Influences of Cattle Grazing on Amphibians

Elizabeth Burton and Chandler Schmutzer
University of Tennessee
Department of Forestry, Wildlife and Fisheries

Amphibian Decline
- Climate changes
  - Global warming
  - UV-B rays
- Invasive species
  - Competition / Predation
    - e.g., Rana catesbeiana in the west
- Water contaminates
  - Pathogens
    - *Aeromonas hydrophila* - *red leg*
    - Chytridiomycosis
    - Iridoviruses
    - *Ribeiroia*
Amphibian Declines and Anthropogenic Stressors

- Habitat Destruction
  - Urbanization
  - Silviculture
  - Agriculture
  - Roads

![Graph showing North America with a timeline from 1960 to 1996 with data points]

<table>
<thead>
<tr>
<th>Biological Conservation</th>
<th>Biotropica</th>
<th>Science</th>
<th>Nature</th>
</tr>
</thead>
</table>

Influences of Cattle on Amphibians

- **Previous research**
  - Healey et al. 1997, Jansen and Healey 2002 - Australia
    - Correlate amphibian abundance with wetland characteristics
    - Suggest cattle indirectly negatively affect amphibians
    - Compare abundance of Columbia Spotted frogs in grazed and ungrazed areas
    - Found no differences in abundance between treatments
  - Knutson et al. 2004 - Minnesota
    - Evaluate agricultural wetlands for value as amphibian habitat
    - Species richness and abundance of some species lower at grazed wetlands

- **Influences of Cattle on Amphibians**
  - Grazing vegetation
    - Vegetation Structure
    - Detritus
  - Trampling egg masses
    - Affects demographics at later life stages
  - Soil Compaction
  - Water Quality
Influences of Cattle
Decrease in Water Quality

- Increases in ammonia, nitrite, nitrate, and phosphate
- Decrease in Dissolved Oxygen
- Change in algal community
- Change in invertebrate composition

Nutrients
Eutrophication

Decrease in growth and survivorship

Planorbid snail

Decrease Water Quality
Increase Stress

Immunocompromised Individual
Mortality

Pathogens
- Ranaviruses (FV3)
- Chytrid fungus
- Aeromonas hydrophila
- Parasites

Pathogen Prevalence
Anthropogenic stressor

Ribeiroia Life Cycle

Influences of Cattle

Bird host
Adult Ribeiroia
Planorbellla snail sp.
Cercaria

Life Cycle
Amphibians and Cattle in Tennessee

Quick Facts

- 40% of land area farmland
- 57% cattle production
- 48,000 cattle operations
- 9th in nation in beef cattle use
- Value of cattle $1.67 billion

Amphibian richness highest in the southeast

- 44 anurans
- 84 caudates

In Tennessee

- 21 anurans
- 45 caudates

Most studies occur out west and along streams


Justification

- It has been reported that cattle negatively affect emergent vegetation and water quality and thus could potentially affect resident amphibians.
- Cattle could potentially increase pathogen occurrence.
- The effect of cattle on adults has rarely been quantified.
- There are no replicated studies for larval amphibians.
- No studies documented in the Southeast, specifically Tennessee.

Study Area

University of Tennessee Plateau Research and Education Center

Crossville, TN

4 Access wetlands
Access > 10 years

4 Non-access wetlands
Never had access

28 March-26 August 2005
Size range 0.153-1.29 ha

27 March-25 August 2006
All ponds have fish
Larval Sampling Design
Objective 1. Determine the influence of cattle on larval amphibian abundance and
richness

- Four Quadrants
- 2 Techniques
- Seine Plots
- Dip Net Transects

- 4.5 m in length
- Dip site every 1.5 m
- 3 sweeps at each dip site

- 10 x 3 m permanent plot
Larval Sampling

Objective 1. Determine the influence of cattle on larval amphibian abundance and richness

- Larvae caught
  - Counted
  - Identified
  - First 5 larvae per species
    - Gosner stage (1960) recorded
    - Measure BL and TL
    - Weighed
  - Any fish and invertebrates caught counted and identified

Water Quality Sampling

Objective 2. Determine the influence of cattle on water quality

- 2.5 m from shore
- Water variables measured at sampling location:
  - Conductivity, temperature, pH and dissolved oxygen
  - Turbidity
- Water collected from sampling location measured for:
  - Ammonia, nitrite, nitrate and phosphate

Invertebrate Sampling

Objective 3. Determine the influence of cattle on macroscopic filamentous algae, detrital biomass and aquatic invertebrate abundance.

