Effect of chemical compounds from invasive species in amphibian diversity

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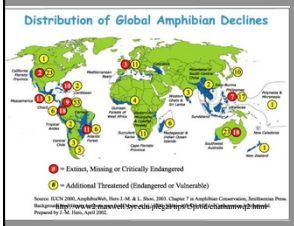
Introduction



Amphibian decline

- Factors:
  - Over-exploitation
  - Loss of habitat
  - Novel infectious diseases (Chytrid fungus and ranavirus)
  - Environmental factors such as pH, UV radiation,
  - Chemical contaminants, or invasive plants

Plant Metabolism

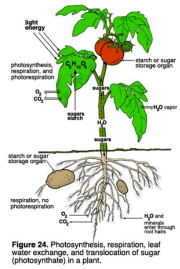


Figure 24. Photosynthesis, respiration, leaf water exchange, and translocation of sugar (photosynthate) in a plant.

Same as animals plant has high metabolic demands

Energy for plant metabolism comes from photosynthesis

Energy allocation and needs varies depending on season, environment, and type of plant

Compound build for an specific function is called **Metabolite**

Photosynthesis

Energy → Metabolites

- Enzymatic activity
- Growth
- Development

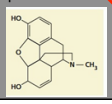
→ Essential for growth and reproduction

Metabolites → Secondary metabolites

- Non Essential for growth or development

→ Terpenes
→ Phenolic compounds
→ Nitrogen compounds Alkaloids

Large complex compounds
Expensive



Secondary metabolites

- Large organic compounds
- Function non related to plant growth or development
- Neurological effects in animals
- Function in protection from herbivores

Representative alkaloids

Energy Produced by plant

→ Metabolites
- Enzymatic activity
- Growth
- Development

Normally most energy is directed to essential functions

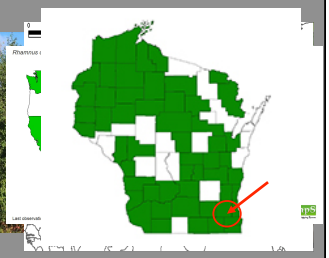
Energy is directed to protection

→ Secondary metabolites

Insect attacks or infestations can detour energy and stimulate production of secondary metabolites

Common buckthorn (*Rhamnus cathartica*)

- Mid size tree
- Introduced from Eu
- Currently distribute Canada
- Widely distribute i



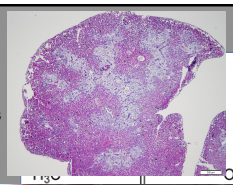
Common buckthorn (*Rhamnus cathartica*)

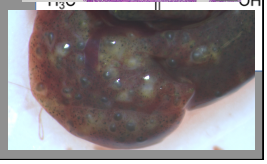
Defense substances → Competition → Allelopathic substances → Inhibit growth of neighboring plants

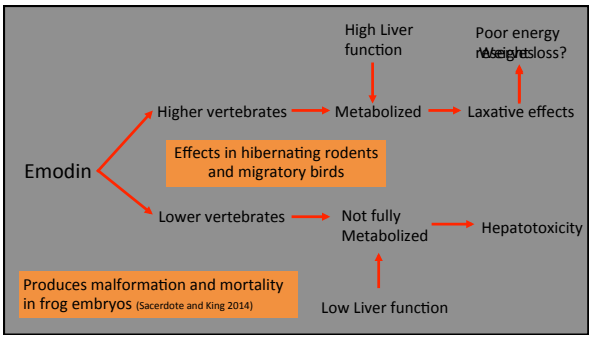
Defense substances → Herbivory → Secondary metabolites → Emodin

Emodin

- Used as a traditional Chinese medicine for over 2000 years
- wide spectrum of pharmacological properties
  - Anticancer
  - Hepatoprotective
  - Antinflammatory
  - antioxidant and antimicrobial activities.
- Must be metabolized in the liver
- Hepatotoxicity
  - Inflammation
  - Hepatocellular congestion
  - Necrosis
- Kidney toxicity

