

Abstract

Triphenyltin (TPT) is a fungicide that is widely used in agriculture on crops such as pecans, potatoes and sugar beets. In areas of the United States, levels as high as 6 µg/L TPT have been measured in the water of rivers and lakes and significant biomagnification of TPT through the food web has been shown. Furthermore, several studies have documented acute toxicity in some amphibian species at concentrations as low as 1.25 µg/L TPT after 48 hours. However, to date no studies have been performed on the sensitivity of the wood frog (*Rana sylvatica*) to TPT despite the continued use of TPT within its range. Thus, the current study was designed to assess the sensitivity of wood frog tadpoles to Triphenyltin chloride (TPTCl) from 1 week post hatch through metamorphic climax. Wood frog tadpoles were exposed to 0.1, 1 and 5 µg/L TPTCl. Endpoints that were examined included mortality, time to metamorphosis and basic morphometrics of tadpoles and metamorphosing froglets. Complete mortality of wood frog tadpoles was observed after 9 days when exposed to 5 µg/L TPTCl. During this same time period, mortality in control treatments was negligible. Furthermore, after seven days the weight and length of wood frog tadpoles treated with 5µg/L TPTCl was significantly less than that of the control. No significant differences were observed in the 0.1 and 1 µg/L TPTCl treatments for weight and length after the same time period.

Table 1: NOAEL, LOAEL and LC50 for TPTCL exposure for three endpoints

Endpoint		NOAEL (µg/L TPTCL)	LOAEL (µg/L TPTCL)	LC50 (µg/L TPTCL)
Mortality	After 9 days	1.0	-	1.793
	After 100 days	0.1	1.0	0.252
Metamorphosis	After 100 days	0.1	1.0	-
	After 7 days	1.0	5	-
Weight and Length	After 7 days	1.0	-	-
	After 100 days	1.0	-	-

Methods

I. Egg Mass Collection & Laboratory Acclimation

- Six egg masses were collected from a pond near Saskatoon, SK with no adjacent agricultural activities on April 8, 2010 (SK Special Permit #10FW059)
- Egg masses were immediately transferred to the Aquatic Toxicology Research Facility (ATRF) at the University of Saskatchewan, and acclimated to laboratory conditions and water
- Eggs began hatching on April 12, 2010 and most larvae were free-swimming on April 15, 2010
- TPT exposure was initiated on April 15, 2010

II. Experimental Design & Maintenance

- Treatments**
- Control and 0.0025% ethanol solvent control (SC)
 - 0.1, 1 and 5 µg/L Triphenyltin Chloride (TPTCl) (Cas #:639-58-7)
- Study Design**
- 50% static water renewal every 24 h
 - Three replicate tanks per treatment group
 - 30-50 tadpoles per tank at experiment initiation
 - Sub-sample of tadpoles were collected at Gosner stage 26 (day 14 post hatch)
 - All tadpoles were grown to metamorphic climax or 108 d post hatch, whichever occurred first



Picture 1: Wood Frog egg mass collection (top) and a close up of a wood frog egg mass (bottom).

III. Experiment Termination

- Tadpole Gosner stage 26 subsamples**
- Gosner stage, weight and snout-vent-length (SVL) determined
 - Flash frozen for molecular analysis
- Tadpole Grow-Out**
- Sacrificed at metamorphic climax (Gosner stage 42+). Days to metamorphosis, gosner stage, weight and SVL was determined
 - Brain and liver flash frozen for molecular analysis. Phenotypic sex determined under dissecting microscope. Body, including gonads, preserved in 10% formalin for determination of histological phenotypic sex and tissue abnormalities
 - Tadpoles that failed to reach metamorphic climax were sacrificed at 108 d post hatch (July 24, 2010) and samples were collected and processed as described for tadpoles that reached metamorphic climax

IV. Statistics

- Survival analysis (Systat 12) was used to determine average DTM. Allowed inclusion of individuals that failed to reach metamorphic climax
- Weight, SVL, and DTM data were analyzed with ANOVA tests. Dunnett's test was used for post hoc testing (SPSS 17; Somers, NY).
- Mortality data were analyzed with T-test (SPSS 17). LC 50 was determined using ToxCalc software (Michigan State University) after 9 d (moving average) and 100 d (probit)
- Due to differences between SC and controls, SC was used as the basis for statistical comparisons.

