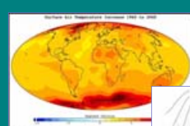
Influences of Cattle Grazing on Amphibians

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
    - Ie. *Rana catesbeiana* in the west
- **Water contaminates**

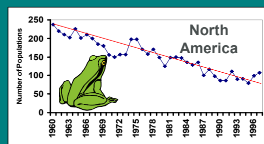


Amphibian Decline

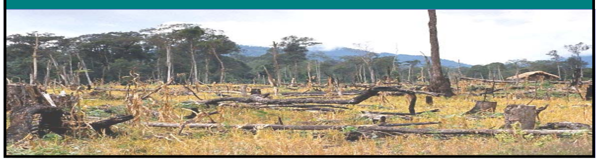
- **Pathogens**
  - *Aeromonas hydrophila* - “red leg”
  - Chytridiomycosis
  - Iridoviruses
  - *Ribeiroia*

Amphibian Declines
and Anthropogenic Stressors

- **Habitat Destruction**
  - Urbanization
  - Silviculture
  - Agriculture
  - Roads



Biological Conservation 125: 271-285 | **Biotropica 37:163-165** | **Science 306:1783-1786** | **Nature 404:752-755**



Influences of Cattle on Amphibians

Previous research

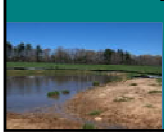
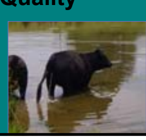
Healey et al. 1997, Jansen and Healey 2002 -Australia
- Correlate amphibian abundance with wetland characteristics
- Suggest cattle indirectly negatively affect amphibians

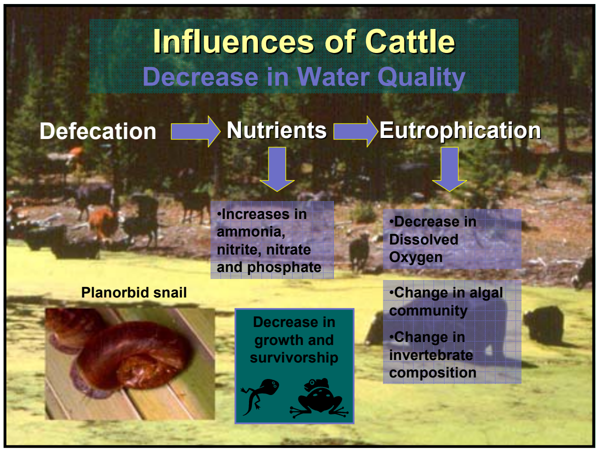
Bull et al. 2001, Bull and Hayes 2000 -Oregon
- Compare abundance of Columbia Spotted frogs in grazed and ungrazed areas
- Found no differences in abundance between treatments

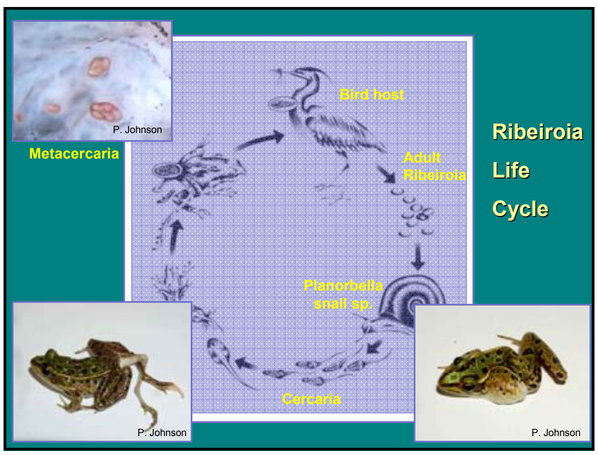
Knutson et al 2004- Minnesota
- Evaluate agricultural wetlands for value as amphibian habitat
- Species richness and abundance of some species lower at grazed wetlands

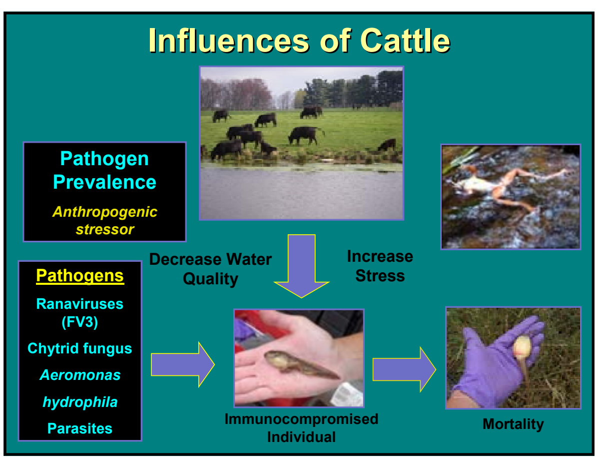
Influences of Cattle on Amphibians

- **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
Defecation
Nutrients
Eutrophication
•Increases in ammonia, nitrite, nitrate and phosphate
•Decrease in Dissolved Oxygen
Planorbid snail
Decrease in growth and survivorship
•Change in algal community
•Change in invertebrate composition


P. Johnson
Metacercaria
Bird host
Adult Ribeiroia
Ribeiroia
Life
Cycle
Planorbella snail sp.
Cercaria
P. Johnson
P. Johnson


