



Ecological Risk Assessment for Amphibians



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Introduction

- Amphibians –salamanders, frogs & toads
- Amphibians are sensitive to tPCBs
- Amphibians present in wetlands with tPCBs
- Characterize & quantify risk to amphibians



Amphibians in Study Area

- **Salamanders**

- Spotted salamander
- Jefferson salamander (State-listed Special Concern)
- Four-toed salamander (State-listed Special Concern)
- Red-spotted newt
- Redback salamander
- Spring salamander

- **Frogs and Toads**

- American toad
- Spring peeper
- Gray treefrog
- Northern leopard frog
- Wood frog
- Green frog
- Bullfrog



Representative Species & Assessment Endpoint

- **Leopard and wood frogs**
- **Community condition, survival, reproduction, and development**





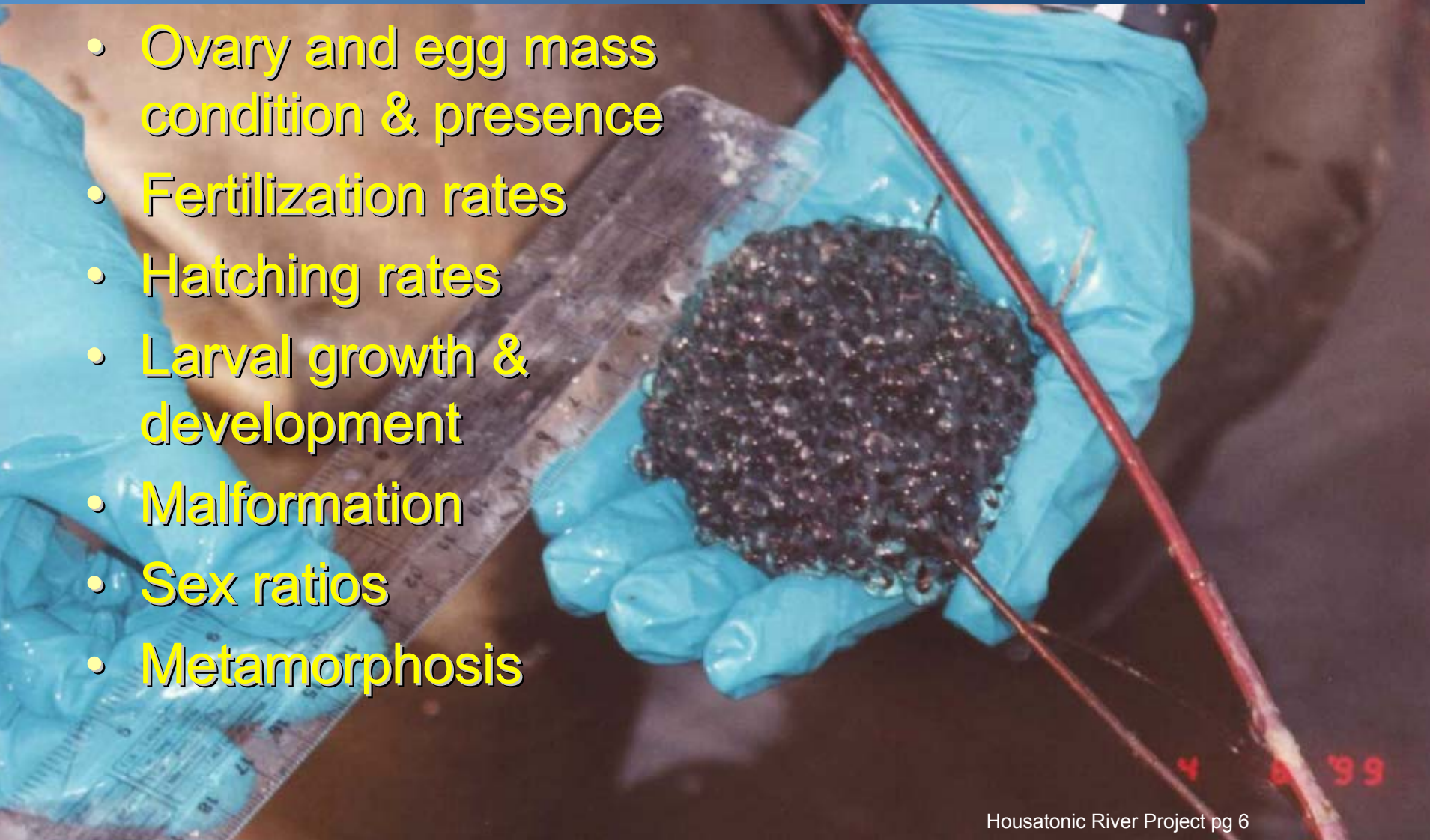
Measurement Endpoints – Community Condition

- Species richness
- Species abundance
- Sex ratios
- Courtship & Breeding
- DELTs
- Growth
- Metamorphosis



Measurement Endpoints – Survival, Reproduction & Development

- Ovary and egg mass condition & presence
- Fertilization rates
- Hatching rates
- Larval growth & development
- Malformation
- Sex ratios
- Metamorphosis

