



Effects of an Aquatic Herbicide Tank Mix on Metamorphic Northern Red-legged Frogs

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Invasive Plants Conference

17 September 2014

Seattle, Washington

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Overview

Herbicides are used in wetland restoration



Specialist surveying large infestation of purple loosestrife



Garden loosestrife infestation at Marymoor Park kingcounty.gov/weeds

Overview

Are there risks to
amphibians?



Specialist surveying large infestation of purple loosestrife



Garden loosestrife infestation at Marymoor Park kingcounty.gov/weeds



Triclopyr TEA Tank Mix



ecologyadventure2.edublogs.org/plant/purple-loosestrife/

What is the best timing of application?



Infestation in Netley-Libau Marsh, Manitoba, 1999.
www.purpleloosestrife.org/faq/

Amphibians

Who's at risk?

Late June-August



Metamorphosis

A whole different ball game in toxicology

- Timing ↑ or ↓ (Howe et al. 2004, Cauble & Wagner 2005)
- Mortality (Greulich & Pflugmacher 2003)
- No food (Chen et al. 2008)
- Increased stress

(Glennemeier & Denver 2002)



www.frog-life-cycle.com/

What are the effects of a triclopyr tank mix on metamorphic northern red-legged frogs?



kingcounty.gov/weeds

Methods

- Tank mix



+



+



- 2 cm water



Methods

- Triclopyr tank mix:

Renovate[®] 3

Specialty Herbicide

SPECIMEN **SePRO**

Aquatic Sites: For control of emerged, submersed and floating aquatic plants in aquatic sites such as ponds, lakes, reservoirs, non-irrigation canals, seasonal irrigation waters and ditches which have little or no continuous outflow, marshes and wetlands, including broadleaf and woody vegetation on banks and shores within or adjacent to these and other aquatic sites.

For use in New York State, comply with Section 24(c) Special Local Need labeling for Renovate[®] 3, SLN NY-060001

Active Ingredient
 triclopyr: 3,5,6-trichloro-2-pyridinyloxyacetic acid,
 triethylamine salt44.4%
 Other Ingredients55.6%



COMPETITOR[®]

Modified Vegetable Oil

U.S. Patent No. 5,631,205

CA Reg. No. 2935-50173

WA Reg. No. AW-2935-04001

EPA Est. NO. 2935-TX-2

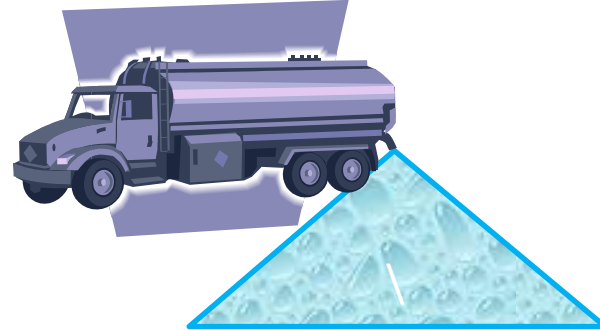
PRINCIPAL FUNCTIONING AGENTS	% By Wt.
Ethyl Oleate, Sorbitan Alkylpolyethoxylate Ester,	98%
Dialkyl Polyoxyethylene Glycol	2%
Constituents Ineffective as spray adjuvant	2%
Total	100%

DIRECTIONS FOR USE

Aquatics: COMPETITOR may be used as an additive with aquatically labeled pesticides. The use rates for COMPETITOR should follow the recommended surfactant rate that is specified on the pesticide product label. If there is no recommended surfactant rate on the pesticide label, COMPETITOR should be used at the rate of 2 to 4 pints per 100 gallons of spray solution.

HI-LIGHT[®]

Industrial Strength Spray Pattern Indicator



47.1 ppm

+



41.3 ppm

+

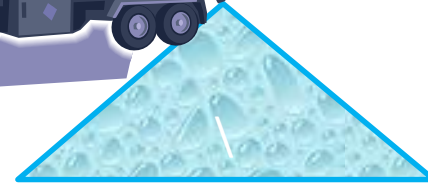
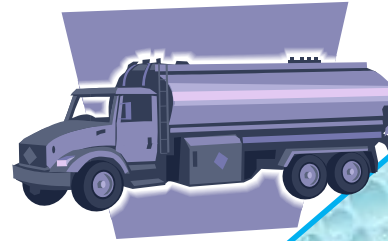


12.9 ppm

Methods



Control (clean water)



96 h
static
renewal



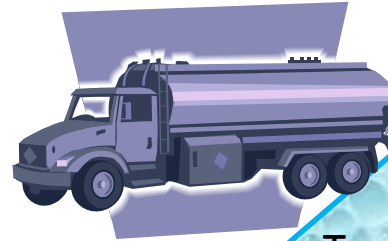
X 15



Methods



Control (clean water)



Tankmix
(clean water)

60-d
grow
out



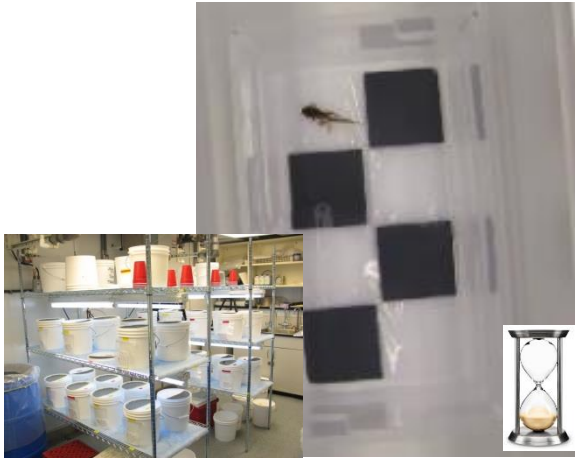
X 15



Methods

- Endpoints

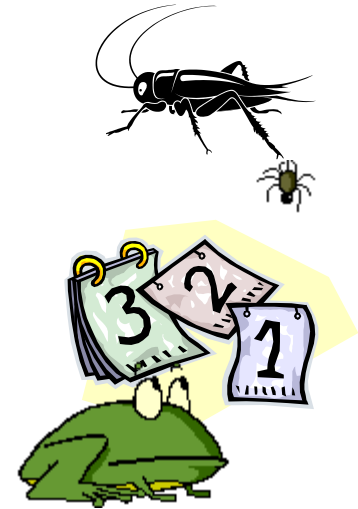
1.