Influences of Cattle
Pathogen Prevalence
Anthropogenic stressor
Pathogens
Ranaviruses (FV3)
Chytrid fungus
Aeromonas hydrophila
Parasites
Decrease Water Quality
Increase Stress
Immunocompromised Individual
Mortality

Amphibians and Cattle in Tennessee

Quick Facts

Most studies occur out west and along streams

(Belsky et al. 1999, Line 2002 Jansen and Healey 2003)

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

NASS and USDA

- Amphibian richness highest in the southeast
  - 44 anurans
  - 84 caudates

Bailey et al. 2006

- In Tennessee
  - 21 anurans
  - 45 caudates

Redmond and Scott 1996

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

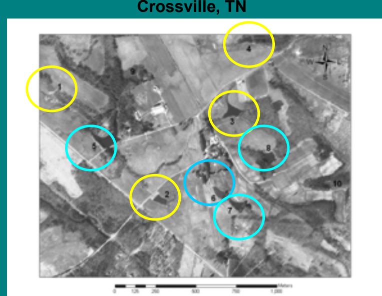
Crossville, TN

4 Access wetlands

Access > 10 years

28 March- 26 August 2005

Size range 0.153-1.29 ha



8 Wetlands

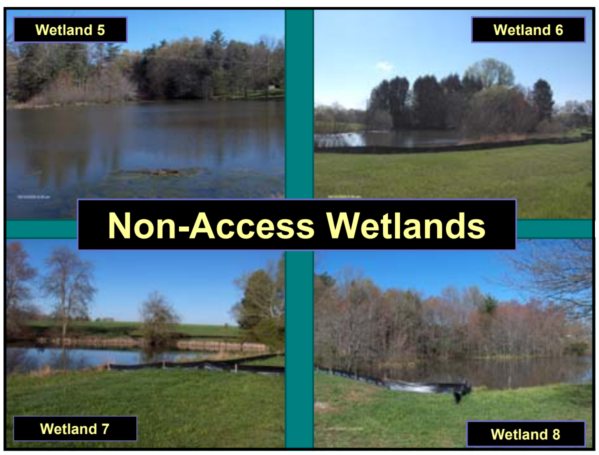
4 Non-access wetlands

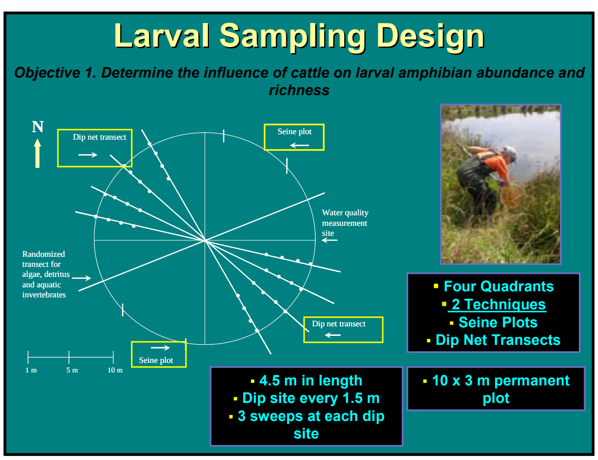
Never had access

27 March- 25 August 2006

All ponds have fish


Wetland 1
Wetland 2
Access Wetlands
Wetland 3
Wetland 4

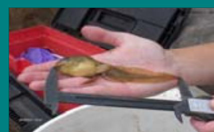

Wetland 5
Wetland 6
Non-Access Wetlands
Wetland 7
Wetland 8

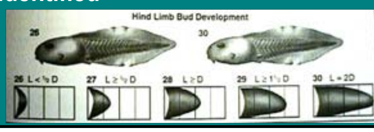

Larval Sampling Design
Objective 1. Determine the influence of cattle on larval amphibian abundance and richness
N
Dip net transect
Seine plot
Water quality measurement site
Randomized transect for algae, detritus and aquatic invertebrates
Dip net transect
Seine plot
1 m
5 m
10 m
▪ Four Quadrants
▪ 2 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

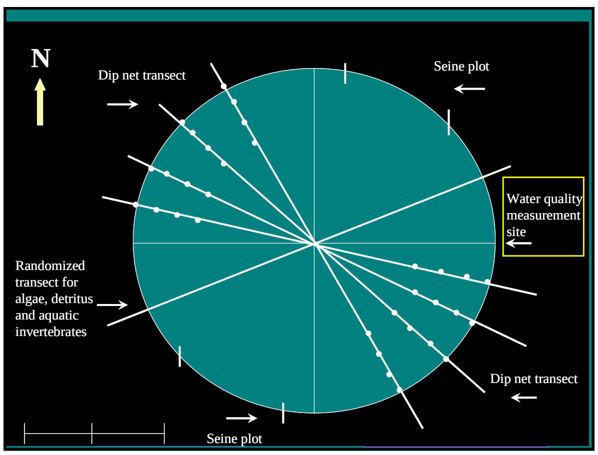
Objective 1. Determine the influence of cattle on larval amphibian abundance and richness