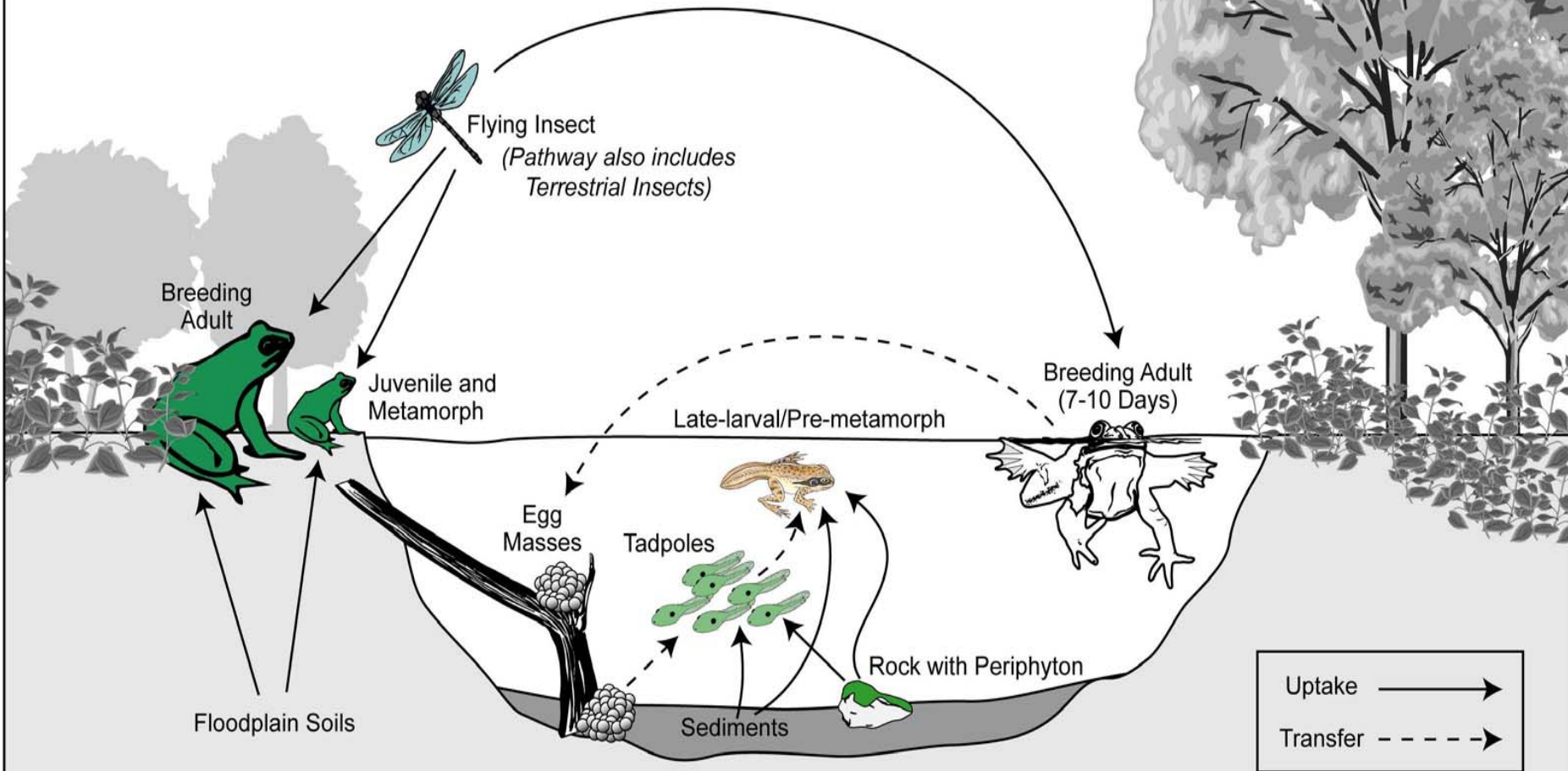




Lines of Evidence

- 1. Field Studies**
- 2. Site-specific toxicity studies**
- 3. Comparison of tissue & sediment chemistry to site-specific & literature effects levels**