2.



3.



4.

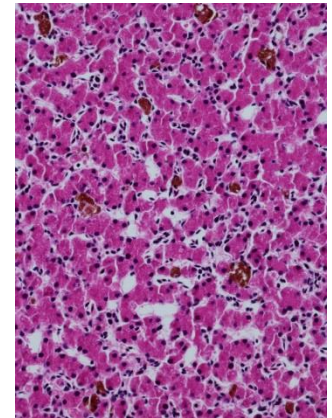


5.



www.mintees.com/tees/3953-liver-going-the-extra-bile/

6.



Results - overall

- No treatment-related mortalities
- No gross anomalies in gonad structure
- No treatment-related anomalies in over-all health



Results – behavior during exposure

- Metamorphs showed evidence of stress during exposure to the tank mix

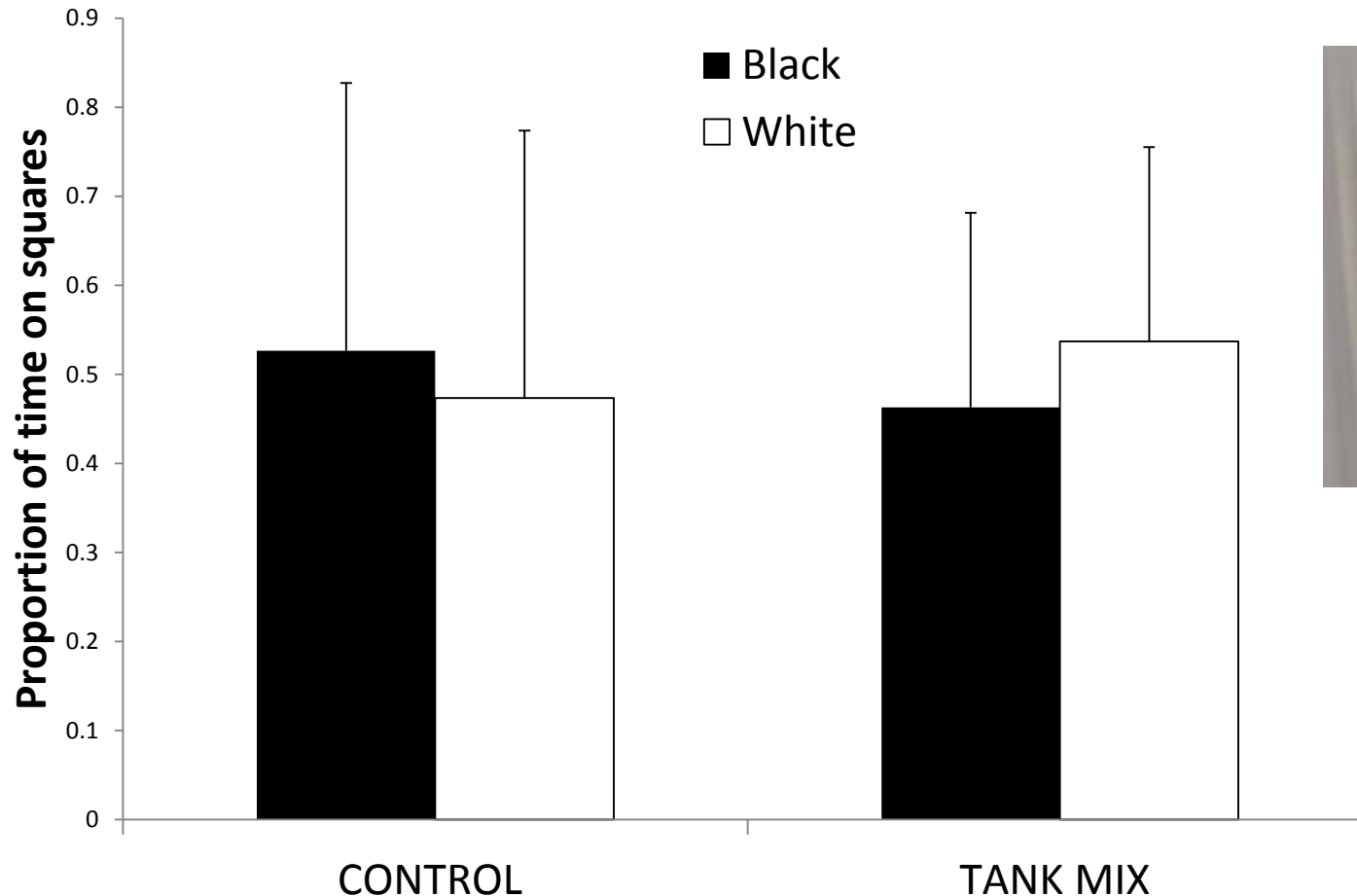
# Observations	Frogs	Legs sprawled
Control	2	2
Tank mix	12	22

