COMBINED EFFECTS OF VIRUS, PESTICIDE, AND PREDATOR CUE ON THE LARVAL TIGER SALAMANDER (*AMBYSTOMA TIGRINUM*)

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Introduction

- **Worldwide**, amphibian populations are rapidly declining with nearly one-third of all species threatened (IUCN, 2006). Emerging infectious diseases and environmental pollutants are two leading hypotheses for these unprecedented declines (Collins and Storfer, 2003).
- **We examined the effects of three stressors** (*Ambystoma tigrinum* virus (ATV), carbaryl and predator-cue) on larval tiger salamanders (*Ambystoma tigrinum*).
- **ATV** is an emerging infectious virus associated with mass mortality events of tiger salamanders across the western United States (Forson and Storfer, 2006).
- **Carbaryl** is a popular broad-spectrum pesticide used worldwide in both urban and agricultural settings (EPA, 2006).
- **Predation** is a ubiquitous natural threat often ignored in toxicological studies.

Hypothesis #1

- Both separately and in combination, carbaryl and predator cue will lead to increased mortality among larval tiger salamanders exposed to virus.

Hypothesis #2

- Both separately and in combination, exposure to virus, carbaryl and predator cue will have sub-lethal impacts on larval tiger salamanders.

Methods

- **Experiment Design**
  - 2 x 2 x 2 factorial design
  - 8 treatments with 15 replicates per treatment
  - Larvae individually housed, with 500 mL filtered aquifer water and a PVC pipe refuge
  - Total duration - 22 days
- **Virus Treatment**
  - Virus - *Ambystoma tigrinum* virus (ATV)
  - LC_{50} dose - 1 x $10^{3.5}$ pfu/mL
  - Introduced via water transmission on day 1
- **Predator Cue Treatment**
  - Predator - green darner dragonfly nymphs *(Anax junius)*
  - Predator cue water taken from container housing predators
  - Applied on days 1, 8, and 15 (once/week)
- **Pesticide Treatment**
  - Pesticide - carbaryl (commercial name - Sevin)
  - Carbaryl treatment dose - 500 ppm (μg/L)
  - Applied on days 1, 8, and 15 (once/week)
- **Behavioral Observations**
  - 3 days/week - total of 20 hours/week
  - 20 spot-checks per larva/week

Figure 1: Proportion dead in all treatments

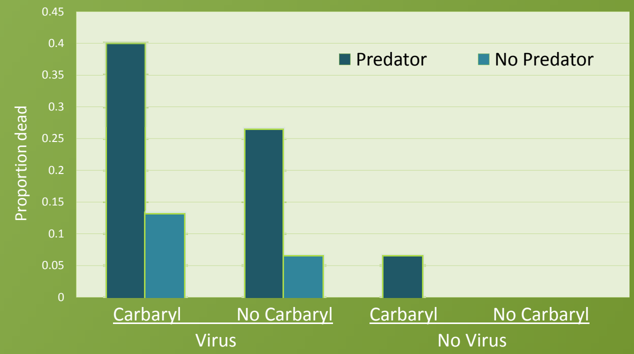


Figure 2: Snout-Vent Length

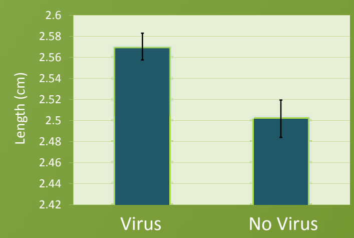


Figure 3: Developmental Stage

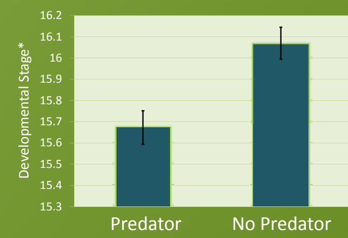
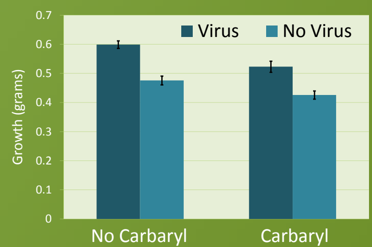
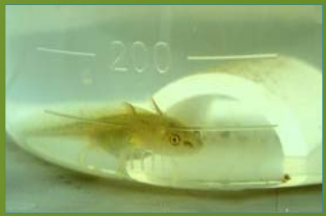