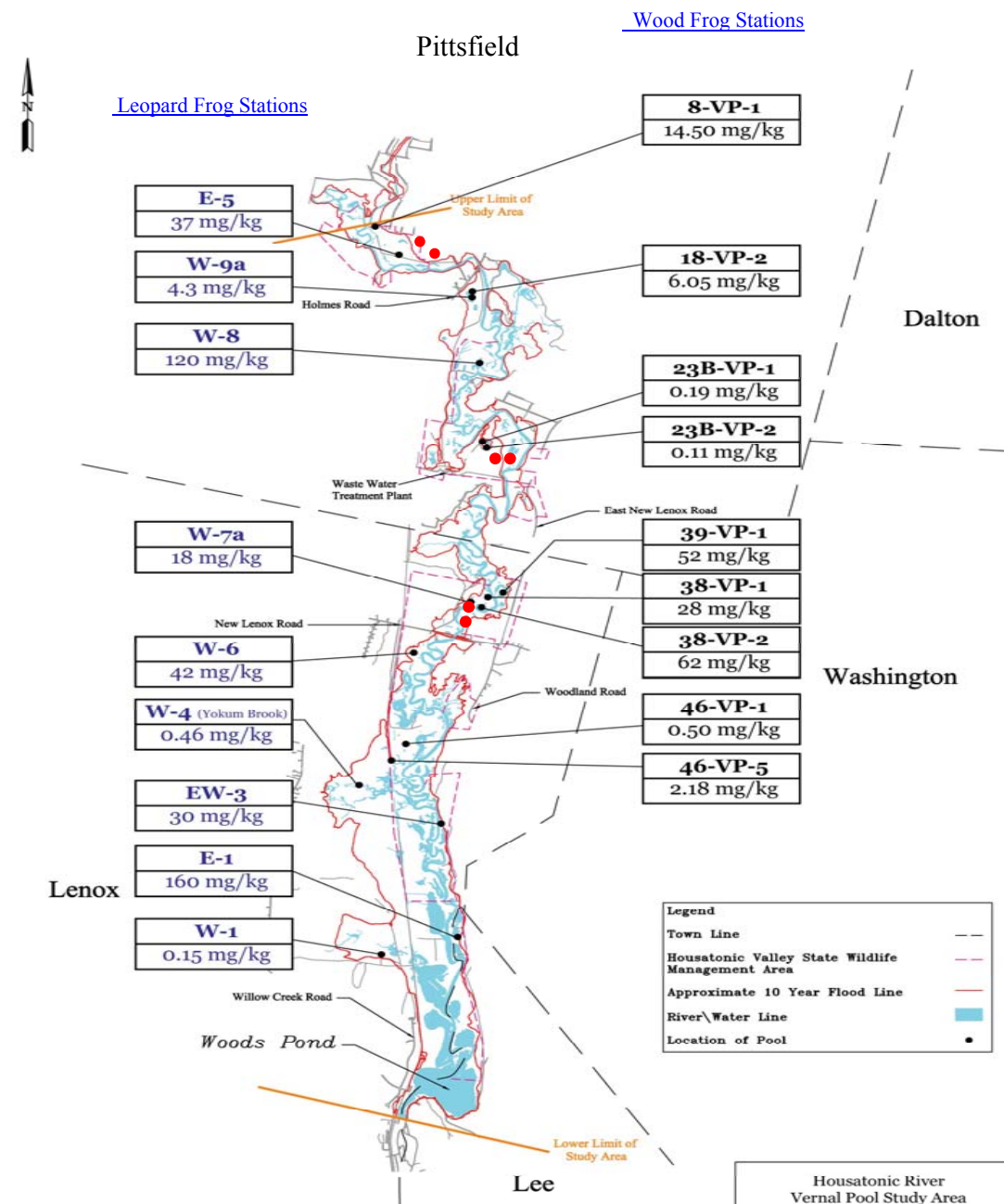
Exposure





Exposure

Leopard Frog & Wood Frog



Notes:
 1) Base Map Information provided by the USEPA.
 2) Placement of Town Lines is approximate. Source USGS Quadrangles.
 3) Pools surveyed include vernal pools, as defined by the Massachusetts Natural Heritage and Endangered Species Program, and other water bodies that contained, or could contain breeding amphibians.

Housatonic River Vernal Pool Study Area

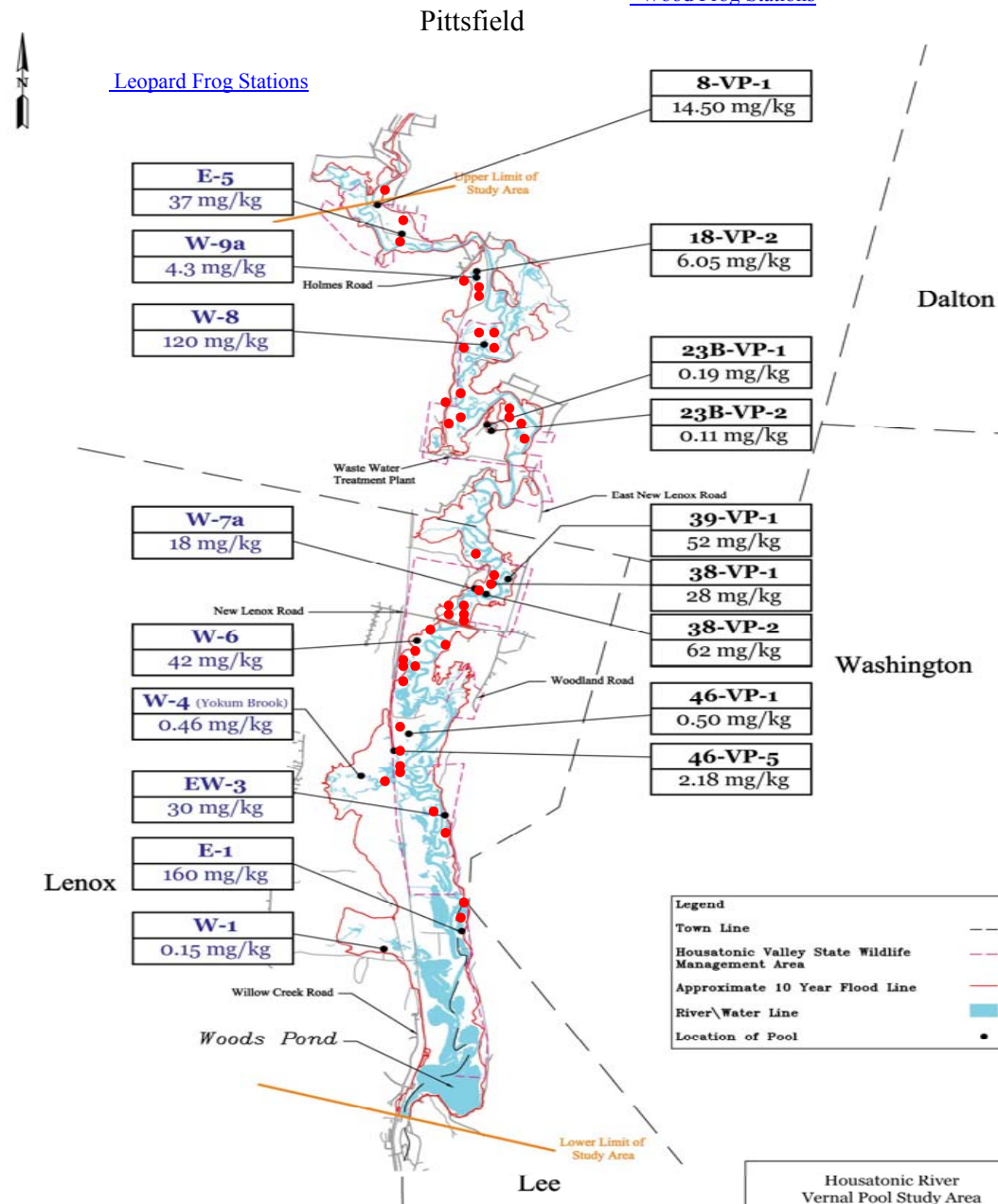
Pools Surveyed for 2000 Rana sylvatica Study with sediment total PCB concentration