$P = 0.013$



Results – behavior post-exposure

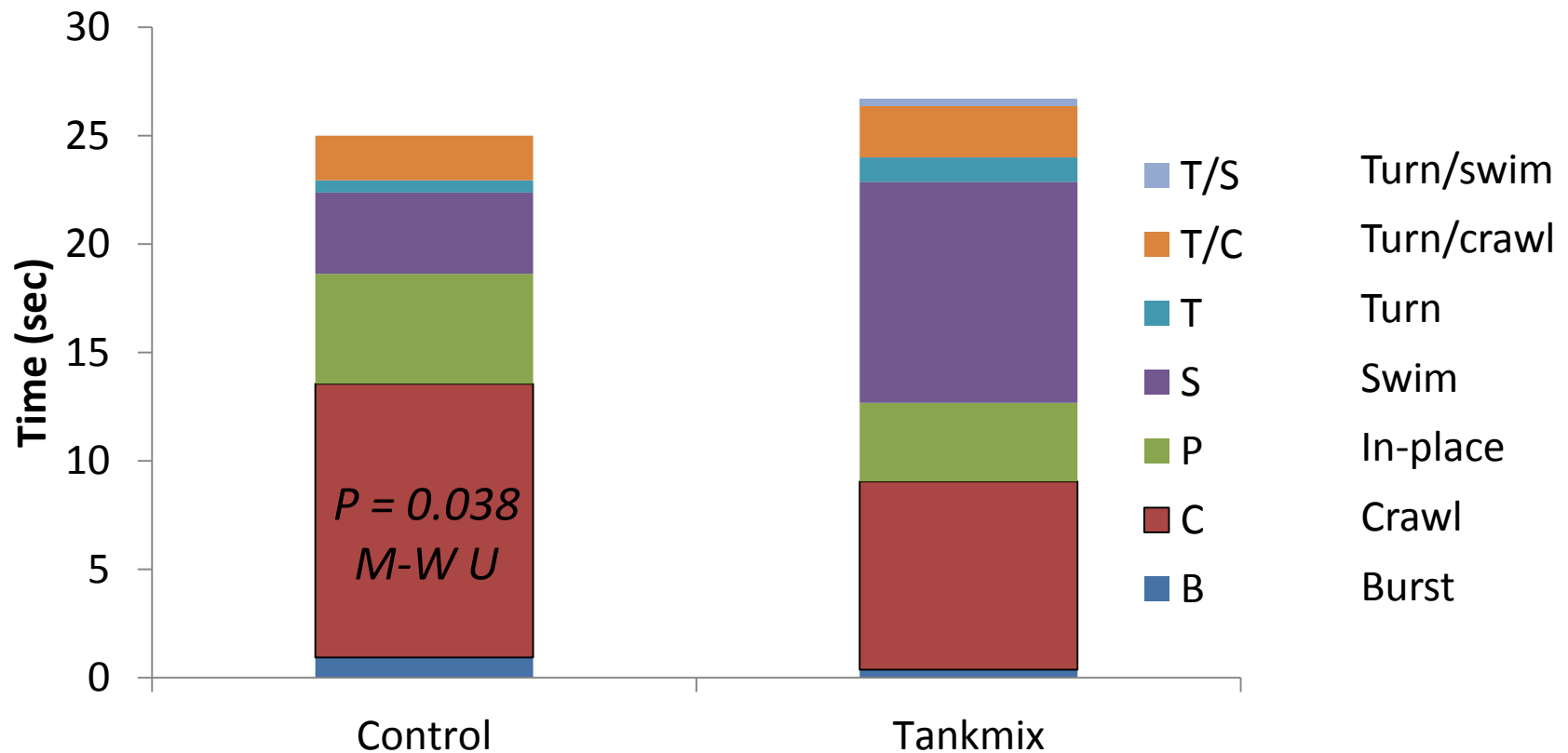
- Metamorphs didn't care what color square they were on