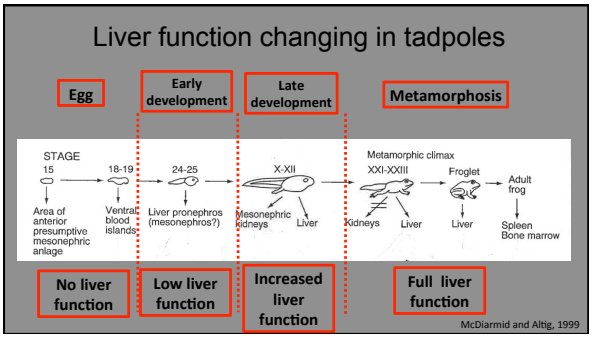


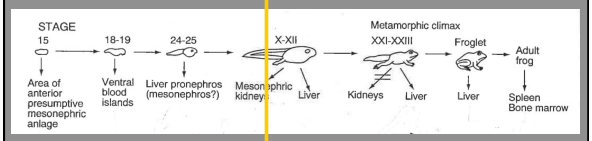




By-phasic life style

- Amphibians have a by-phasic life style
  - Part of their life as an aquatic organism
- During metamorphosis
  - Restructuration of vital organs
    - Kidney
    - Liver





Low or no liver or kidney function

Low Capacity to deal with complex compounds like Emodin

Produces malformation and mortality in frog embryos (Sacerdote and King 2014)

Higher to full liver or kidney function

Higher capacity to deal with complex compounds like Emodin

Can more developed tadpoles metabolize Emodin?

Greene Field Station, Waukesha, WI

- Diversity surveys at Field station and nearby Natural Area
  1) Two main communities expected
     - Temporal pool dwelling
     - Permanent pool dwelling
  2) Low diversity
  3) Only one abundant species
     - Frogs inhabiting temporal pools gone
     - Frogs inhabiting permanent ponds abund

Why some species remain and other disappear?

Figure 3. Number of individuals per species recorded at the Fen State Natural Area and the Greene Field Station (Genesee depot, WI)

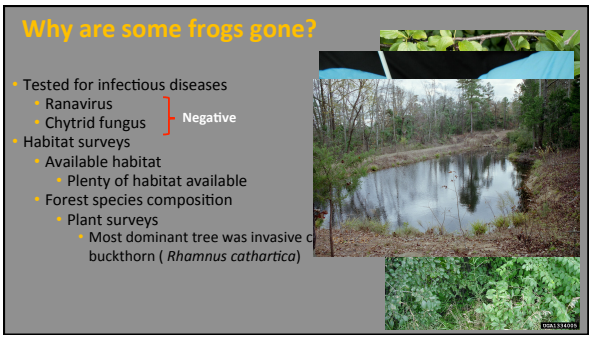
Green frogs (*Lithobates Clamitans*)

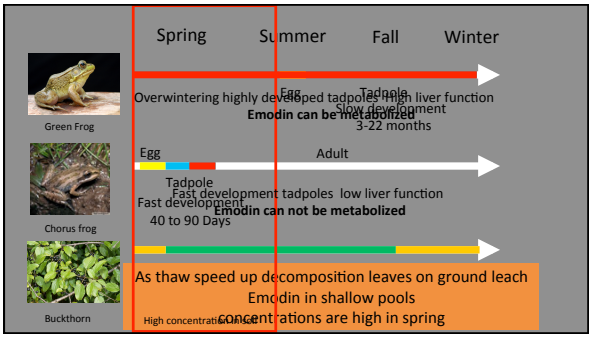
- Spring fed permanent pools
- Breeding in Fall
- Large tadpoles develop slowly
- 3-22 months