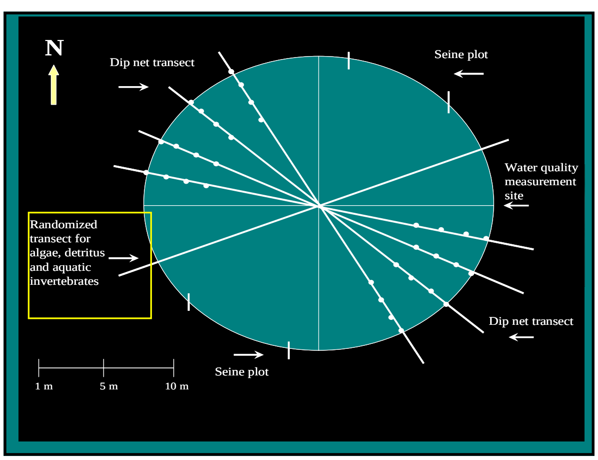
- Larvae caught
  - Counted
  - Identified
  - First 5 larvae per species
    - Gosner stage (1960) recorded
    - Measure BL and TL
    - Weighed
- Any fish and invertebrates caught counted and identified





Gosner (1960)





Filamentous Algae, Detritus and Invertebrate Sampling

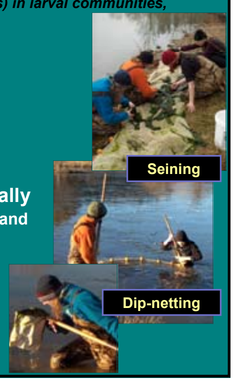
Objective 3. Determine the influence of cattle on macroscopic filamentous algae, detrital biomass and aquatic invertebrate abundance.

- 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

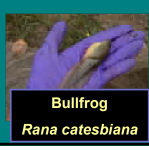
Objective 4. Determine the influence of cattle on the presence of pathogens (viruses, bacteria and parasites) in larval communities,

- 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



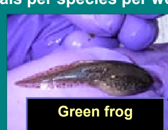
Seining

Dip-netting



Bullfrog

Rana catesbiana



Green frog

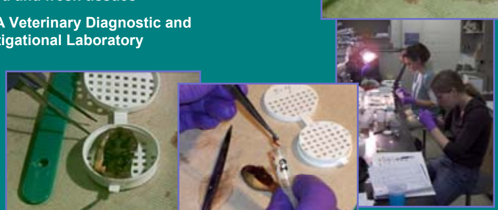
Rana clamitans

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

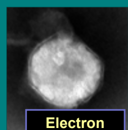
  Gosner 1960
- Fixed and fresh tissues
- UGA Veterinary Diagnostic and Investigational Laboratory



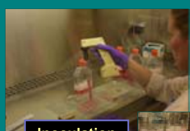
Pathogen Sampling

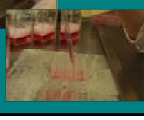
Objective 4. Determine the influence of cattle on the presence of pathogens (viruses, bacteria and parasites) in larval communities,

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

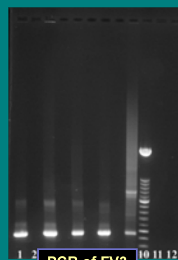


Electron microscopy of FV3

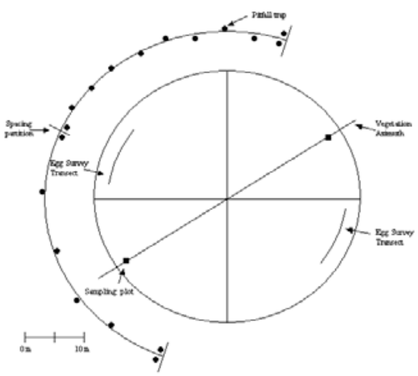




Inoculation into cell lines

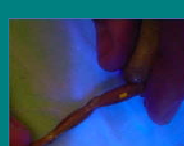


PCR of FV3



Processing Captured Individuals

- **Measure (SVL)**
- **Weigh**
- **Tag-VIA tags®**
- **Mark-Toe clipping**



Methods

Breeding Call Surveys

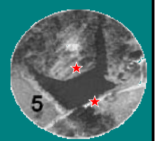
Objective 1. Determine the influence of cattle on species richness and relative abundance of postmetamorphic amphibians

Surveys followed North American Amphibian Monitoring Program (NAAMP) protocol

- 2 survey durations
  - 5 minutes (0-5:00)
  - 10 minutes (0-10:00)
- 2 Permanent listening stations