- Each sample taken at a 0.5 m depth
- A cylinder (0.25-m²) placed in sample area
- All contents collected using a dip net (20 cm x 20 cm)
- Contents collected and transported on ice in plastic bags back to UT
- Once per month
- Measured at one location in two opposing quadrants in each wetland
Filamentous Algae, Detritus and Invertebrate Sampling

Objective 3. Determine the influence of cattle on macroscopic filamentous algae, detrital biomass and aquatic invertebrate abundance.

- All samples sorted
- Algae and detritus separated and dried at 80 °C for 48 hours
- Weighed and dry mass recorded
- All invertebrates identified to family

Pathogen Sampling

Objective 4. Determine the influence of cattle on the presence of pathogens (viruses, bacteria and parasites) in larval communities.

- Pathogens measured
  - Winter-February 15th 2005
  - Summer-June 15th 2005
  - Fall-October 12th 2005
- 2 species
  - Bullfrog (Rana catesbiana)
  - Green frog (Rana clamitans)
- Larvae collected opportunistically
  - 5 individuals per species per wetland

Pathogen Processing Methods

Objective 4. Determine the influence of cattle on the presence of pathogens (viruses, bacteria and parasites) in larval communities.

- Transported back to UT
- Benzocaine hydrochloride
- Body mass and length, development stage
  - Gosner 1960
- Fixed and fresh tissues
- UGA Veterinary Diagnostic and Investigational Laboratory
Pathogen Sampling

Objective 4. Determine the influence of cattle on the presence of pathogens (viruses, bacteria and parasites) in larval communities.

FV3 Identification Techniques:
- Virus isolation
- Electron microscopy
- PCR

Processing Captured Individuals
- Measure (SVL)
- Weigh
- Tag-VIA tags®
- Mark-Toe clipping
Methods

Breeding Call Surveys

Objective 1: Determine the influence of cattle on species richness and relative abundance of postmetamorphic amphibians

Surveys followed North American Amphibian Monitoring Program (NAAMP) protocol

- 2 survey durations
  - 6 minutes (0:0-0:06)
  - 10 minutes (0:0-0:10)
- 2 Permanent listening stations
- Began ≥30 minute after sunset
- Upon arrival waited 1 minute
- Species occurrence and ranked abundance

Methods

- Ranking species-specific abundance
  - 1 = individuals can be distinguished and calls do not overlap
  - 2 = individuals can be distinguished and calls do overlap
  - 3 = full chorus (individuals cannot be distinguished and calls do overlap)

Methods

Sampling Techniques

Objective 2: Determine the influence of cattle on egg mass abundance
Methods
Sampling Techniques

Objective 4: Determine the influence of cattle on pathogen and malformation prevalence in amphibians

Pathogen prevalence
- 5 metamorphs *Rana clamitans* collected from each wetland on June 15, 2005
- Individuals euthanized via transdermal exposure to benzocaine hydrochloride
- Comprehensive histological and parasitological analysis of tissue samples performed at the Tifton Veterinary Diagnostic and Investigational Lab
- Bacteria, viruses, parasites and other pathogens

Trematode prevalence
- Malformed individuals opportunistically collected
- Malformation classified using USGS Field Guide to Malformations of Frogs and Toads
- Humanely euthanized via transdermal exposure to benzocaine hydrochloride
- Fixed in 10% buffered formalin and Cleared
- Light microscopy used to detect presence of encysted trematode metacercariae
Statistical Analyses

Repeated Measures ANOVA: Amphibians
- **Response:** Relative Daily Abundance
- **Effects:** Access Treatment, Month
  - Two-sample T-tests (Trt*Month, P<0.1)

Repeated Measures ANOVA: Egg Mass
- **Response:** Mean Total Abundance
- **Effect:** Access Treatment, Month

Statistical Analyses

Repeated Measures ANOVA: Vegetation
- **Response:** Mean Vegetation Structure
  - Vegetation Variables: Percent Vertical & Horizontal Cover, Height
- **Effects:** Access Treatment, Month

Two Sample Z-test:
- **Pathogens and Malformations**

Results

Larval Abundance

- RACA 2005: 2.9X greater
- RACL 2006: 5X greater

All other p ≥ 0.11

**Analysis**
- SAS®
- Repeated measures ANOVA
Results
Larval Diversity and Richness

Analysis
Diversity = Shannon-Weiner Diversity Index Algorithm
Repeated Measures ANOVA

Results
Species Composition

Analysis
\( \alpha = 0.10 \)
SAS®

\( p < 0.05 \)

\( p > 0.13 \)