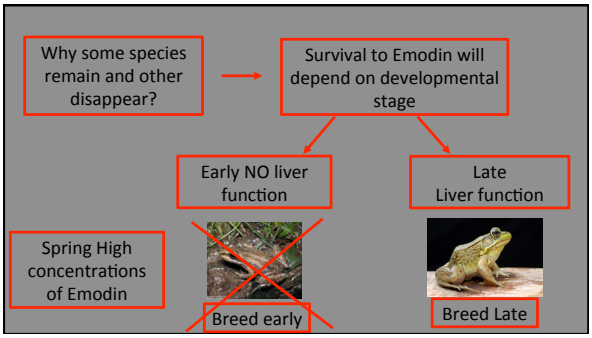
Chorus frogs (*Pseudacris maculata*)

- Shallow temporal pools
- Breeding in Spring
- Small tadpoles develop fast
- 40 to 90 Days

http://www.reptilesofaz.org/Turtle-Amphibs-Subpages/h-p-triseriata.html


Why are some frogs gone?
Tested for infectious diseases
Ranavirus
Chytrid fungus
Negative
Habitat surveys
Available habitat
Plenty of habitat available
Forest species composition
Plant surveys
Most dominant tree was invasive c
buckthorn (Rhamnus cathartica)


Spring
Summer
Fall
Winter
Green Frog
Egg
Tadpole
Slow development
3-22 months
Overwintering highly developed tadpoles High liver function
Emodin can be metabolized
Chorus frog
Egg
Adult
Tadpole
Fast development tadpoles low liver function
Fast development
Emodin can not be metabolized
40 to 90 Days
Buckthorn
As thaw speed up decomposition leaves on ground leach
Emodin in shallow pools
concentrations are high in spring


Why some species remain and other disappear?
Survival to Emodin will depend on developmental stage
Early NO liver function
Late Liver function
Spring High concentrations of Emodin
Breed early
Breed Late

Why some species remain and other disappear?

Survival to Emodin will depend time of exposure

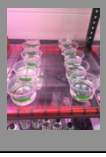
Early stages

Later stages

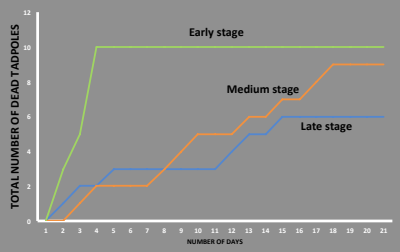
Tadpoles at later developmental stages would be able to withstand Emodin while the earlier stage tadpoles would succumb to the toxin.

lesion but will survive

- Exposed Green frog tadpoles of different developmental stages to emodin (0.5 ppm)
- complete randomized blocked design
- 20 replicates
  - Negative control
- 21 Days
- Necropsy
  - Liver
  - Kidney



Results



$F_{(3,17)}=342.3$, $P<0.01$

Differences in time of mortality among size classes

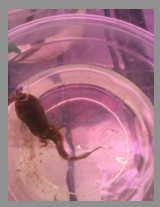
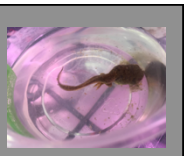
Pearson=0.595, $P<0.01$

Earlier stage animals died at a faster rate than later stage

Figure 1. Cumulative number of dead tadpoles when exposed to 0.5pmm of emodin in a 21-day control experiment. *Early development stage data was obtained from published data from Sacerdote *et al.* 2014

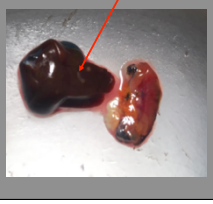
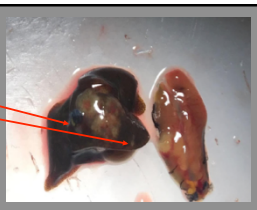
If tadpoles at later stages survive exposure to Emodin
What are the effects in the liver and kidney?