Figure 4: Growth of Surviving Larvae



Larva in treatment cup (near refuge)



Some of the symptoms of ATV include: edema, skin sloughing, and hemorrhaging

Figure 5: Activity level

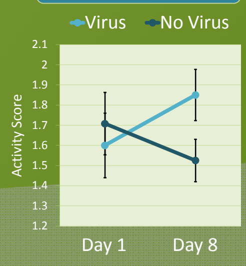
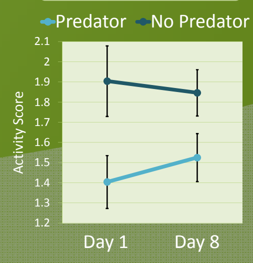


Figure 6: Activity level



Results

- **Lethal effects:**
  - **Significant effect** of virus (X^2 = 15.09, df = 1, p = 0.0001) and predator-cue (X^2 = 7.92, df = 1, p = 0.0049) on mortality, and a near significant effect of carbaryl (X^2 = 3.32, df = 1, p = 0.0683); Fig. 1
  - **Synergistic effect** on larval mortality exists between virus and predator-cue (X^2 = 4.19, df = 1, p = 0.0406); Fig. 1
- **Sub-lethal effects:**
  - **Snout-Vent Length:** increased in virus treatments ($F_{1,98}$ = 9.232, p = 0.003); Fig. 2
  - **Developmental Stage:** decreased in predator treatments ($F_{1,98}$ = 11.850, p = 0.001); Fig. 3
  - **Mass:** increased in viral treatments ($F_{1,98}$ = 44.574, p = 0.000) and decreased in carbaryl treatments ($F_{1,98}$ = 19.390, p = 0.000); Fig. 4
  - **Activity:** increased in virus treatments (day x virus interaction, $F_{1,112}$ = 3.968, p = 0.049) and decreased in predator treatments ($F_{1,112}$ = 6.441, p = 0.013); Figs. 5 & 6 (Nearly all infected larvae died by day 15 so activity data from week 3 was excluded from this analysis.)

Discussion

- **Hypothesis #1**
  - **Predator cue had a significant effect on mortality; Fig. 1**
  - Carbaryl separately and/or in combination with predator cue did not have a statistically significant effect on mortality (contrary to Relyea and Mills 2001); Fig. 1
  - **A synergistic relationship between ATV and predator cue on mortality is clearly evident; Fig. 1**
  - All larvae that died tested positive for ATV infection; no surviving larvae tested positive.
    - This suggests that predator cue and/or carbaryl alone may not directly cause mortality, but may increase larval susceptibility to ATV infection.
- **Hypothesis #2**
  - **ATV exposed larvae had significantly increased snout-vent lengths, growth rate, and activity levels; Figs. 2, 4 & 5**
    - Three possible explanations for the differences in length and growth rate:
      - (1) Smaller larvae were more likely to die from viral infection than larger larvae.
      - (2) Larvae grew faster to more fully develop their immune system.
      - (3) Larvae grew faster to expedite metamorphosis and escape the high-risk environment.
    - Increased activity levels may have resulted from ATV acting at the individual level to increase the conspecific contact rate, which may, in turn, increase disease transmission.
  - **Carbaryl significantly reduced growth rate of larvae; Fig. 4**
    - A reduction in growth rate could lengthen the time to metamorphosis potentially resulting in decreased larval survival since breeding ponds are highly ephemeral.
  - **Predator cue caused a significant decrease in developmental stage and activity level; Figs. 3 & 6**
    - Delayed development may be correlated with the decreased activity level, which likely reflects a reduction in foraging time due to the implied threat of predation.

Acknowledgements

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