Results
Larval Species Captured
**Results**

**Fish Composition**

<table>
<thead>
<tr>
<th>Fish Species</th>
<th>Access (%)</th>
<th>Non-access (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF</td>
<td>0.22</td>
<td>0.08</td>
</tr>
<tr>
<td>RE</td>
<td>0.70</td>
<td>0.27</td>
</tr>
<tr>
<td>RB</td>
<td>0.36</td>
<td>0.34</td>
</tr>
<tr>
<td>MF</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>LMB</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>HATCH</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>GS</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>BG</td>
<td>0.50</td>
<td></td>
</tr>
</tbody>
</table>

**Water Quality**

- **DO = 28.2% > non-access (2006)**
- **SPCOND = 67.8% > in access (2005)**
- **TURB = 3.7X > access (2005)**
  - **3.5X > access (2006)**
- **All other water quality variables**
  - **NH3, NO2, NO3 and TEMP**
  - **Higher in access**

**Analysis**

Repeated Measures ANOVA, p≤0.10 SAS®
Results
Algae and Detritus

![Graph showing comparison between Algae and Detritus biomass.

**Analysis**
Repeated Measures ANOVA

\[ \alpha = 0.10 \]

SAS®

Results
Invertebrate Abundance

![Graph showing invertebrate abundance with taxa.

**Analysis**
Repeated measures ANOVA

\[ \alpha = 0.10 \]

SAS®

All other p-values were \( p \geq 0.11 \)

Aquatic Invertebrates Captured

- Dragonfly larvae
- Damselfly larvae
- Small Squaregill
- Physid Snail
- Planorbid snail
- Non-Biting Midge Larvae
- Aquatic worm
- Pea Clam
- Leech
Results
Regression Model

\[ BUFO = 0.098 \text{(TURB)} - 5.076 \text{(NH3)} \]
\[ RAPA = 0.004 \text{(DIFISH)} \]

\[ 2006 \]
\[ RACA = 0.393 \text{(OFISH)} \]
\[ RACL = -0.026 \text{(SPCOND)} + 0.556 \text{(SR)} \]
\[ RAPA = 0.001 \text{(PREDF)} \]

standardized coefficients presented

Results
Pathogen Prevalence

Treatment

Analysis
Logistic Regression and Maximum Likelihood Estimation

Pathogen Prevalence
Seasonal Effects

Analysis
Logit and Logistic Regressions and Maximum Likelihood Estimation
Results
Developmental Stage Effect
Bullfrog

- 25% Decrease in the Predicted Odds of Infection with each unit increase in Gosner stage.

- FV3 Prevalence

- Gosner Stage (1960)

- Bullfrogs n = 102 tadpoles

- *P*=0.005

Results
Developmental Stage Effect
Green Frog

- No detectable trend.

- FV3 Prevalence

- Gosner Stage (1960)

- Green Frogs n = 80 tadpoles

- *P*=0.872

Summary of Results

- Larval Abundance, Richness and Diversity
  - Bullfrog and green frog abundance was greater in non-access.
  - Species richness was greater in non-access wetlands
  - No significant difference in species diversity

- Water Quality
  - Specific conductivity and turbidity were higher and dissolved oxygen lower in cattle-access wetlands
  - No significant difference in other water quality variables

- Detritus and Algae
  - Detritus was greater in non-access wetlands
  - No significant difference in algae biomass between treatments
Summary of Results

- **Invertebrates**
  - Dragonfly larvae abundance was greater in non-access.
  - Aquatic worm abundance was greater in cattle-access.

- **Regression Model**
  - Specific conductivity explained 82% of variation in green frog larval abundance.
  - Other fish (non-predators) explained 73% of variation in bullfrog larval abundance.

- **FV3**
  - Green frog larvae were more likely to be infected with FV3 in cattle-access wetlands.
  - FV3 prevalence was higher in cooler months for both species.
  - As development progressed FV3 prevalence decreased in American Bullfrog larvae.

Discussion

- **Larval Abundance**
  - It was documented that cattle access wetlands negatively impacted *American bullfrog* and *green frog* tadpole populations.
  - Water quality and fish abundance were important predictors of abundance.
  - It appears that *American* and *Fowler’s toad* tadpoles were not negatively impacted by cattle access.
  - Higher resistance to water quality.
  - Exploitation of habitat where there is lower abundance of ranids.

- **Detritus and Algae**
  - Detritus > in non-access wetlands.
  - Indirect effects from lack of grazing pressure.
  - Provided better habitat for ranids.
  - Algae trend toward being > in cattle-access wetlands.
  - Trend toward higher nutrients.