Results

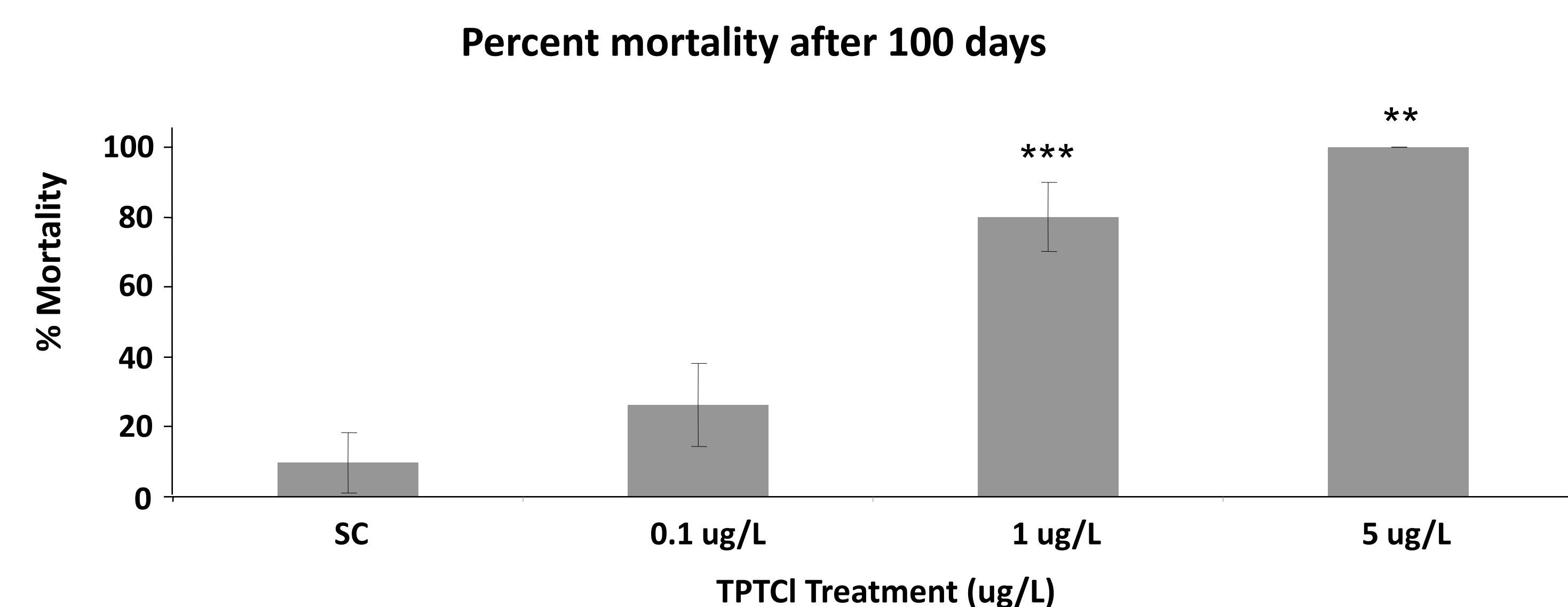


Figure 1: Percent Wood Frog mortality (mean ± SD) after exposure to TPTCl for 100 days. *Indicates significant differences from SC (P<0.05)