$P > 0.75$
 $\chi^2_c = 0.10$

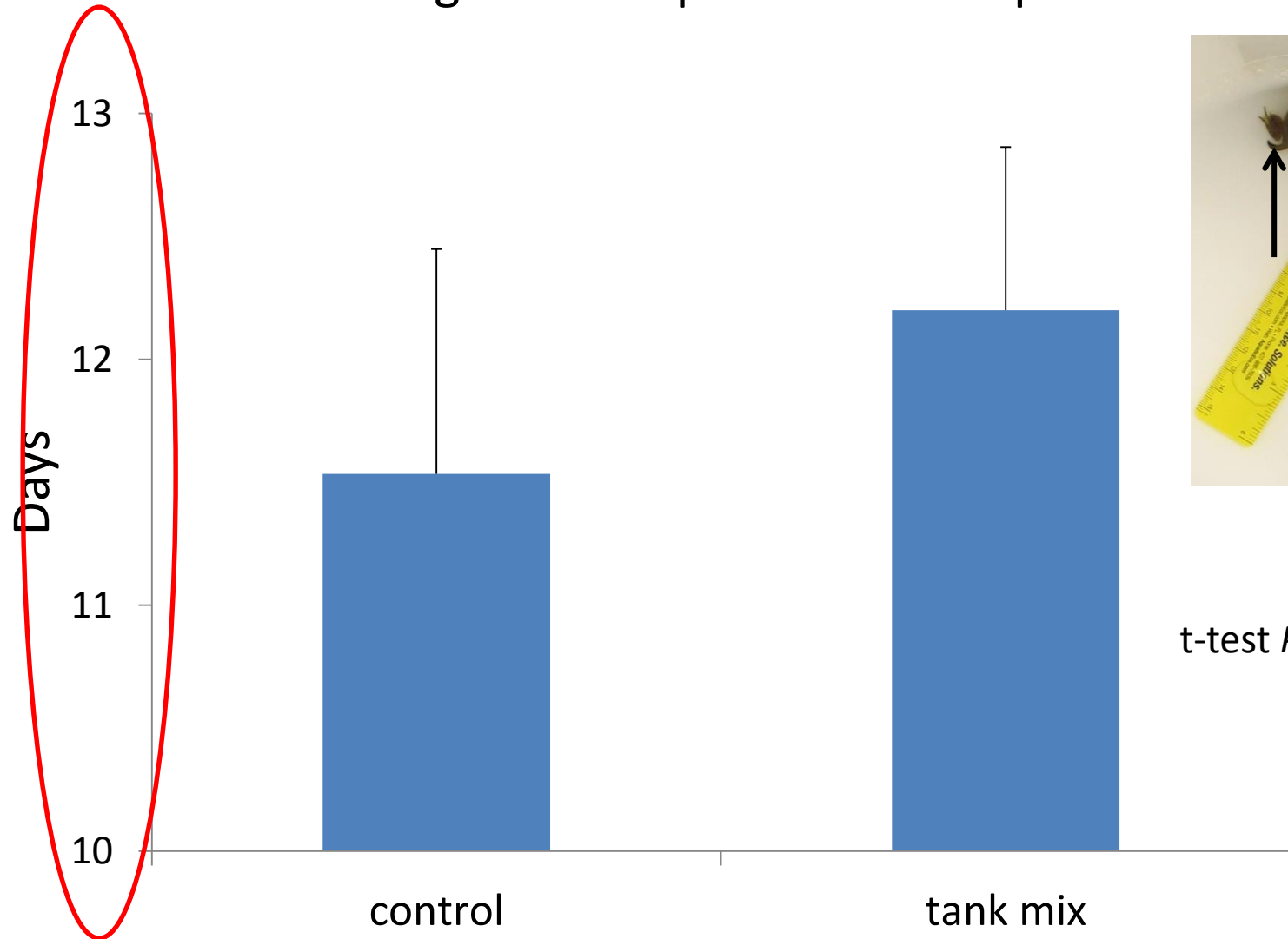
Results – behavior post-exposure

- Controls crawled more than tank mix metamorphs



Results – development timing

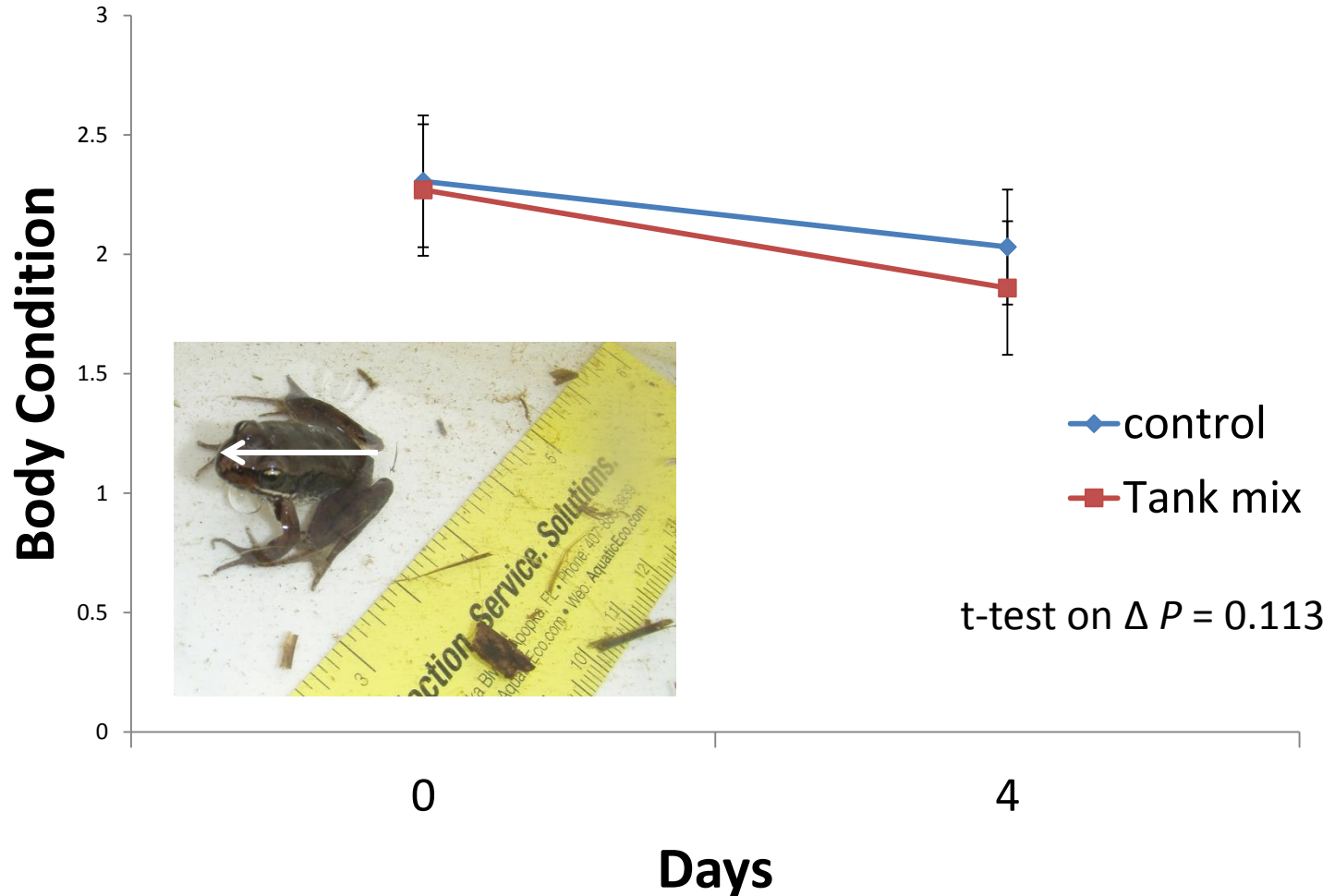
- Tank mixes took longer to complete metamorphosis



