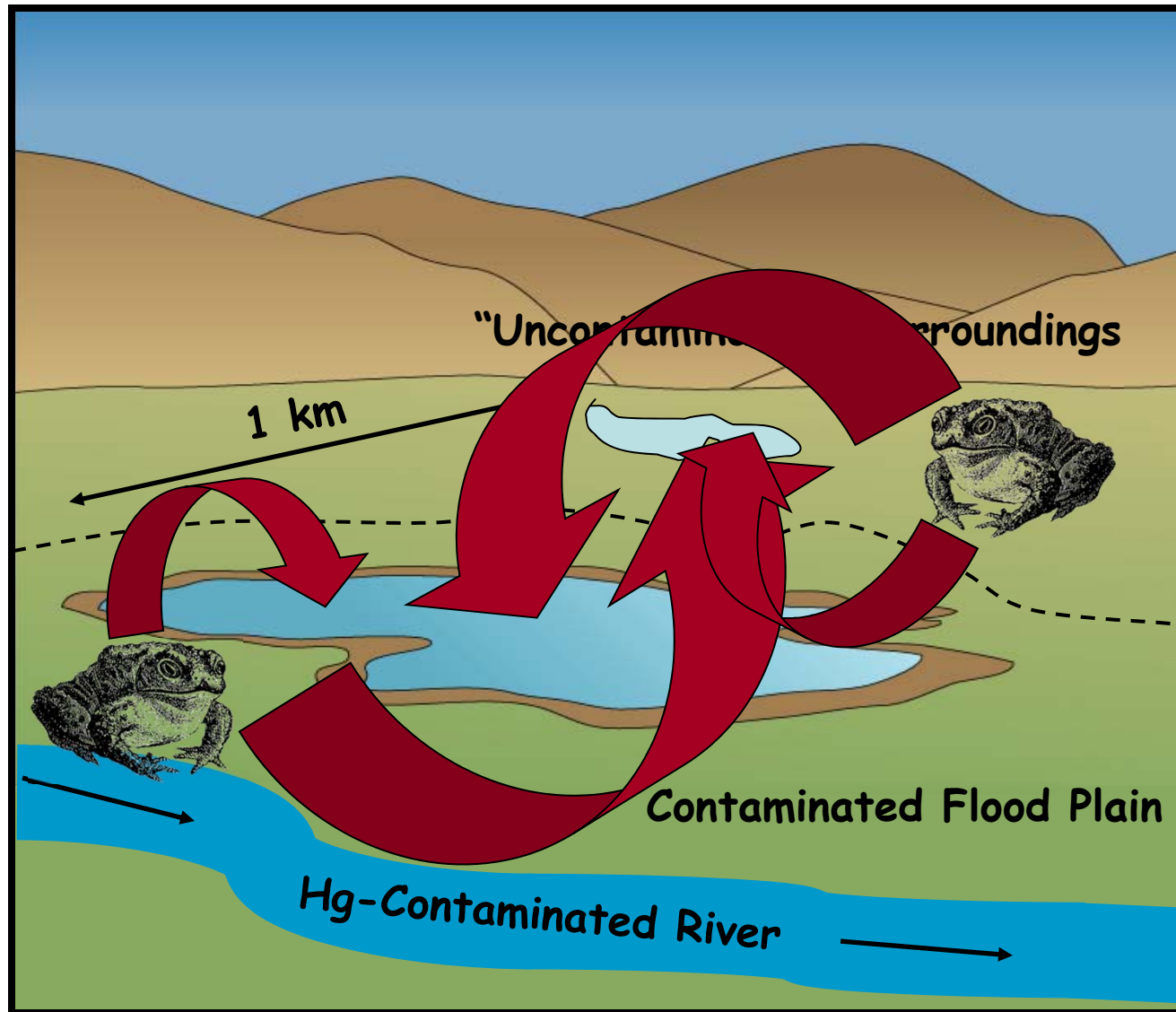


Spring 2007

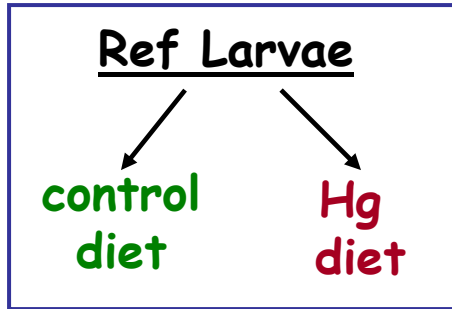


Spring 2008

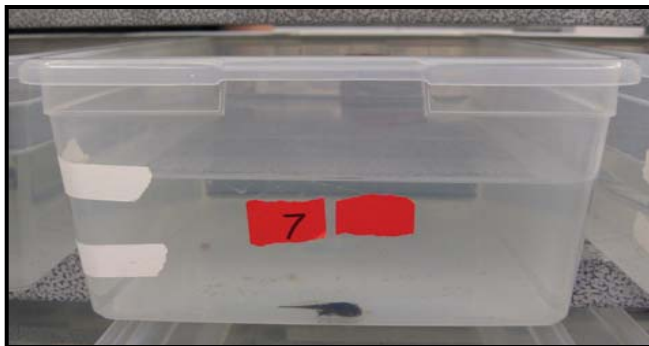
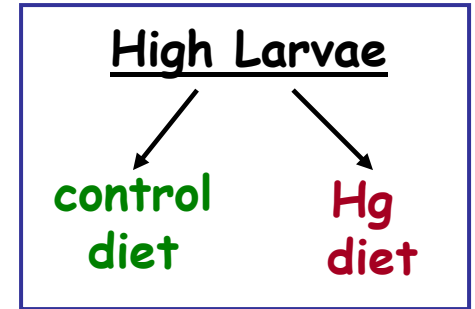
Individual and interactive effects of maternally and trophically derived mercury on larval development