Tail deformations

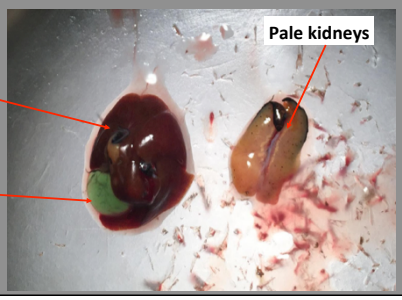

signs of metabolic detoxification

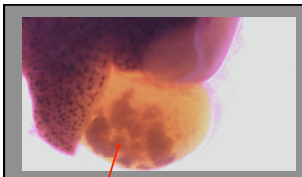
Hepatic cyst

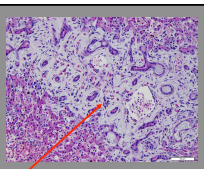



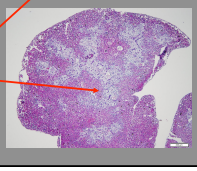
Typical in detoxification of alcohols

signs of metabolic detoxification



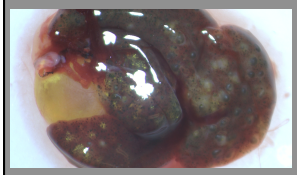


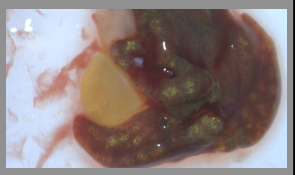




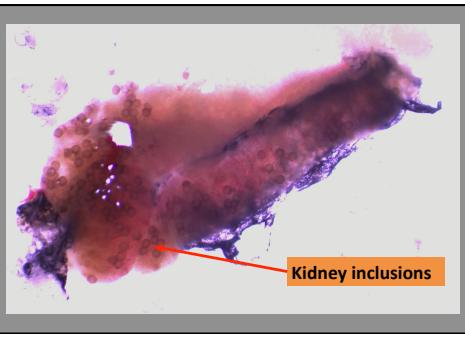
Sediment in gallbladder

Fibrosis in cystic bile duct





Emodin trapped in liver



What do we know?

- Tadpoles at later stages with higher liver function can metabolize emodin
  - Histopathological signs of metabolite synthesis are consistent with reports for mammals (Chen et al., 2014; Ma et al., 2016)
  - Survival of tadpoles will depend on time of exposure
    - Early development with low liver function are more likely to die
    - Later development with higher liver function are more likely to survive

Can Emodin Amphibian Metamorphosis

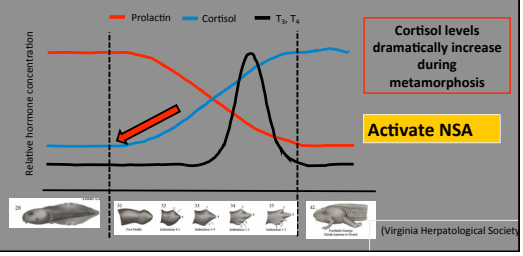
Amphibian Metamorphosis

- Time and speed can be affected by biotic and abiotic factors
- High stress = increased rate of metamorphosis
- Hormones:
  - Thyroid hormones T_3 and T_4
  - Prolactin
  - Corticosterone (corticotropine-releasing factor)

Feeding-Growing Stage		Start of Metamorphosis
High prolactin	→	Low prolactin
Low corticosterone	→	High corticosterone
Low T_3 and T_4	→	High T_3 and T_4

(Denver, 1997; Riley and Moudley, 2012; Lorenzi et al., 2009; Werner, 1996; Cabrera-Guzman et al., 2013; Wells, 2007; Ambulgery et al, 2006; Walne et al., 2011; Harvey et al., 1997; Newman, 1998; Morey and Reznick, 2000; Morey and Reznick, 2001; Doughty and Roberts, 2003)

Hormone regulation on tadpole metamorphosis



Our Objective

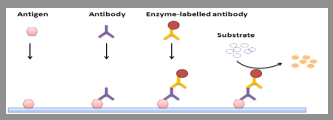
- To determine if emodin (toxin) will induce stress and result in early metamorphosis of tadpoles
- Our hypothesis:
  - Emodin (stress) → activation of NSA → propel metamorphosis
  - More emodin (more stress) → earlier activation of NSA and faster rate of metamorphosis

