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

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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?
What is the best timing of application?

www.purpleloosestrife.org/faq/
Amphibians

Who’s at risk?

Late June-August

www.frog-life-cycle.com/
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)
What are the effects of a triclopyr tank mix on metamorphic northern red-legged frogs?
Methods

• Tank mix

• 2 cm water
Methods

• Triclopyr tank mix:

**Renovate® 3**
Specialty Herbicide

**COMPETITOR®**
Modified Vegetable Oil

**HI-LIGHT®**
Industrial Strength Spray Pattern Indicator

47.1 ppm + 41.3 ppm + 12.9 ppm
Methods

Control (clean water)
Methods

Control (clean water)

Tankmix (clean water)

60-d growth out

X 15
Methods

- Endpoints

1. ![Image 1]
2. ![Image 2]
3. ![Image 3]
4. ![Image 4]
5. ![Image 5]
6. ![Image 6]

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

<table>
<thead>
<tr>
<th># Observations</th>
<th>Frogs</th>
<th>Legs sprawled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Tank mix</td>
<td>12</td>
<td>22</td>
</tr>
</tbody>
</table>

\[ P = 0.013 \]
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

![Bar chart showing days for control and tank mix with a t-test P = 0.031]
Results – body condition

- Body condition was not statistically different at 96 h

![Graph showing body condition over days with control and tank mix lines, and a t-test value of Δ P = 0.113]
Results – body condition

![Graph showing body condition over days for control and tank mix groups. At 30 days post-metamorphosis, body condition is significantly different between the two groups. Metamorphosis complete is indicated by an arrow and the graph.]
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

\[ t\text{-test } P = 0.942 \]
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
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- School of Aquatic & Fishery Sciences
- SePro
- Woodland Park Zoo
## Liver histology

<table>
<thead>
<tr>
<th>Lesion Severity</th>
<th>#Lesions per 20x field</th>
<th>Control</th>
<th>Tankmix</th>
<th>Total # frogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>mild</td>
<td>1-4</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>mild to moderate</td>
<td>1-7</td>
<td>5</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>moderate</td>
<td>5-7</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>moderate to severe</td>
<td>7-15</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>severe</td>
<td>10-15</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>