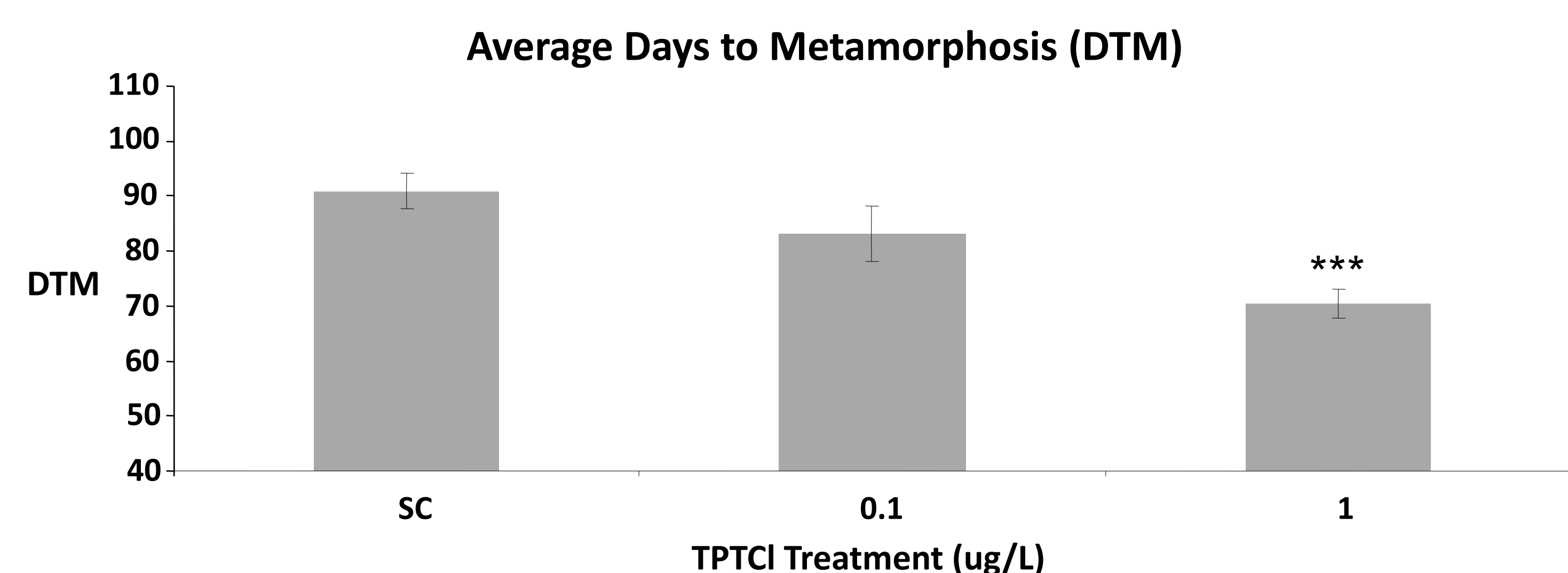


Figure 2: Average number of days to metamorphosis (mean ± SD) after exposure to TPTCl for 100 days. * Indicates significant differences from SC (P<0.05)