SCALE: 1" = 3000'



Exposure

Leopard Frog



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Housatonic River Vernal Pool Study Area

Pools Surveyed for 2000 *Rana sylvatica* Study with sediment total PCB concentration

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LEOPARD FROG REPRODUCTION STUDY

Evaluation of Adult Leopard Frog Reproductive Condition



Field Collection of Adults (Male, Female) from 9 Breeding Areas of PSA

Endpoints:

MALE:

Sperm Count,
Sperm Head Abnormality,
Testes Weight,
Body Weight

FEMALE:

Ovary Weight,
Egg Count, Necrotic Eggs,
Oocyte Maturity,
Body Weight

Tissue:

Adult Whole Body

•
Female Offal
(Whole Body Minus Egg
Mass/Ovary)

•
Egg Mass/Ovary

LEOPARD FROG DEVELOPMENTAL STUDY

Laboratory Culture of Field-Collected Egg Masses

Main Study
Culture of Target and
External Reference Site Egg
Masses in Natal Pool
Sediment/Water



Endpoints:

LARVAE:


Survival,
Growth,
Malformation

METAMORPHS:


Incidence,
Malformation


Tissue:

Late Larval/Metamorph

Supplemental Investigations


Cross-over Study

Exposure:


Reference Site
Larvae to
Contaminated Site
Media

Aroclor 1260 Spiking Study

Exposure:


Reference Site
Larvae in Clean
Sediment Spiked
with 30 mg/kg
Aroclor 1260

Endpoints:
Mortality,
Malformation,
Metamorphosis,
Growth

Tissue:

Larvae

Wood Frog Study

PHASE I

Laboratory Culture of Field-Collected Egg Masses

Supplemental Investigations

Cross-over Study

Exposure:



Reference Site Larvae to Target VP Media



Target Site Larvae to Reference VP Media

Endpoints:
Mortality,
Malformation,
Growth
(Length, Weight),
Metamorphosis

Tissue:
Individual Larvae

Aroclor 1260 Spiking Study

Exposure:



Reference Site Larvae to Sediment Spiked with 30 mg/kg Aroclor 1260



Reference Site Larvae in Reference Site VP Media ("Clean")

Endpoints:
Malformation,
Mortality,
Metamorphosis,
Growth (Length)

Tissue:
Individual Larvae

Main Study

Culture of Target and Reference Site Egg Masses in Natal Pool Sediment/Water

Endpoints:
EGG MASSES:



Count, Weight,
% Fertilized, % Necrotic,
Hatching Success

LARVAE:



Mortality,
Malformation,
Developmental Stage,
Growth (Length)
Metamorphosis

METAMORPHS:



Malformation,
Growth (Weight)

Tissue:
Individual Egg Mass,
Larvae,
Metamorph

PHASE II

Field Collection of Larvae

Four Field-sampling Events, Each Several Weeks Apart

Endpoints:



Malformation,
Growth (Length) at
Each Sampling Event

Tissue:
Individual Larvae
(from Events 1 & 3)

PHASE III

Field Collection of Metamorphs

One Sampling Event

Endpoints:

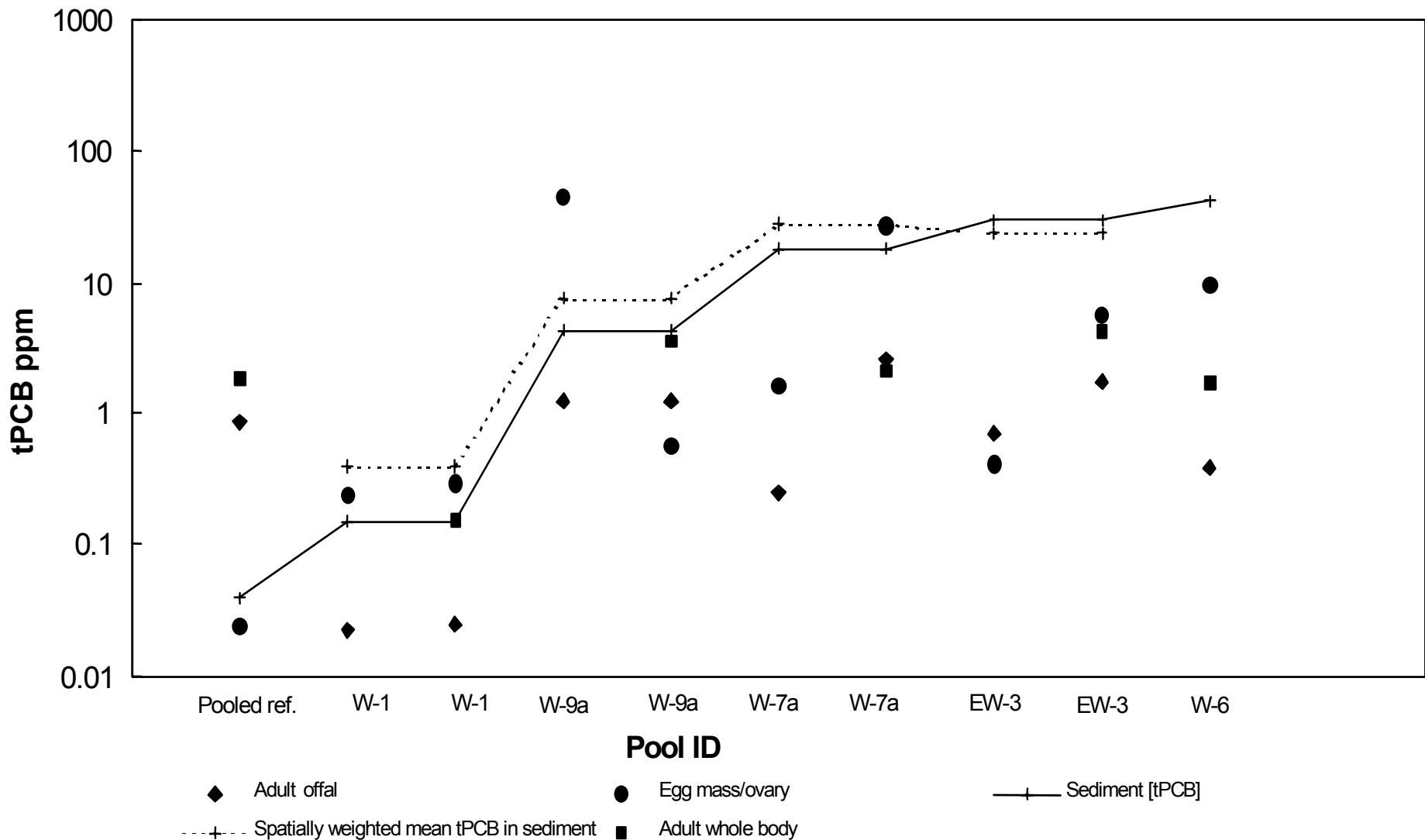


Malformation,
Sex Ratio,
Growth (Weight),
Necropsy of Internal
Organs

Tissue:
Metamorph
Composite



Exposure Leopard Frog (FEL 2002)





Effects Leopard Frogs (FEL 2002)

- Abnormal sperm
- Females had immature eggs
- Higher larval mortality
- Higher malformation rates
- Delayed development



Effects Leopard Frogs (FEL 2002)





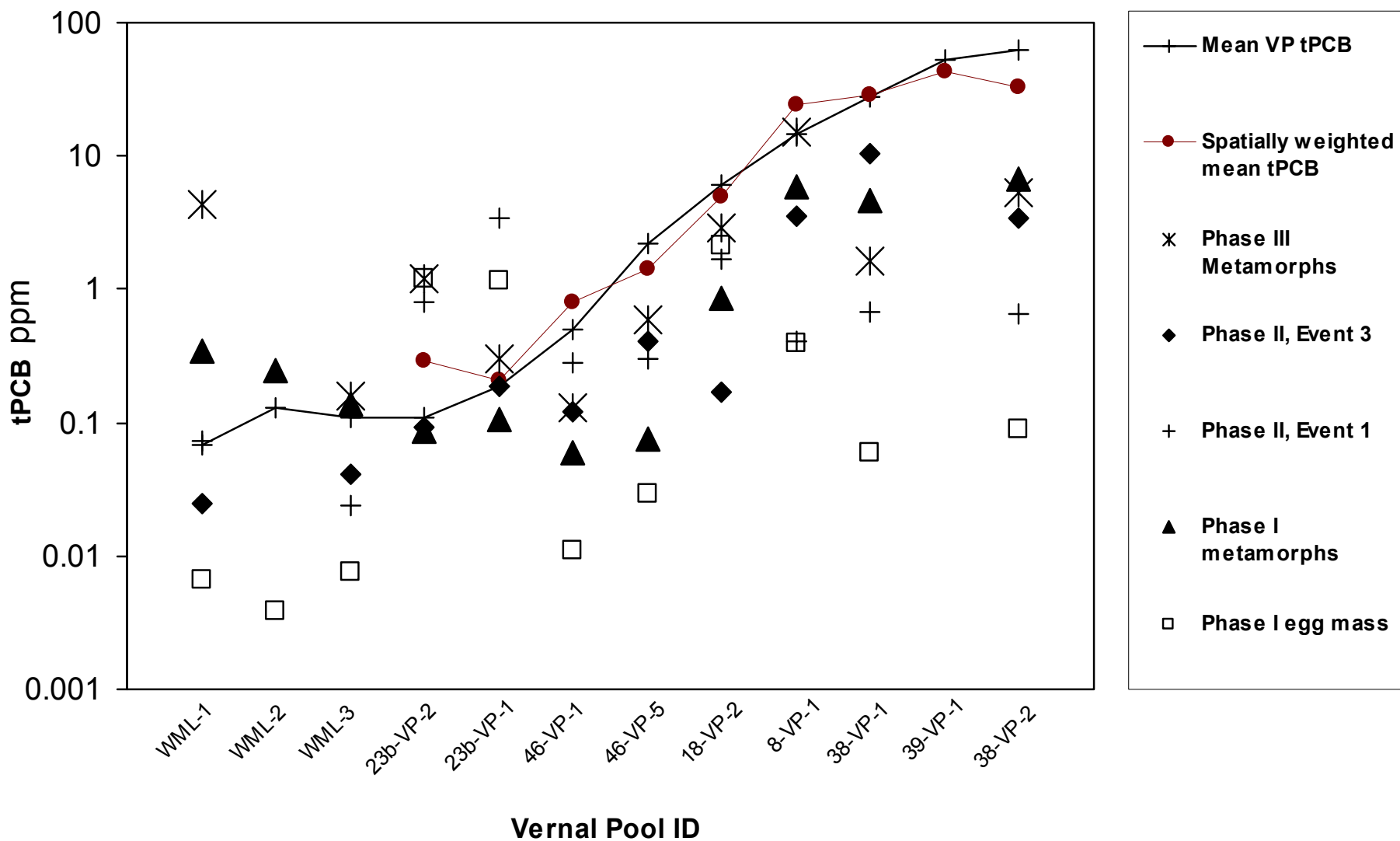
Effects Leopard Frogs (ARCADIS G&M, 2003)