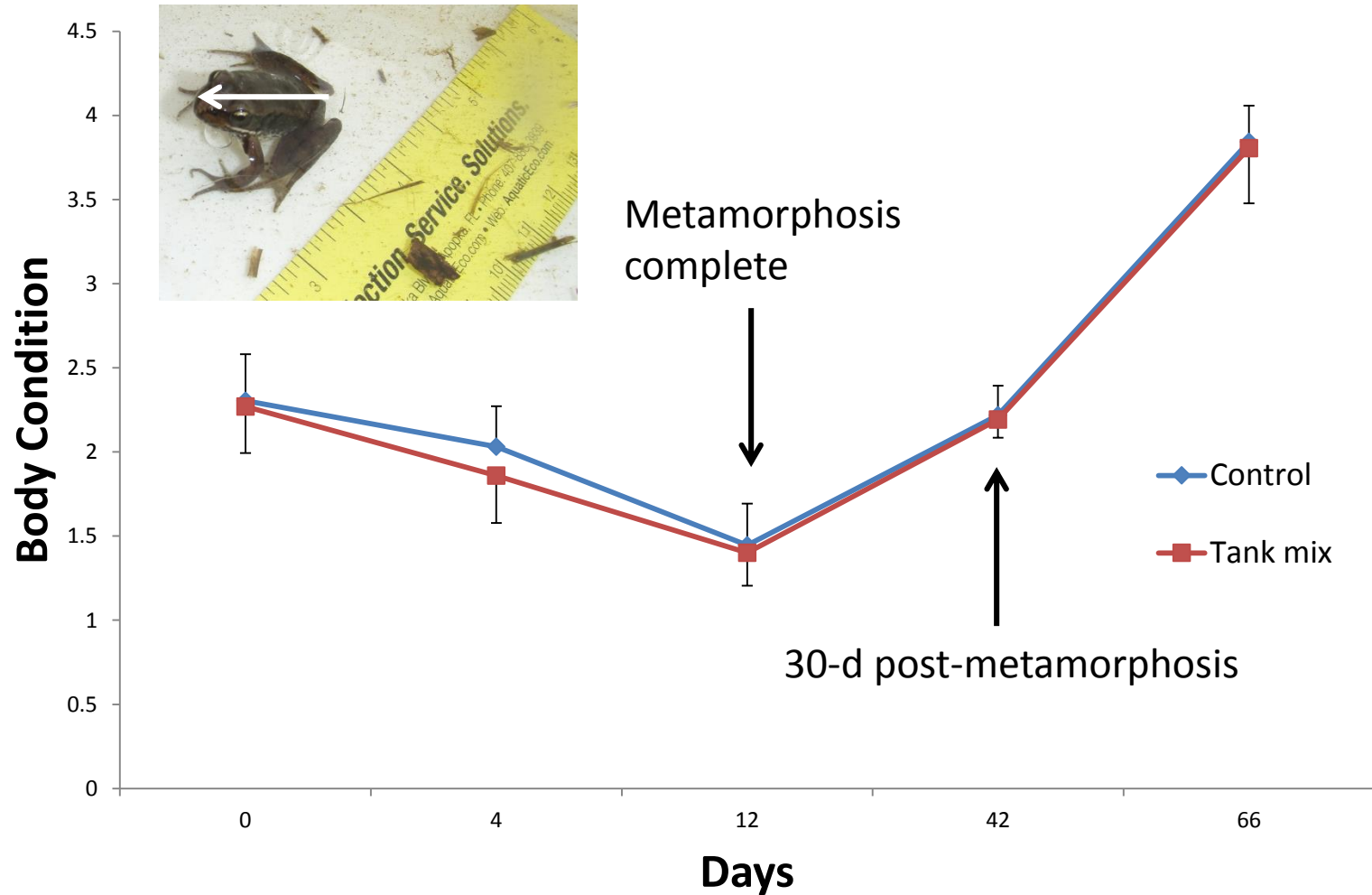
t-test $P = 0.031$

Results – body condition

- Body condition was not statistically different at 96 h

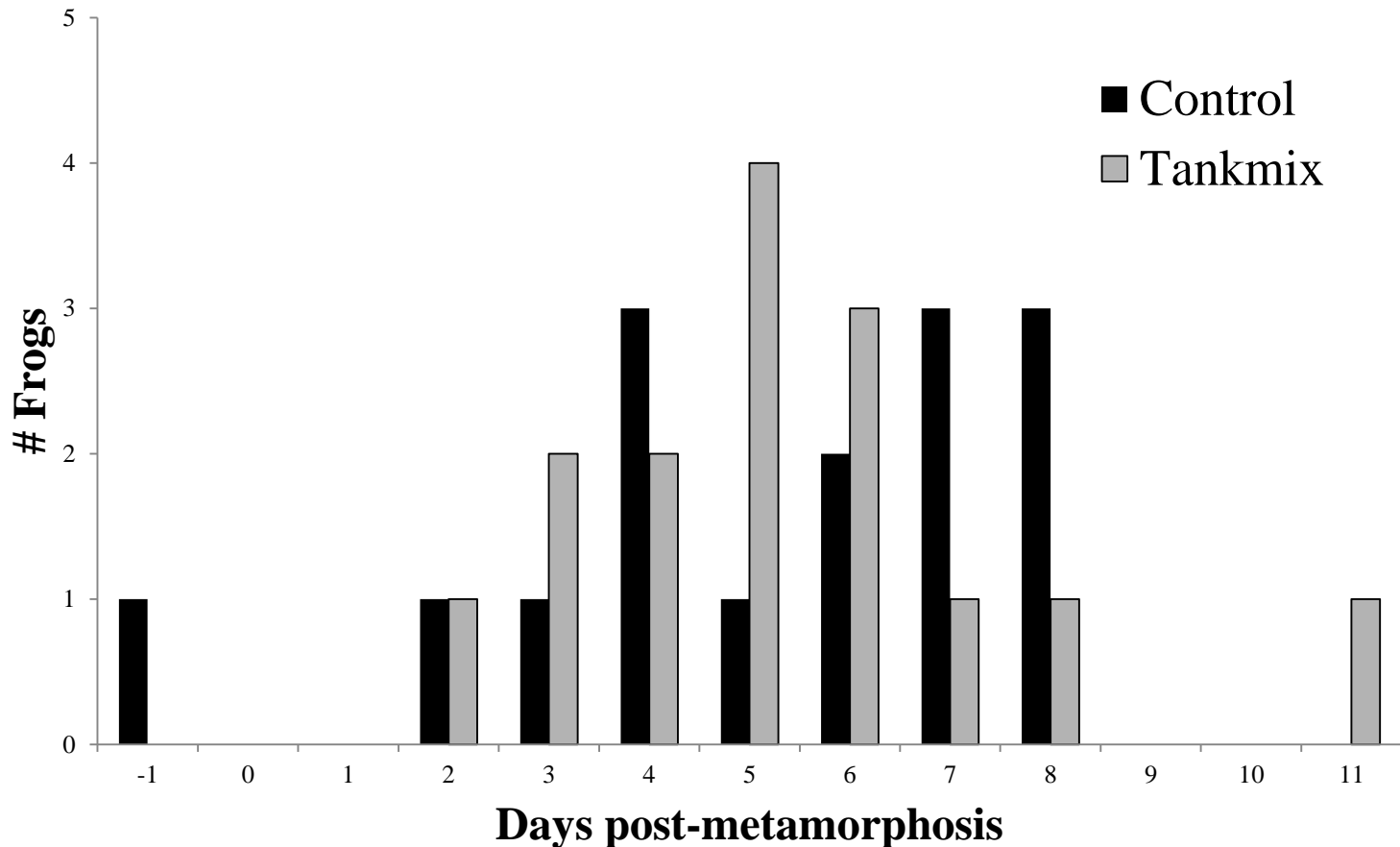


Results – body condition