Observers did not share survey results

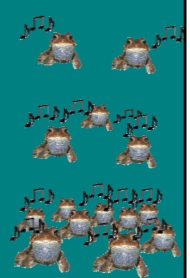
Exposed to the same chorus

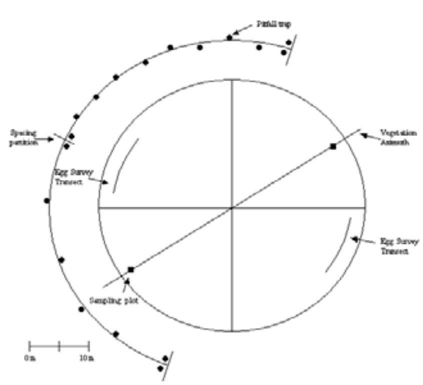


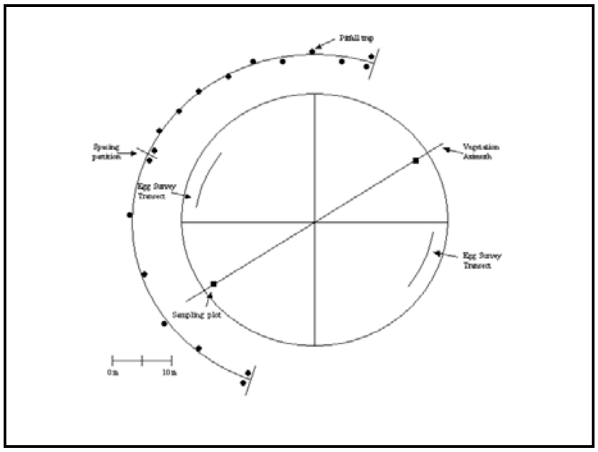
- Began ≥30 minute after sunset
- Upon arrival waited 1 minute
- Species occurrence and ranked abundance

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)





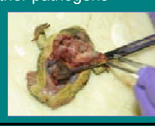


Methods

Sampling Techniques

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

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

Methods

Sampling Techniques

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

- 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

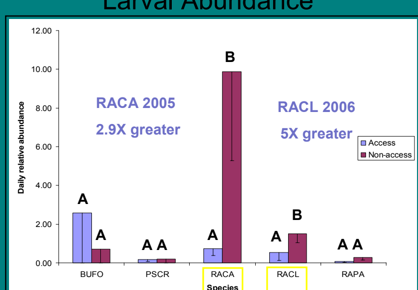
- Response: Mean Vegetation Structure
  - Vegetation Variables: Percent Vertical & Horizontal Cover, Height
- Effects: Access Treatment, Month

Two Sample Z-test:

- Pathogens and Malformations

Results

Larval Abundance

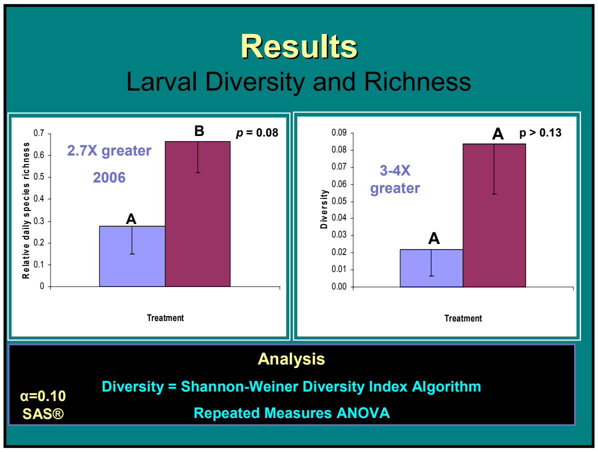


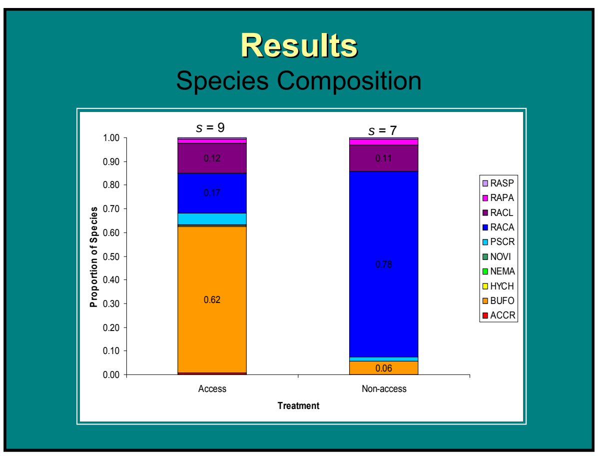
All other $p \geq 0.11$

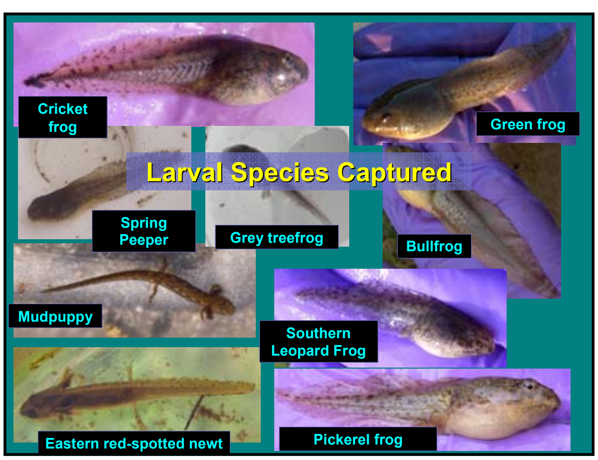
α=0.10 SAS®

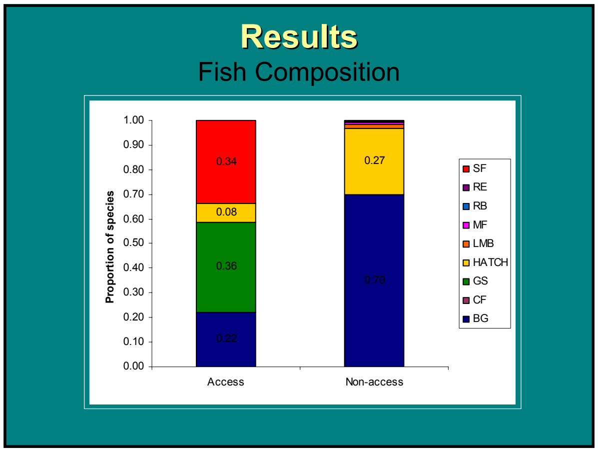
Analysis

Repeated measures ANOVA


Results
Larval Diversity and Richness
Relative daily species richness
2.7X greater
2006
B
p = 0.08
A
Treatment
Diversity
3-4X
greater
A
p > 0.13
A
Treatment
Analysis
α=0.10
SAS®
Diversity = Shannon-Weiner Diversity Index Algorithm
Repeated Measures ANOVA

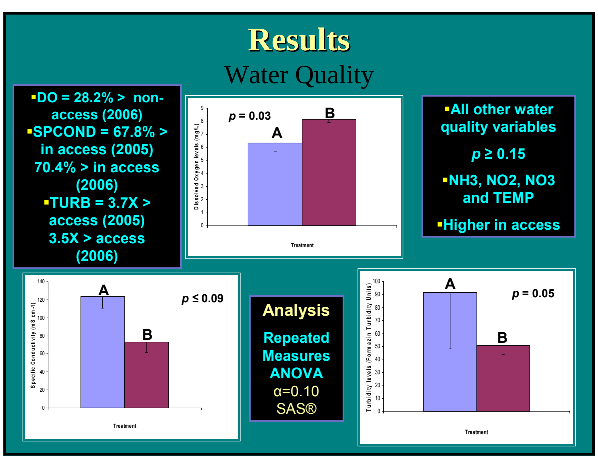

Results
Species Composition
s = 9
s = 7
Proportion of Species
0.12
0.17
0.62
0.11
0.78
0.06
RASP
RAPA
RACL
RACA
PSCR
NOVI
NEMA
HYCH
BUFO
ACCR
Access
Non-access
Treatment


Larval Species Captured
Cricket frog
Green frog
Spring Peeper
Grey treefrog
Bullfrog
Mudpuppy
Southern Leopard Frog
Eastern red-spotted newt
Pickerel frog

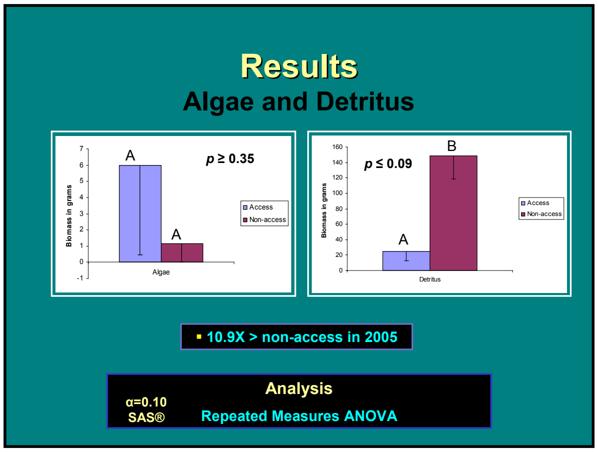


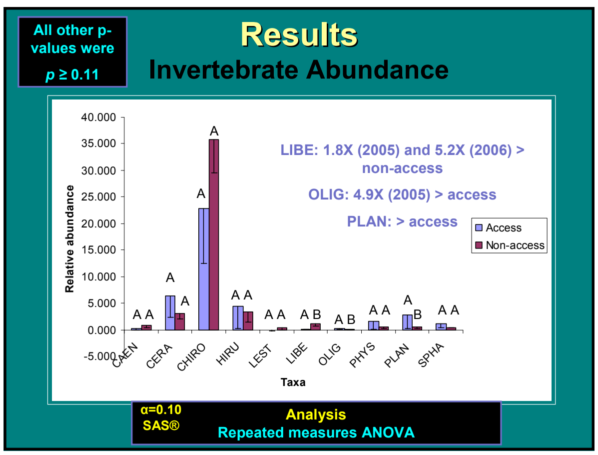
Results
Fish Composition
Proportion of species
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.34
0.08
0.36
0.27
Access
Non-access
SF
RE
RB
MF
LMB
HATCH
GS
CF
BG

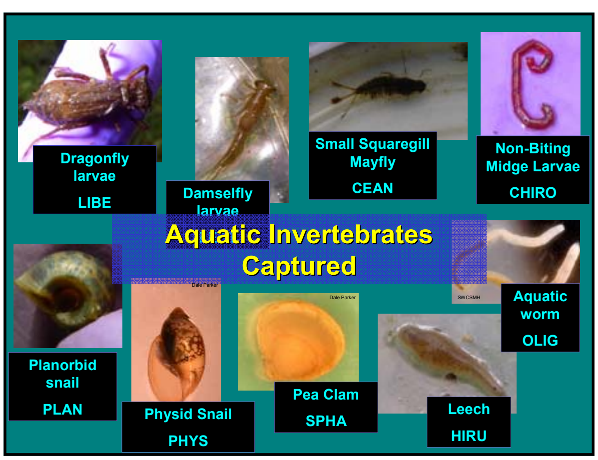
Red-eared Sunfish
Largemouth Bass
Fish Species Captured
Bluegill
Golden Shiner
Green Sunfish