- 44 ponds with suitable habitat were extensively searched
- Egg masses not found in 61% (27 of 44) of ponds with suitable habitat





Exposure Wood Frogs (FEL 2002)





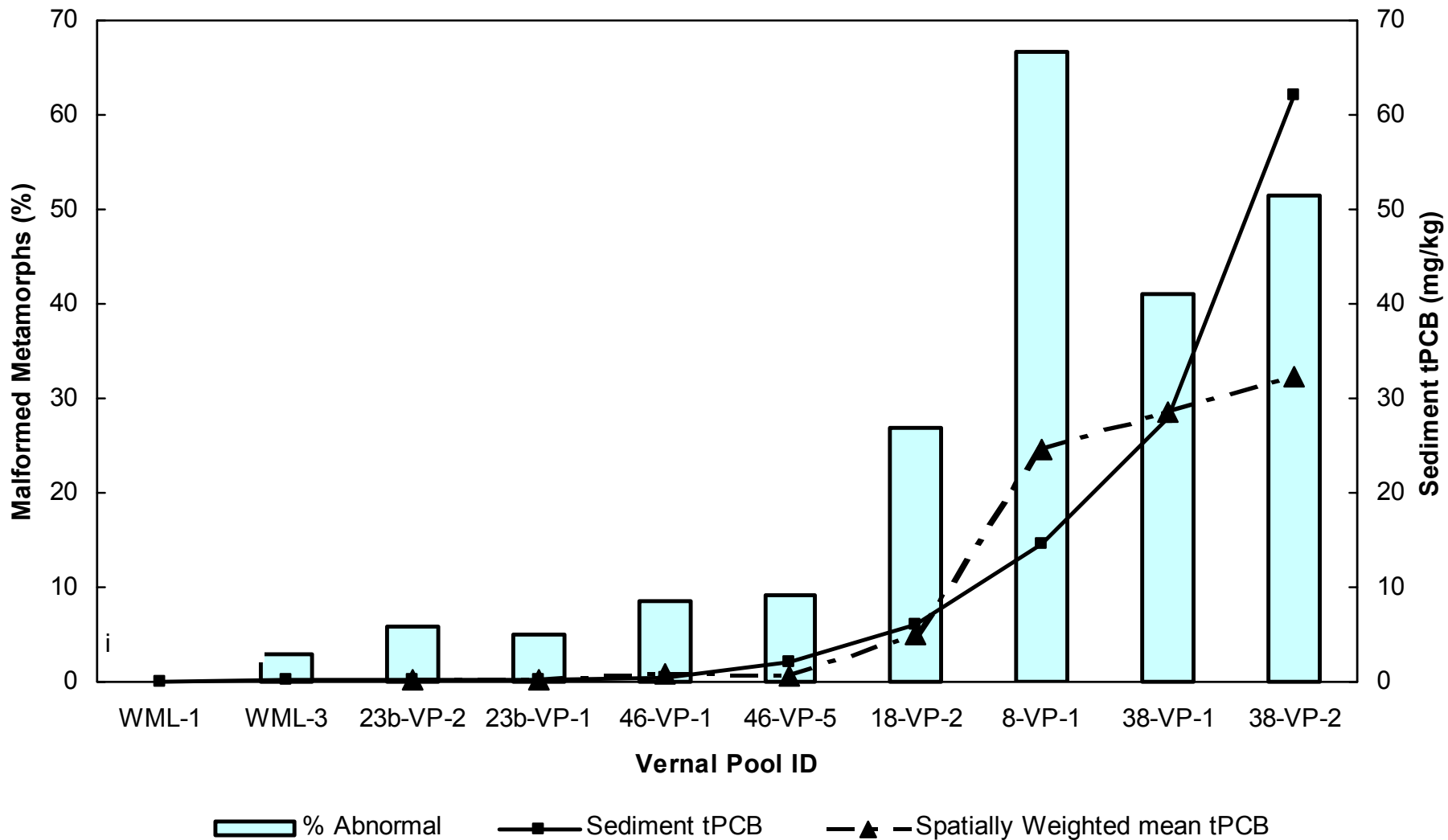
Effects Wood Frogs (FEL 2002)