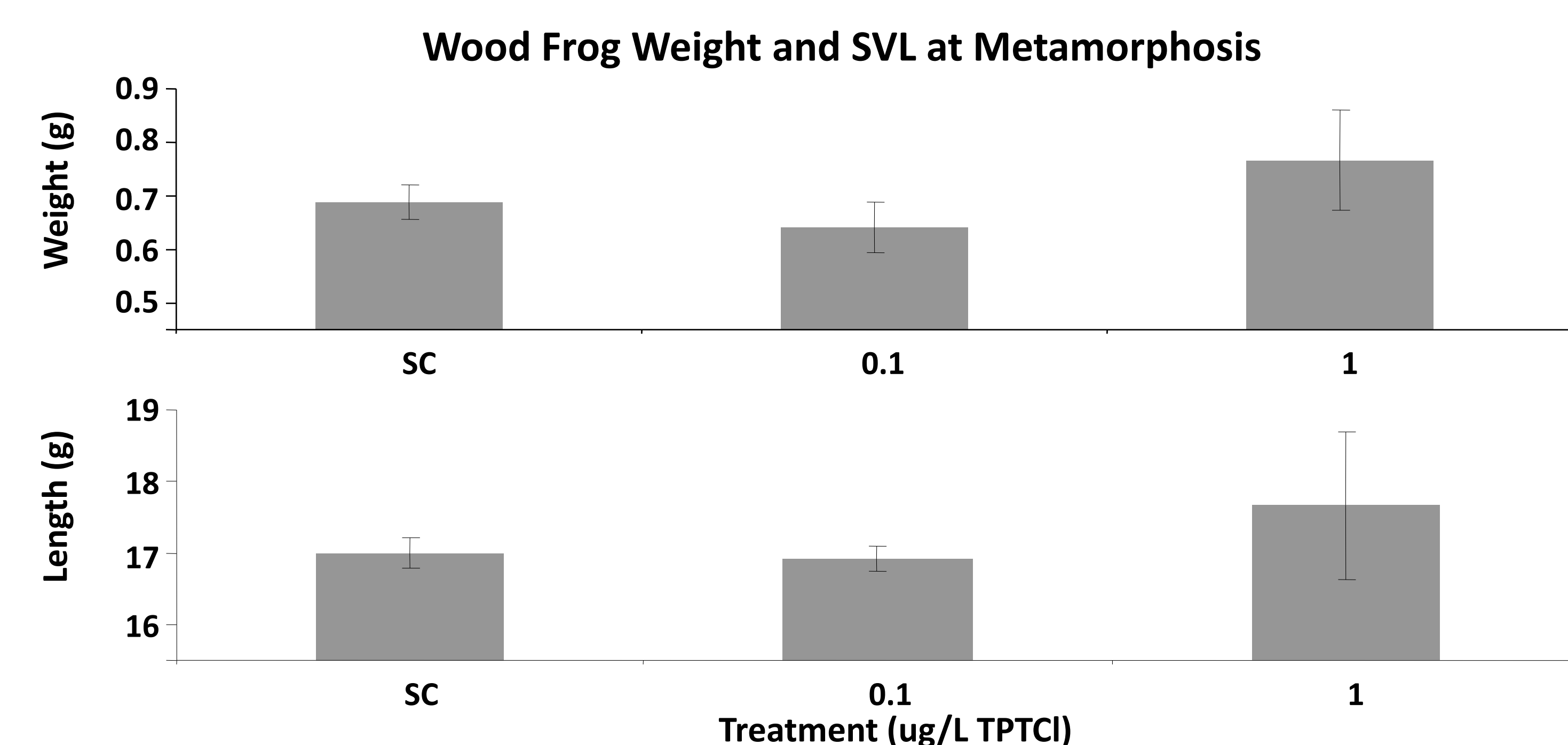


Figure 3: Wood frog weight and length (SVL) measurements (mean ± SD) at metamorphosis. No significant differences were observed (P>0.05)