Results
Water Quality
▪DO = 28.2% > non-access (2006)
▪SPCOND = 67.8% > in access (2005)
70.4% > in access (2006)
▪TURB = 3.7X > access (2005)
3.5X > access (2006)
p = 0.03
A
B
Treatment
▪All other water quality variables
p ≥ 0.15
▪NH3, NO2, NO3 and TEMP
▪Higher in access
A
B
p ≤ 0.09
Treatment
Analysis
Repeated Measures ANOVA
α=0.10
SAS®
A
B
p = 0.05
Treatment


Results
Algae and Detritus
A
$p \geq 0.35$
A
Biomass in grams
Algae
Access
Non-access
B
$p \leq 0.09$
A
Biomass in grams
Detritus
Access
Non-access
▪ 10.9X > non-access in 2005
Analysis
α=0.10
SAS®
Repeated Measures ANOVA


All other p-values were
$p \geq 0.11$
Results
Invertebrate Abundance
LIBE: 1.8X (2005) and 5.2X (2006) > non-access
OLIG: 4.9X (2005) > access
PLAN: > access
Access
Non-access
Relative abundance
40.000
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0.000
-5.000
CAEN
CERA
CHIRO
HIRU
LEST
LIBE
OLIG
PHYS
PLAN
SPHA
Taxa
α=0.10
SAS®
Analysis
Repeated measures ANOVA


Dragonfly larvae
LIBE
Damselfly larvae
Small Squaregill Mayfly
CEAN
Non-Biting Midge Larvae
CHIRO
Aquatic Invertebrates Captured
Aquatic worm
OLIG
Planorbid snail
PLAN
Physid Snail
PHYS
Pea Clam
SPHA
Leech
HIRU

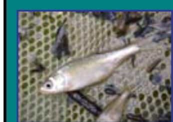
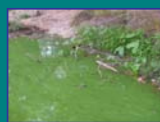
Results

Regression Model

2005 BUFO = 0.098 (TURB) – 5.076 (NH_3)
RAPA = 0.004 (OFISH)

2006 RACA = 0.393 (OFISH)
RACL = – 0.026 (SPCOND) + 0.556 (SR)
RAPA = 0.001 (PREDF)

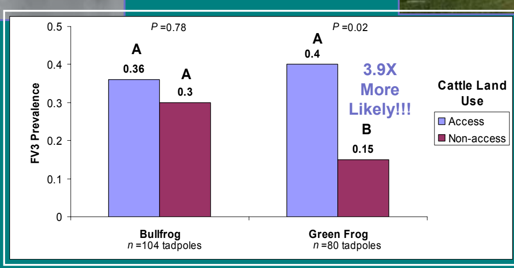
standardized coefficients presented


Results

Pathogen Prevalence

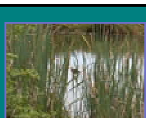
Treatment



Analysis

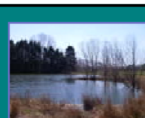
Logistic Regression and Maximum Likelihood Estimation

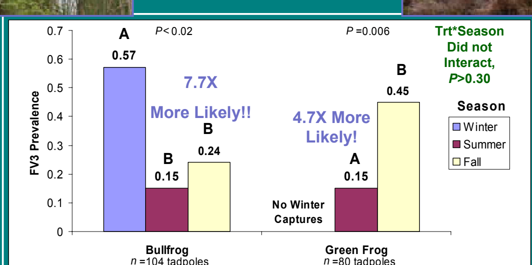
Results



Pathogen Prevalence

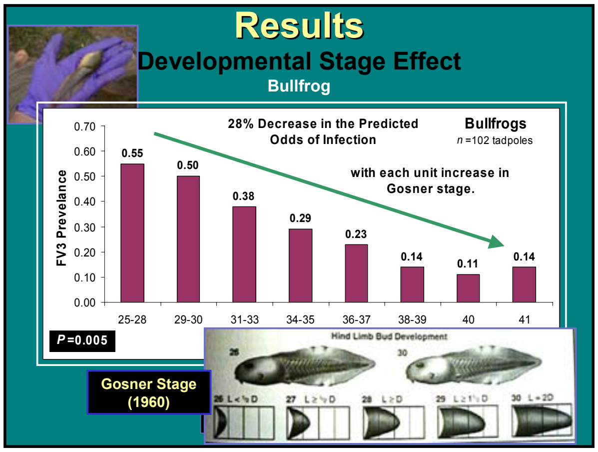
Seasonal Effects

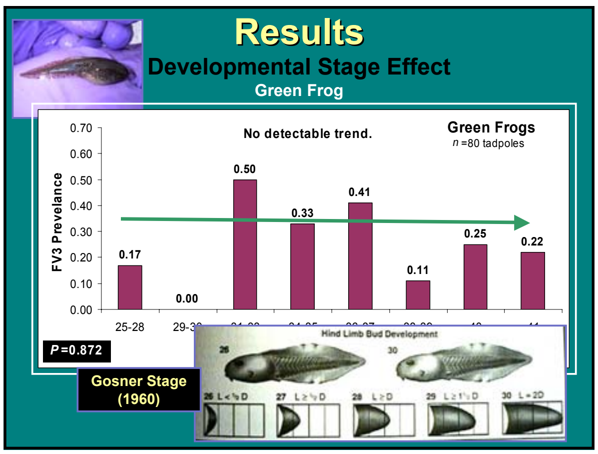




Analysis

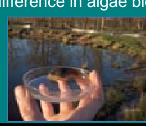
Logit and Logistic Regressions and Maximum Likelihood Estimation





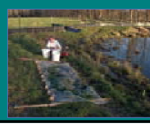
Summary of Results

- **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.