- Effects related to length of exposure and life stage
- Early life-stages showed no concentration-response relationships
- Late life-stage malformations significantly correlated with sediment, water, and tissue tPCB
- Skewed sex ratios (increase in females) significantly correlated with sediment and tissue tPCB



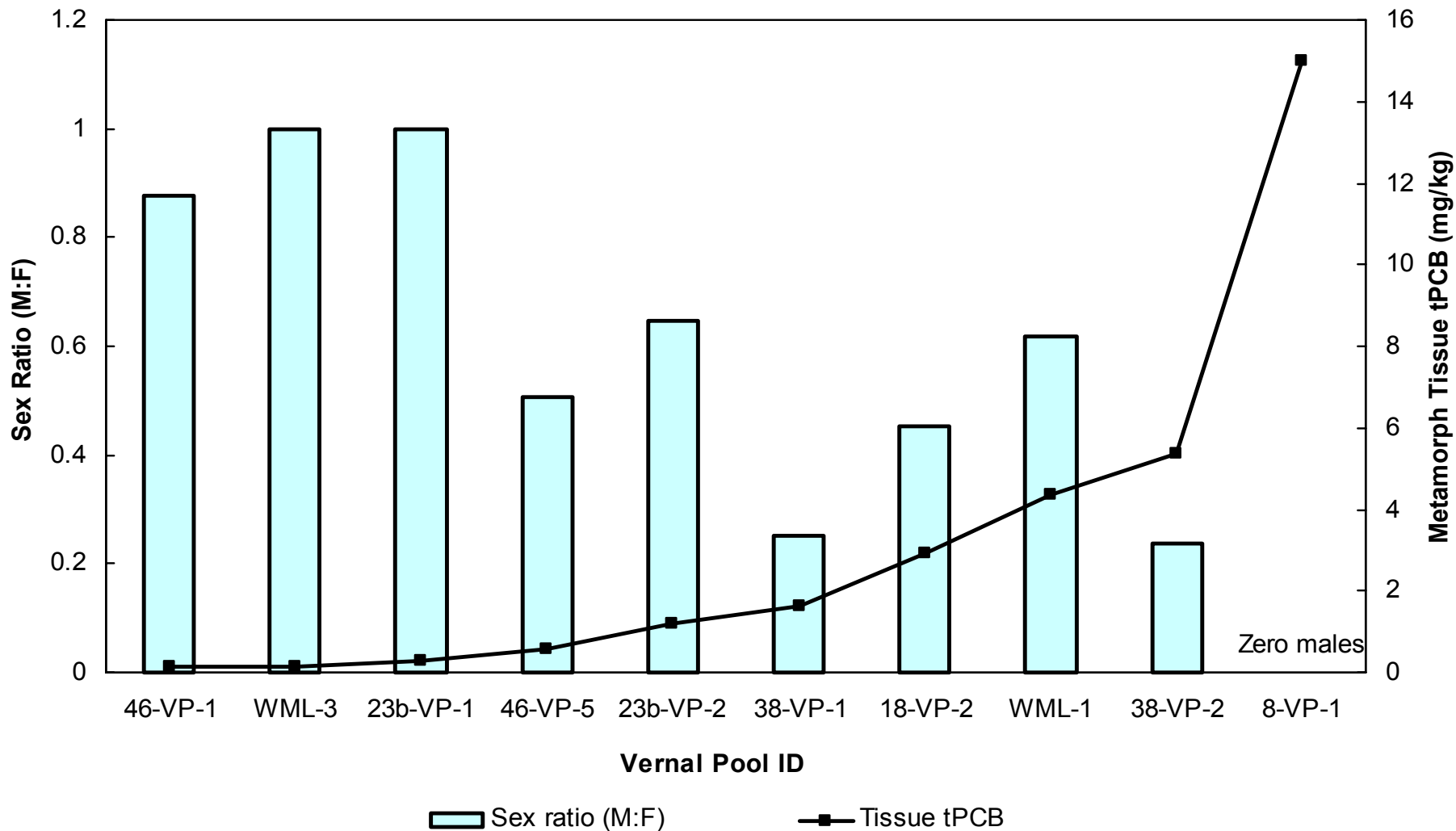


Effects Wood Frogs (FEL 2002)