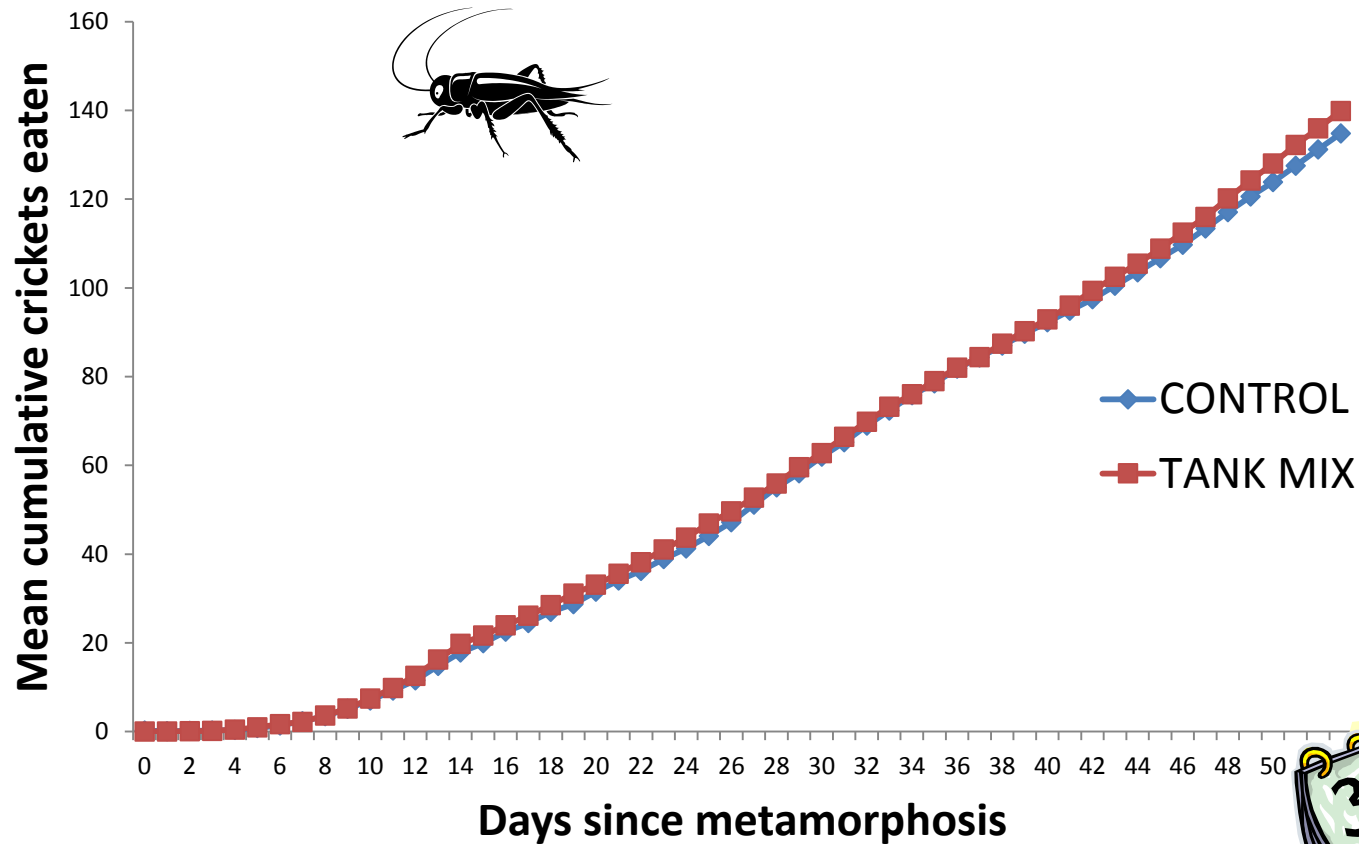
Results – feeding behavior

- Everyone started eating at the same time



Results – feeding behavior

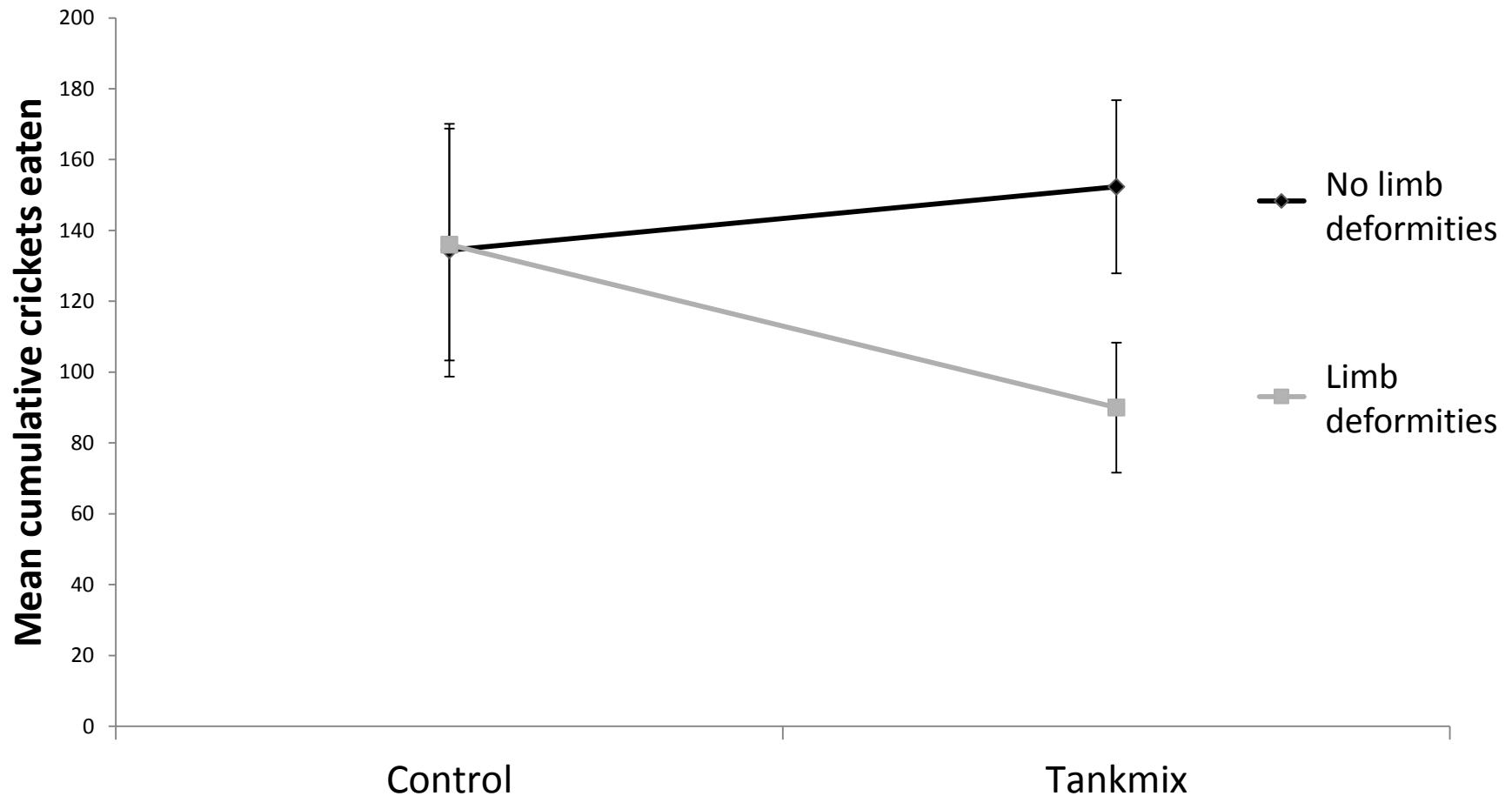
- Everyone ate the same amount



LMM day 53, $P = 0.199$