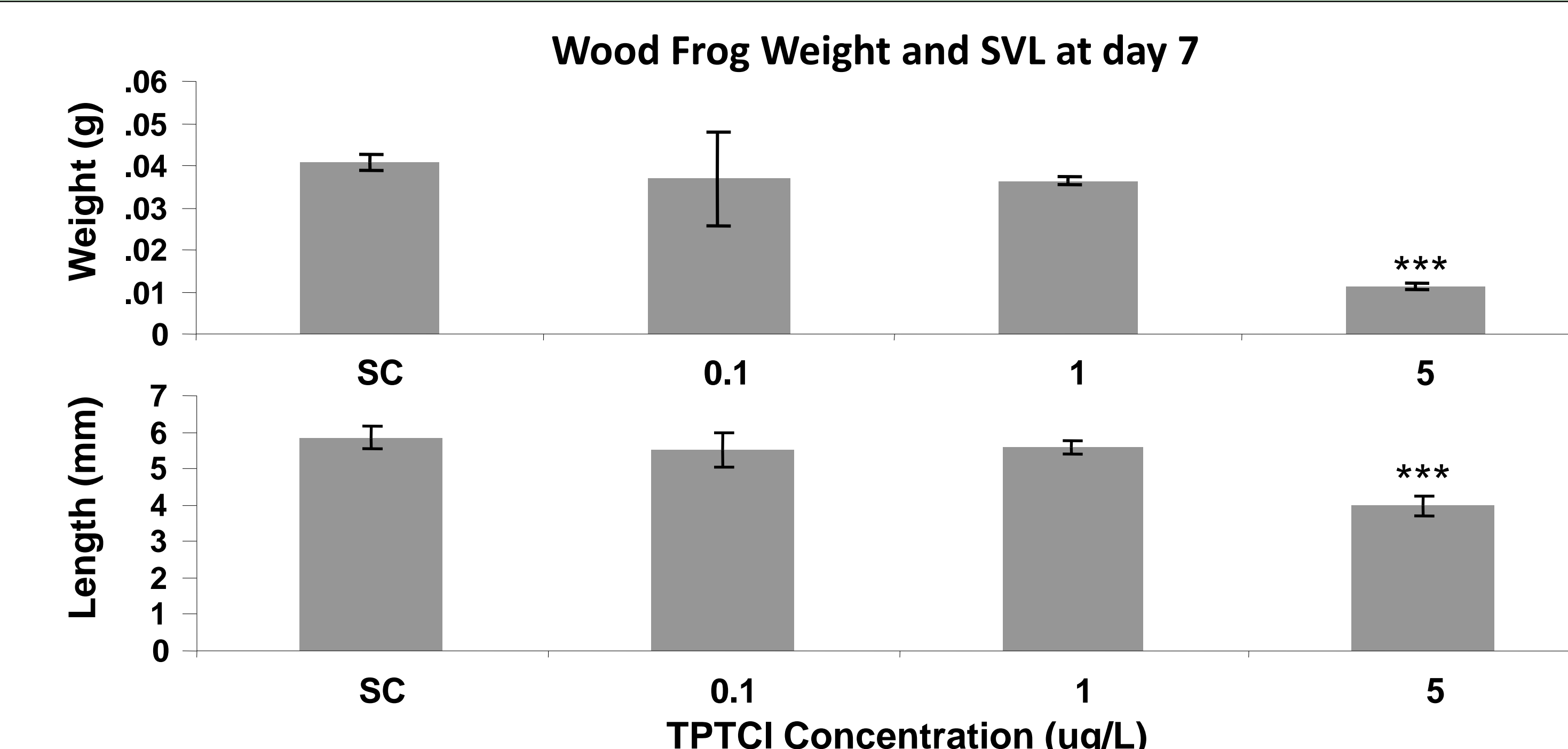


Figure 4: Wood frog weight and length (SVL) measurements (mean ± SD) after exposure to TPTCl for 7 days. * Indicates significant differences from SC (P<0.05)



Picture 2: Experimental setup (left) and close up of the low and medium TPT tanks (right).

Conclusions

Mortality

- Wood frog tadpoles exposed to 5 µg/L TPT were significantly smaller than control animals after 7 days. Furthermore, in the 5 µg/L TPT treatment group all tadpoles died within 9 days. No differences in mortality were observed in the 1 and 0.1 µg/L TPT treatments in the same time period.
- Only two other TPT chronic amphibian studies could be found in the literature. One study examined the effects of TPT on the streamside salamander (*Ambystoma barbouri*) and the other on *Rana lessonae* and *Rana esculenta*. We found that the Wood frog is more sensitive than *A. barbouri*, *R. lessonae* and *R. esculenta* to TPT (Table 1).

Table 2: Species comparison to long term exposure of TPT

Species	TPT Concentration	% Mortality
<i>Ambystoma barbouri</i>	1 µg/L	No effect
<i>Rana lessonae</i> and <i>Rana esculenta</i>	1.87 µg/L	62 %
<i>Rana sylvatica</i>	1 µg/L	80 %

- Similar to *A. barbouri* study the majority of mortalities in the 1µg/L TPT treatment group occurred midway through the exposure (days 31-43). It is possible that the stress of transitioning to exogenous feeding could play a role in the increase mortality at this time. This is in contrast to the *R. lessonae* and *R. esculenta* study that found that most mortality occurred later in metamorphosis during tail resorption.

Metamorphosis

- The significant decrease in time to metamorphosis at 1µg/L TPT is in contrast to both the *Ambystoma* and *Rana* studies that observed an increase in days to metamorphosis compared to control when exposed to 1 µg/L and 1.87 µg/L TPT, respectively. The reasons for this difference are under investigation at this time.

Body mass and length

- Body mass and SVL at metamorphosis for the 0.1 and 1 µg/L TPT treatments were similar to the controls. Although, a trend of increasing body mass and SVL was observed in the 1 µg/L treatment. This trend is similar to the results found in the 1.87 µg/L treatment for the *R. lessonae* and *R. esculenta* studies but no differences were measured in the *A. barbouri* studies.

Future work

- Molecular endpoints including gene expression in the liver or whole body.
- Gonadal histology to determine sex and presence of any abnormalities.