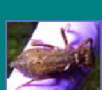


Discussion

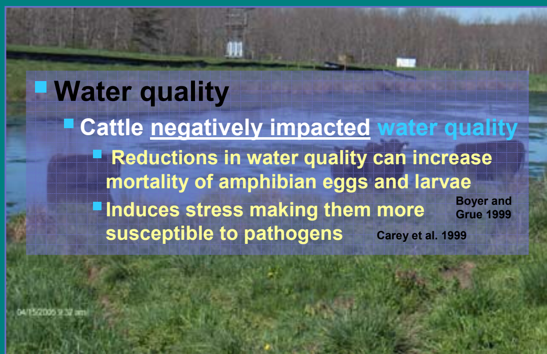
- **Larval Abundance**
  - It was documented that cattle access wetlands negatively impacted **American bullfrog** and **green frog** tadpole populations
    - Water quality and fish abundance were important predictors of abundance
  - It appears that **American** and **Fowler's toad tadpoles** were **not negatively impacted** by cattle access
    - Higher resistance to water quality   Jofre and Karasov 1999
    - Exploitation of habitat where there is lower abundance of ranids

Discussion

- **Detritus and Algae**
  - **Detritus > in non-access wetlands**
    - **In-direct 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**   Voshell 2002
  - **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
    - Reductions in water quality can increase mortality of amphibian eggs and larvae
    - Induces stress making them more susceptible to pathogens

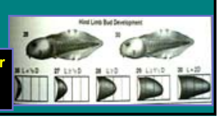
Boyer and Grue 1999

Carey et al. 1999

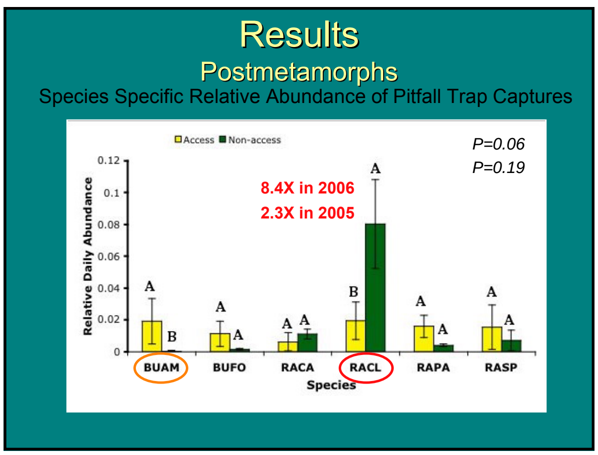
Discussion

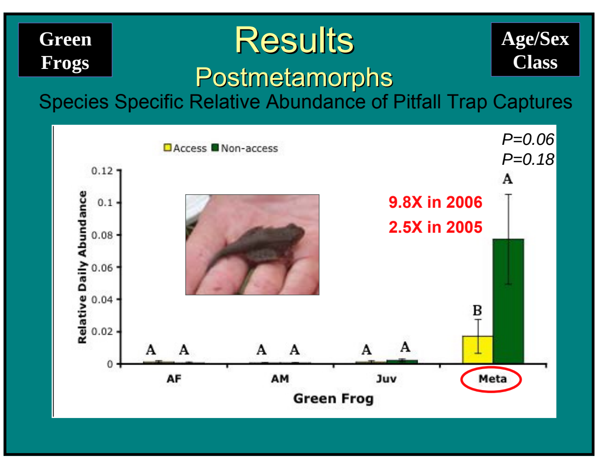
- Pathogen Prevalence- Frog Virus 3 (FV3)
  - Water quality
    - Effected green frog survival — Jofre and Karasov 1999
    - Potentially compromised immunity
  - Seasonal Effects
    - Low temperatures increases pathogen prevalence
    - Low temperatures cause a decrease in overall immune function — Maniero and Carey 1997
  - Developmental Stage
    - Immunity could increase in bullfrogs — Brunner et al., 2004
    - Susceptible tadpoles at earlier stages experienced mortality

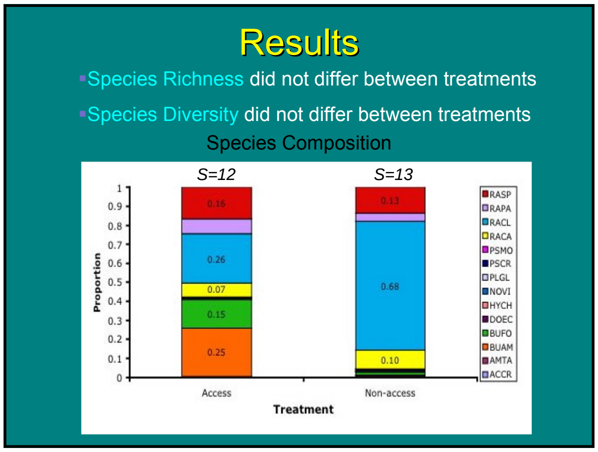
Gosner Stage (1960)