Results – feeding behavior

- **Pre-existing** limb deformities made it harder for tank mix metamorphs

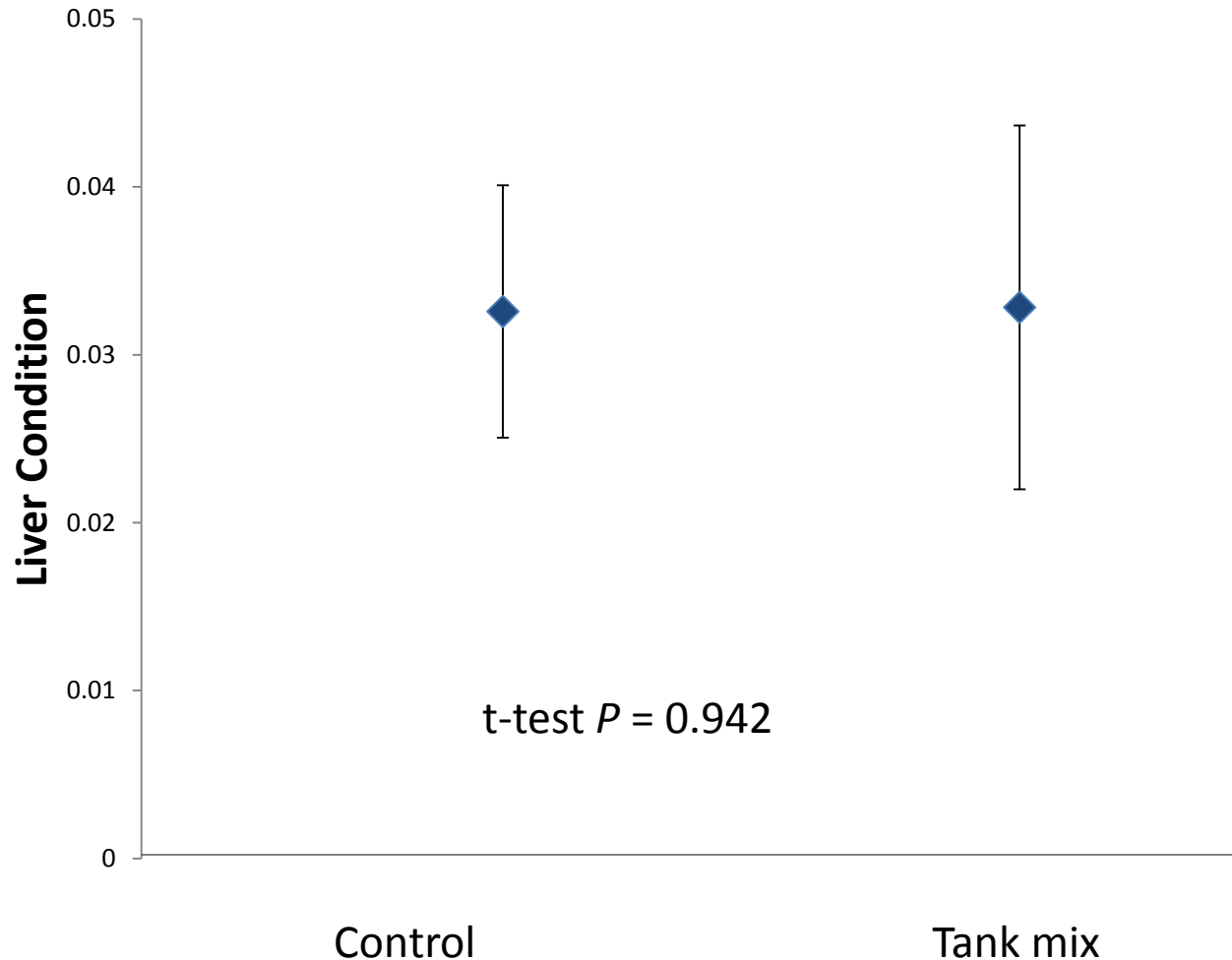


Improvement over simple model: LMM $X^2_1 = 3.99$, $P < 0.05$;