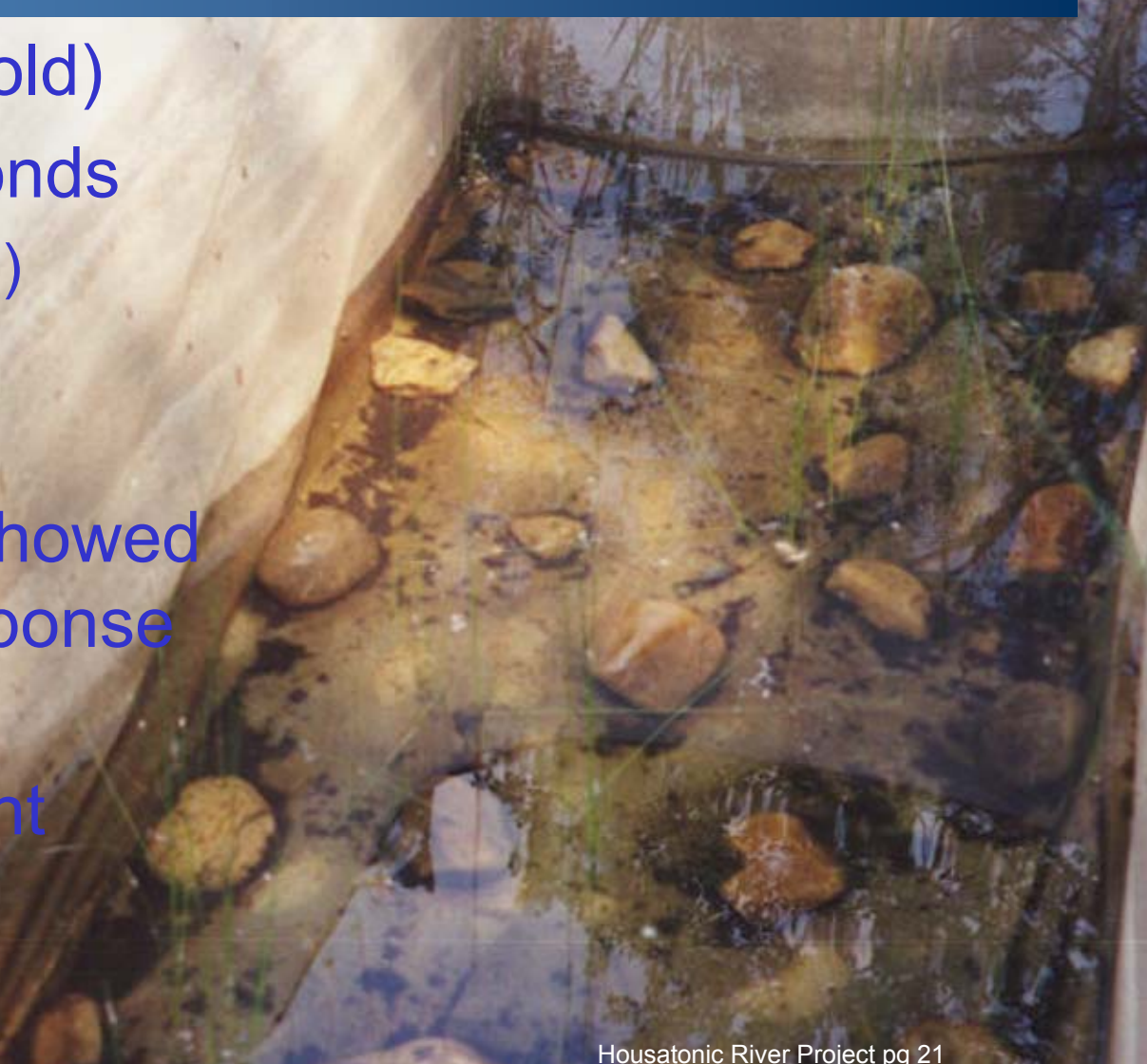
Effects Wood Frogs (FEL 2002)





Effects Wood Frogs (Resetarits, 2002)

- Larvae (1-2 days old) selected from 6 ponds
 - Very low (0.9 ppm)
 - Low (3.3 ppm)
 - High (11.2 ppm)
- Early life-stages showed indeterminate response relationships – insensitive endpoint





Effects Vernal Pool Field Study

Vernal Pools with higher sediment tPCBs had:

- **Lower species richness**
- **Lower density and biomass**

**Larval malformation rates
high in all pools**

Measurement Endpoints	Weighting	Evidence of Harm (Yes/No/Undetermined)	Magnitude (High/ Moderate/Low)
C. Chemical Measures			
C. Concentration of PCB in frog tissues in relation to levels reported to be harmful to amphibians.	Moderate	Yes	Low
W. Wood Frog Toxicological Measures			
W-1. Sediment toxicity to hatchling/late embryo life	Mod/High	No	-
W-2. Sediment toxicity to larval life stages.	Mod/High	Yes	Intermediate
W-3. Sediment toxicity to late larval/metamorph life	Mod/High	Yes	High
W-4. GE Study (juvenile wood frogs)	Low	Undetermined	-
L. Leopard Frog Toxicological Measures			
L-1. Sediment toxicity to hatchling/late embryo life	Mod/High	Yes	Low
L-2. Sediment toxicity to larval life stages.	Mod/High	Yes	High
L-3. Sediment toxicity to late larval/metamorph life	Mod/High	Yes	High
L-4. Sediment toxicity to adult leopard frogs	Mod/High	Yes	High
B. Biology			
B-1. Vernal pool community study.	Mod/High	Yes	Low
B-2. GE leopard frog egg mass survey	Low	Undetermined	-
B-3. Anecdotal observations during collections for	Moderate	Yes	Low



Risk Characterization

Risks to Individuals



Risks to Local
Populations



Population Modeling

- tPCBs have a negative impact on wood frog population growth & abundance
- tPCBs hasten population decline
- Increased tPCB concentrations lead to decreased density



Risk Characterization Other Species

- **Salamanders may be at greater risk**
 - **Longer larval periods**
 - **Bottom dwelling**
 - **Longer-lived**
 - **Carnivorous**





Downstream Risk

Lenoxdale to Rising Pond

Below Rising Pond