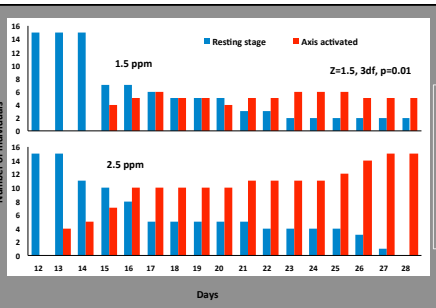
Methods: Set-Up

- Trials: 24 days
- 18 tadpoles in 1 Liter containers
  - Control group (6): dechlorinated water
  - Experimental Groups:
    - 1.5 ppm (6)
    - 2.5 ppm (6)
- Feeding and water changes every other day
  - Tadpole diet

Methods: Chemical Data Collection

- Corticosterone levels among the three groups
- Plasma collection via centrifuging
  - Pithing followed by blood collection from heart
- Competition ELISA to determine corticosterone levels





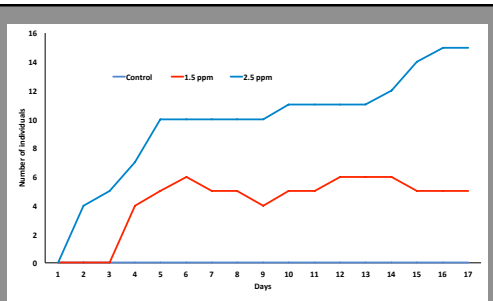
Red = starts metamorphosis

*2.5ppm group started metamorphosis 5 days faster

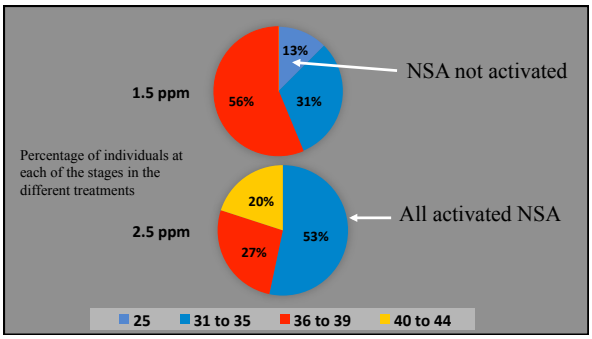
*2.5ppm: ratio of blue and red changes fast

*2.5ppm has faster rate of metamorphosis

Relationship between emodin concentration and number of individuals going through metamorphosis



Number of individuals going through metamorphosis



Discussion

- **Emodin is a source of stress**
  - Stress activates the NSA
    - Increase of corticosterone, T_3 and T_4
  - Endocrine disruption induces early metamorphosis
    - Tadpoles come out of the water early
  - **Consequences of earlier onset of metamorphosis:**
    - Lowered fitness, survivorship, etc.
- Higher concentrations (2.5ppm) induce faster activation of the NSA
- ELISA assay:
  - Corticosterone levels found to increase from start to middle of metamorphosis

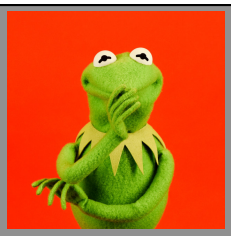
(Hayes et al, 2006; Hayes et al., 2010; Relyea, 2004; Amburgey et al., 2016; Boone et al.; McDiarmid and Altig, 1999)

So what?

- The secondary metabolite Emodin produced by the common Buckthorn could be responsible of the decline of spring breeding species observed at the Greene field station
- Similar patterns of spring breeding species decline is observed across Southeastern Wisconsin
- Late stage tadpoles would have better chance to survive because of liver function
- Exposure to Emodin can cause liver and kidney damage
- Exposure to Emodin can cause stress resulting in the activation of the **Neuroendocrine-stress axis (NSA)** resulting in early metamorphosis

What's next?

- What are the effects of liver and kidney damage in juvenile and adult survivorship?
- What is the effect of Emodin in othe vertebrate species?



Questions?

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