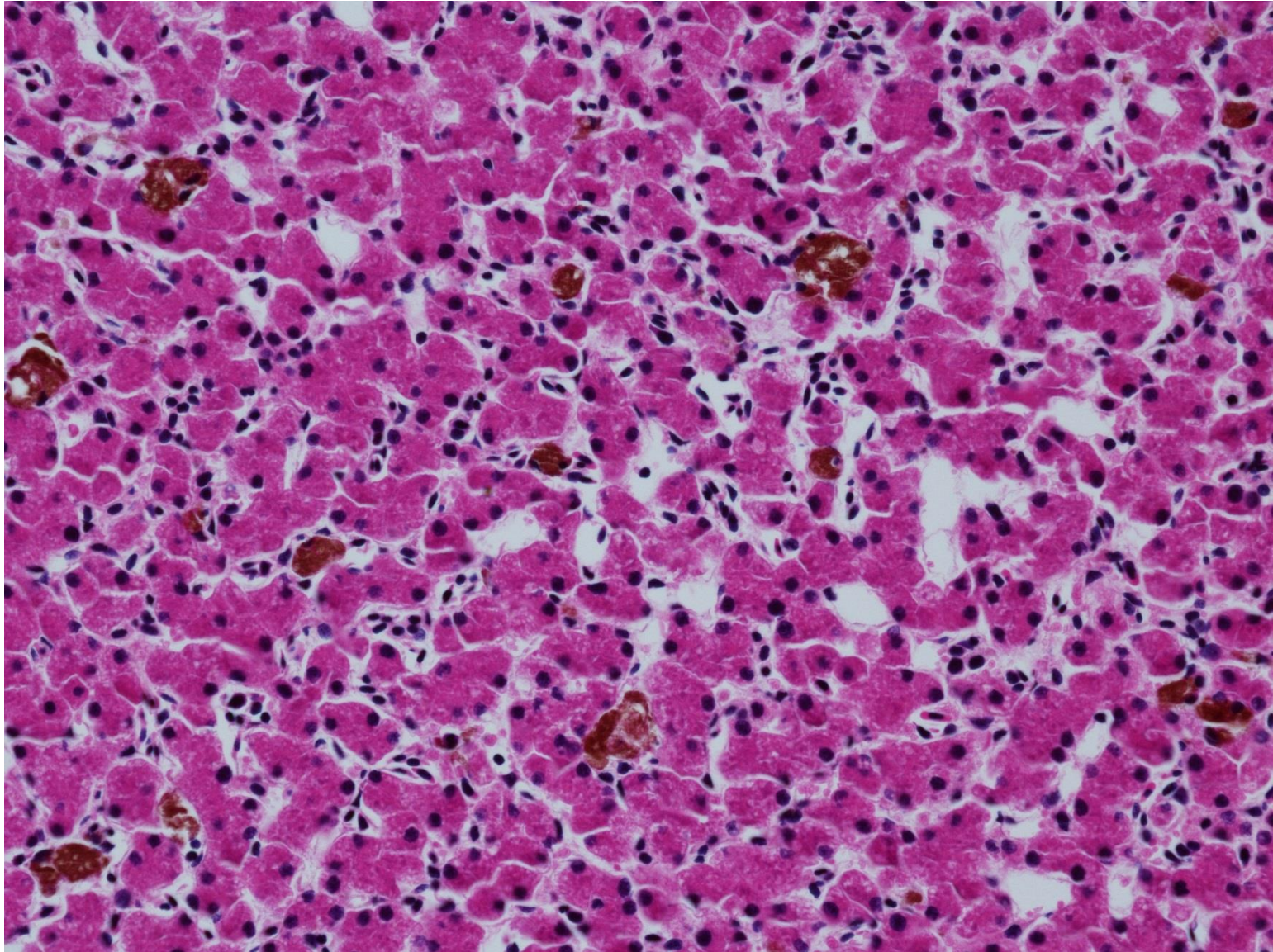
64% of variance explained by interaction of treatment with limb deformities

Results – liver condition

- Everyone had the same liver condition



Results – liver histology



Summary

- Minimal effects observed
- Potential interaction with stressors



Conclusions

- Triclopyr can be a little stressful, but NRLF metamorphs get over it
 - What is the real exposure in the field; is the risk acceptable?
- This information is important for informing policy and the public

Thank you!

- UW PhD Committee
C Grue; D Beauchamp, E Faustman , M Hayes, A Wirsing
- Washington Department of Ecology, Aquatic Weeds Management Grants
- Washington Department of Fish and Wildlife
M Hayes, D Heimer, T Hicks, C Roberts, K Tidwell, J Tyson
- UW Hatchery & UW Veterinarian Services
J Wittouck Dr. G Sanders
- Washington Cooperative Research Unit, University of Washington
R Fisk, JM Grassley, C Grue, S Pearman-Gillman, M Smith, A Troiano
- School of Aquatic & Fishery Sciences
- SePro
- Woodland Park Zoo



Washington Department of
FISH and WILDLIFE

A photograph of two frogs in a pond. The frog in the foreground is brown with dark spots and large, prominent eyes, looking directly at the camera. It is partially obscured by green reeds. Another frog is visible in the background, also partially hidden by reeds. The water is dark and still. The word "Questions?" is written in white text in the upper right quadrant of the image.

Questions?

Liver histology

Lesion Severity	#Lesions per 20x field	Control	Tankmix	Total # frogs
mild	1-4	5	5	10
mild to moderate	1-7	5	6	11
moderate	5-7	2	0	2
moderate to severe	7-15	0	2	2
severe	10-15	2	1	3