- **Invertebrates**
  - LIBE – somewhat tolerant.
  - OLIG – tolerant to water pollution.
  - In General.
  - More snails in cattle-access.
  - Slight change in composition – difference in abundance.
  - More “sensitive” species in non-access wetlands.
**Discussion**

- **Water quality**
  - Cattle negatively impacted water quality
    - Reducing in water quality can increase mortality of amphibian eggs and larvae
    - Induces stress, making them more susceptible to pathogens
    - **Boyer and Grue 1999**
    - **Carey et al. 1999**

**Discussion**

- **Pathogen Prevalence - Frog Virus 3 (FV3)**
  - **Water quality**
    - Effect on green frog survival
    - Potentially compromised immunity
  - **Seasonal Effects**
    - Low temperatures increase pathogen prevalence
    - Low temperatures cause a decrease in overall immune function
    - **Maniero and Carey 1997**
  - **Developmental Stage**
    - Immunity could increase in bullfrogs
    - Susceptible tadpoles at earlier stages experienced mortality
    - **Brunner et al., 2004**
    - **Gosner Stage (1960)**
Desmognathus ocoee
Pseudotriton montanus
Notophthalmus viridescens
Ambystoma talpoideum
Plethodon glutinosus

Results
Postmetamorphs
Species Specific Relative Abundance of Pitfall Trap Captures

8.4X in 2006  
2.3X in 2005  
P=0.06  
P=0.19

Results
Postmetamorphs
Species Specific Relative Abundance of Pitfall Trap Captures

9.8X in 2006  
2.5X in 2005  
P=0.06  
P=0.16

Green Frogs
Age/Sex Class

Results
Postmetamorphs
Species Specific Relative Abundance of Pitfall Trap Captures
Results

- Species Richness did not differ between treatments
- Species Diversity did not differ between treatments

Species Composition

Egg Masses

Results

Vegetation Responses

Access

Non-access

Percent Vertical Structure

84%
84%
2006
2005

Percent Horizontal Cover

25%
25%
Results: Pathogens
- No differences in bacterial, viral or parasitic prevalence between treatments
- 35 malformed individuals
- 2% malformation rate
- 11 Malformation types

<table>
<thead>
<tr>
<th>Malformation Type</th>
<th>Access</th>
<th>Non-Access</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amelia Anophthalmia</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Polydactyly</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Brachydactyly</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Polymelia</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Trematode metacercariae</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Access Non-Access

Treatment Percent Malformations

Access 41% Non-Access 59%
P = 0.14

No differences in bacterial, viral or parasitic prevalence between treatments

Summary of Results
- Green frog metanmorphs are negatively affected by cattle presence
- Vegetation structure, horizontal cover and height are reduced in cattle access wetlands
- Egg mass abundance did not differ between treatments
- Prevalence of pathogens and malformations did not differ between treatments

Discussion
- Vegetation Structure:
  - Breeding sites
  - Foraging and escape cover
  - Egg deposition
  - Jansen & Healey (2003), Healey et al. (1997)
- Green Frog Metamorphs driving the trends
  - Higher Abundance
  - More sensitive to disturbance
  - Heavily impacted by vegetative cover and water quality
- Toads- going against the trend
  - Reduced Competition / Out competing other species
  - Better suited to areas with less vegetation
  - Resistance, movement
  - Can withstand lower water quality than other species
**Discussion**

- **Water Quality**
  - Ammonia
  - Turbidity
  - Specific Conductivity
  - Cattle Access

- Reduced immunocompetence increased FV3 prevalence
- Tadpoles affect later demographic stages

**Modeling Postmetamorphic Amphibian Abundance**

Environmental Cofactors: vegetation, water quality, cattle density, tadpole abundance

<table>
<thead>
<tr>
<th>Year</th>
<th>Formula</th>
<th>$R^2_{adj}$</th>
<th>% Explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>$0.0002(Cattle) - 0.39(NO2) - 0.0002(Turbidity) - 0.03(P04)$</td>
<td>0.79</td>
<td>82% = Specific Conductivity</td>
</tr>
<tr>
<td>2006</td>
<td>$0.0004(\text{Turbidity}) - 0.0002(\text{NH3}) + 0.002(\text{Temperature})$</td>
<td>0.99</td>
<td>90% = Turbidity</td>
</tr>
<tr>
<td>2006</td>
<td>$0.0001(\text{Turbidity}) - 0.006(\text{NH3}) + 0.002(\text{Temperature})$</td>
<td>0.99</td>
<td>90% = Turbidity</td>
</tr>
</tbody>
</table>

**Conservation Implications**

- Cattle grazing may be contributing to amphibian declines
- Separation of cattle and amphibians
- Providing alternative food and water sources

**Acknowledgements**

**Funding:**
- UT Dept. of Forestry, Wildlife and Fisheries
- Tennessee Wildlife Resources Agency

**Assistance:**
- Walt Hitch
- PREC Staff
- Volunteers