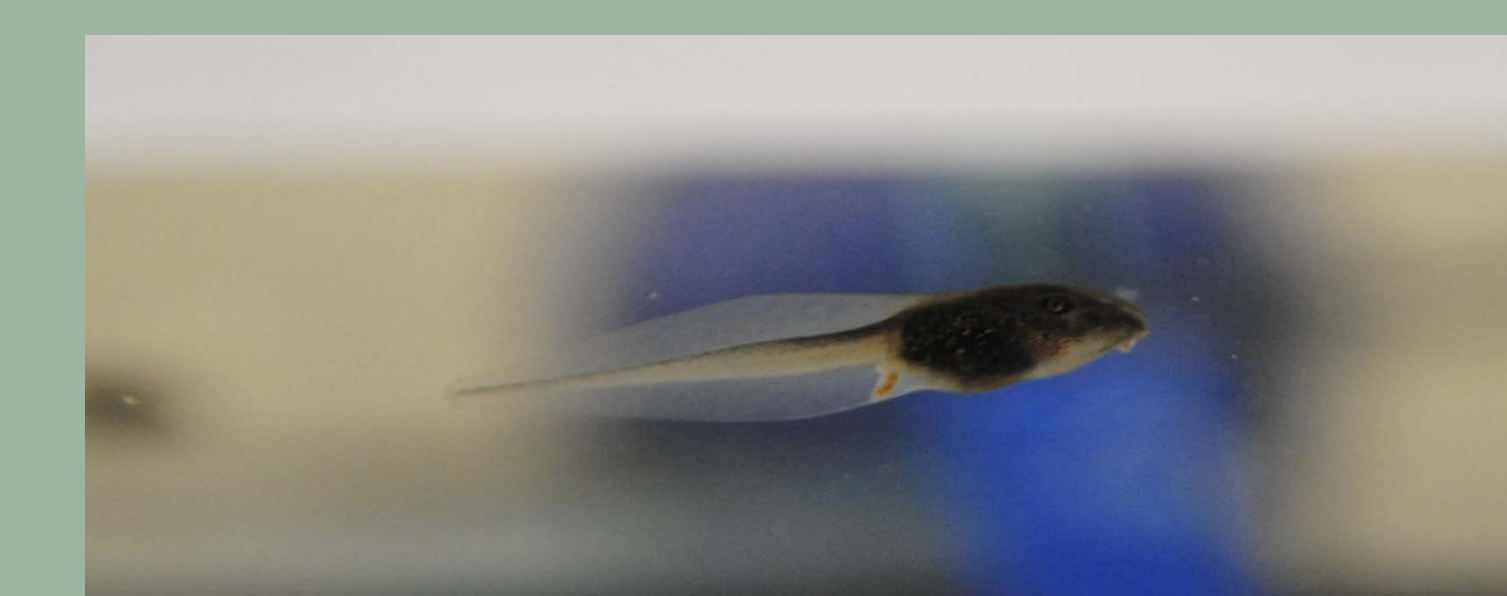
References and Acknowledgements

References

- Fioramonti, E., Semlitsch, R.D., Reyer H., Fent, K. 1997. Effects of triphenyltin and pH on the growth and development of *Rana lessonae* and *Rana esculenta* tadpoles. *Environ. Tox. and Chem.* 16(9):1940-1947.
- Rehage J.S., Lynn S.G., Hammond J. I., Palmer B.D., Sih, A. 2002. Effects of larval exposure to triphenyltin on the survival, growth, and behavior of larval and juvenile *Ambystoma barbouri* salamanders. *Environ. Tox. and Chem.* 21(4):807-815.
- Schriks M., van Hoom, M.K., Faassen, E.J., van Dam, J.W., Murk, A.J. 2006. Real-time automated measurement of *Xenopus laevis* tadpole behavior and behavioral responses following triphenyltin exposure using the multispecies freshwater biomonitor (MFB). *Aquatic Toxicology* 77:298-305.
- Semlitsch, R.D., Foglia, M., Mueller, M., Steiner, I., Fioramonti, E., Fent, K. 1995. Short-term exposure to triphenyltin affects the swimming and feeding behavior of tadpoles. *Environ. Tox. and Chem.* 14(8):1419-1423.

Acknowledgements

- All experimental procedures were approved by the University Committee on Animal Care and Supply (UCACS) at the University of Saskatchewan (Protocol #2010066). Collection of wood frog egg masses for scientific research was approved by the Saskatchewan Ministry of Environment (Permit #10FW059). The Environmental Toxicology Laboratory at the U of S is supported by a Canada Research Chair to Dr. John P. Giesy.



Picture 3: Close up of wood frog tadpole (left) and group of wood frog tadpoles (right).