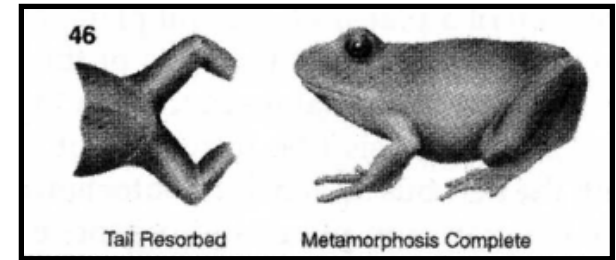
Diet Study



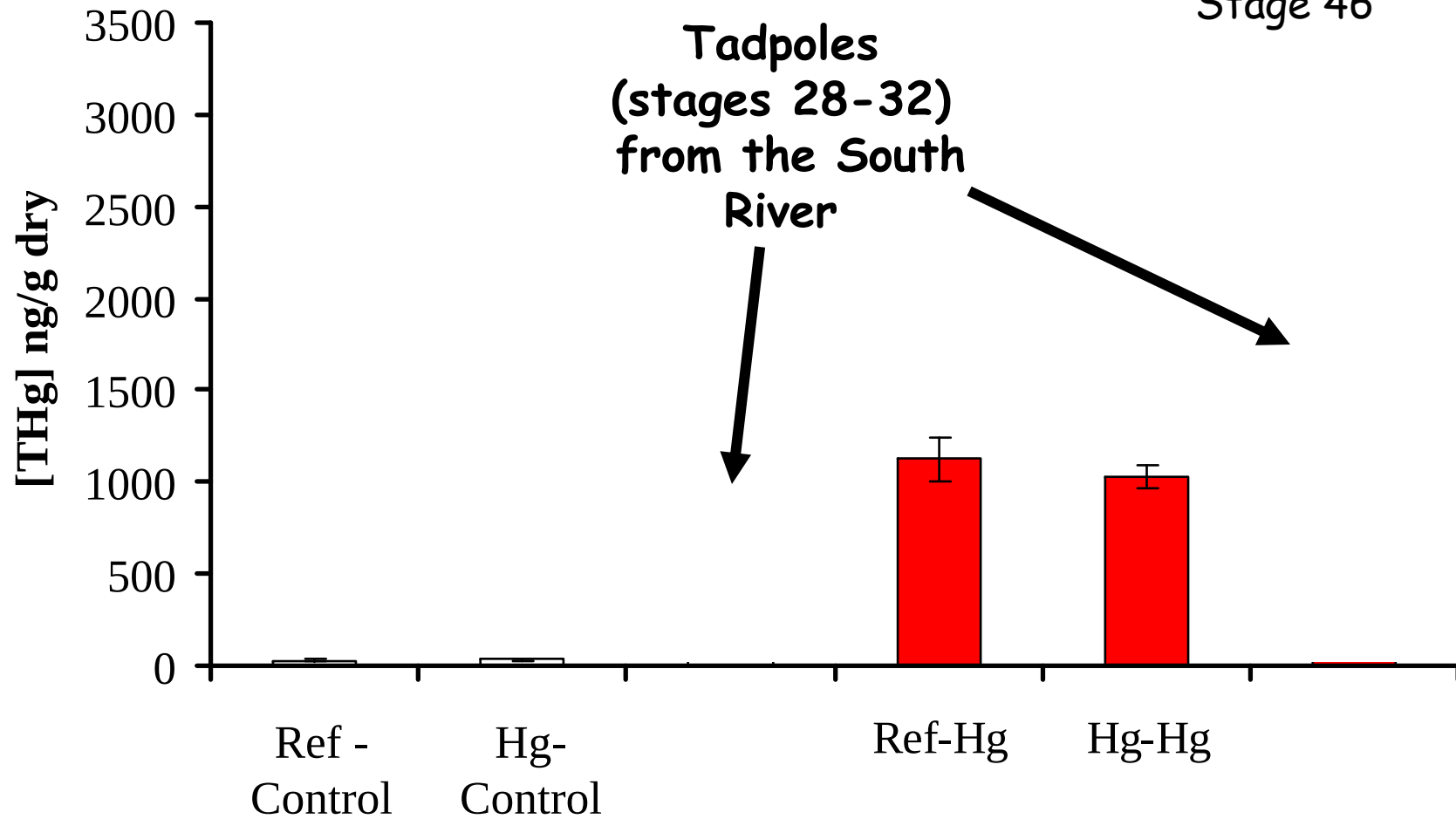
Diet	[THg], dry wt	%MMHg
Control	4.96 ng/g	78%
Hg	2,571 ng/g	4%



Diet Study: Total Hg Concentrations



Stage 46



Terrestrial Field Enclosures



Southern toad

BIOLOGICAL CONSERVATION 133 (2006) 178–185



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**Assessing quality of clearcut habitats for amphibians:
Effects on abundances versus vital rates in the southern
toad (*Bufo terrestris*)**

Brian D. Todd*, Betsie B. Rothermel

The University of Georgia, Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29802, USA

Acknowledgements

Hopkins Lab: Sarah Budischak, John Burke
Sarah DuRant, Britney Hopkins, Sarah
Orlofske, Brian Todd, Haruka Wada

Dan Cristol and Lab

Heather Brandt, SREL

Scott Gregory, SRST office

Mike Liberati, SRST

Ralph Stahl, SRST

Erin Mack, SRST

John Schmerfeld, US Fish and Wildlife

Don Kain, VA DEQ

Landowners along the South River

Waynesboro Parks and Recreation Department



Approaching Toadal Eclipse