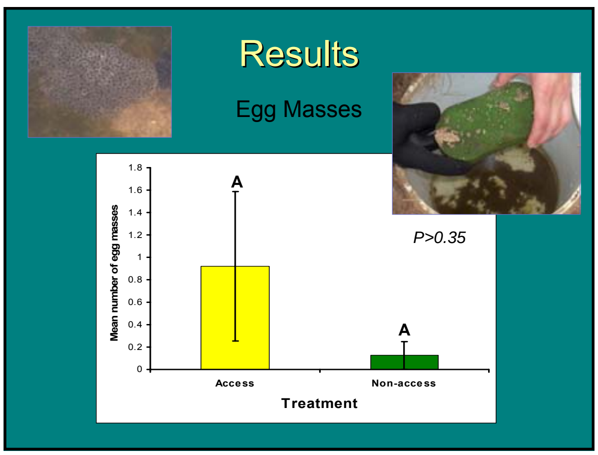


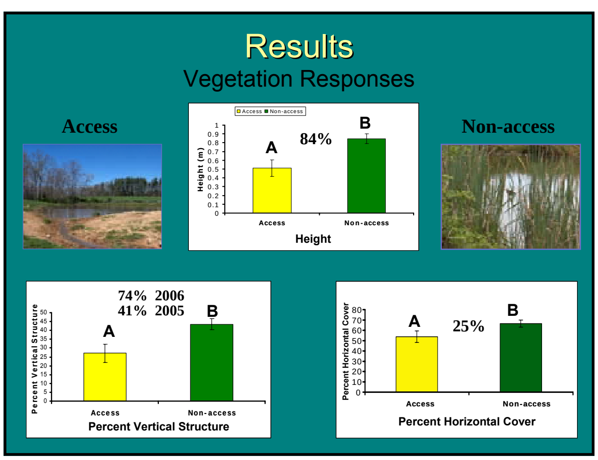

Ambystoma talpoideum
Desmognathus ocoee
Pseudotriton montanus
Notophthalmus viridescens
Plethodon glutinosus


Results
Postmetamorphs
Species Specific Relative Abundance of Pitfall Trap Captures
Access
Non-access
P=0.06
P=0.19
8.4X in 2006
2.3X in 2005
Relative Daily Abundance
0.12
0.1
0.08
0.06
0.04
0.02
0
A
B
A
A
A
A
B
A
A
A
A
A
BUAM
BUFO
RACA
RACL
RAPA
RASP
Species


Green Frogs
Results
Age/Sex Class
Postmetamorphs
Species Specific Relative Abundance of Pitfall Trap Captures
Access
Non-access
P=0.06
P=0.18
9.8X in 2006
2.5X in 2005
Relative Daily Abundance
0.12
0.1
0.08
0.06
0.04
0.02
0
A
A
A
A
A
A
B
A
AF
AM
Juv
Meta
Green Frog


Results
▪Species Richness did not differ between treatments
▪Species Diversity did not differ between treatments
Species Composition
S=12
S=13
Proportion
0.16
0.26
0.07
0.15
0.25
0.13
0.68
0.10
Access
Non-access
Treatment
RASP
RAPA
RACL
RACA
PSMO
PSCR
PLGL
NOVI
HYCH
DOEC
BUFO
BUAM
AMTA
ACCR

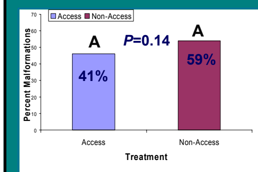

Results
Egg Masses
Mean number of egg masses
A
A
P>0.35
Access
Non-access
Treatment


Results
Vegetation Responses
Access
Non-access
Height (m)
A
B
84%
Height
Percent Vertical Structure
74% 2006
41% 2005
A
B
Percent Vertical Structure
Percent Horizontal Cover
A
B
25%
Percent Horizontal Cover

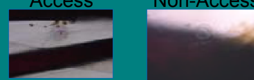
Results: Pathogens

- No differences in bacterial, viral or parasitic prevalence between treatments
- 35 malformed individuals
- 11 Malformation types

2% malformation rate



Access Non-Access



2 individuals with trematode metacercariae

Summary of Results

- Green frog metamorphs 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

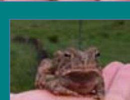
Discussion

- **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
    - Desiccation, 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

2006 RACL = -0.002(Specific Conductivity)

$R^2_{adj} = 0.79$ 82% = Specific Conductivity

2005 BUAM = 0.0004(Cattle) – 0.39(NO_2) – 0.0002(Turbidity) – 0.03(PO_4)

$R^2_{adj} = 0.99$ 83% = Cattle Density

2006 BUAM = 0.0001(Turbidity) – 0.006(NH_3) + 0.002(Temperature)

$R^2_{adj} = 0.99$ 90% = Turbidity

Conservation Implications

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

Acknowledgements

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